Table of Content / Supplemental Material to:

Sonabend et al: Repeated blood-brain barrier opening by an implantable ultrasound device for enhanced delivery of albumin-bound paclitaxel – a phase 1 trial in recurrent glioblastoma. Lancet Oncology 2023

Methods, Supplementary Details

Anatomical considerations related to inclusion criteria:

At time of screening, the principal investigator studied each MRI using DICOM images in 3 planes and assessed whether a rectangular prism derived from the dimensions of the SC9 projected from a plane parallel to the skull surface, can be positioned to cover the peri-tumoral brain. This considered the surgical plan. For instance, if a lobectomy was planned, the targeted region only included the un-resected peri-tumoral brain that would remain after the lobectomy.

Surgical implantation of the SC9 device:

The localization of this cranial window was optimized using neuronavigation to maximize the coverage of peri-tumoral brain after resection, as the dimensions of the device and field of sonication related to SC9 is the same across patients. When necessary, the previous craniotomy was expanded to accommodate optimal positioning of the implant.

LIPU-MB-based BBB opening with SC9:

For each sonication, the ultrasound emitters were each sequentially activated to emit a 25 ms pulse every two seconds for 4½ minutes (270 seconds) at a maximum pressure output of 1.03 MPa, as this pressure was deemed safe and effective for BBB opening in a previous trial with a first-generation of the device with a single emitter(1, 2). The previously used implantable ultrasound device (SonoCloud-1) is equivalent to one of the emitters of the SonoCloud-9 (10 mm diameter, 1 MHz ultrasound transducer). The reported pressure is the pressure amplitude in water at the free-field maximum and is

approximately linear. The delivery of ultrasound energy to the brain tissue was started at the time of IV injection of MB (bolus over 20 seconds, followed by a saline IV flush of 10 ml over 30 seconds). The MB were used as ultrasound resonators (10 µl/kg, Definity®, Lantheus, Billerica, MA).

Phase 1 trial design statistics:

The BOIN design is optimized for identifying the MTD while minimizing the risk of assigning patients to sub-therapeutic or overly toxic doses.**(3)** Decision for dose level allocation was assessed at the completion of the DLT period (cycle 1, 21 days) of the previous patient based on occurrence or absence of DLT. Once a total of 12 patients had been safely treated at a given dose level, the phase 1 trial was considered concluded.

Imaging analysis of BBB closure:

Gadolinium enhancement attributable to BBB opening induced by the SC9 implant was evaluated as previously described.(4) Enhancement maps were computed from non-rigidly registered pre- and post-sonication T1w images. Sonicated regions of interest (ROI) were defined by 10-mm diameter x 75-mm length cylinders in front of each of the 9 emitters of the implant, considering only brain tissue that was not enhanced prior to sonication. The volume with detectable ultrasound-induced gadolinium enhancement was determined in the sonicated ROI by thresholding the enhancement map (threshold level: 1st centile of non-sonicated control ROI). The percentage of the sonicated volume with detectable BBB disruption was then calculated taking into account the volume of brain tissue that did not enhance prior to sonication.

Intraoperative pharmacokinetic study:

The use of corticosteroids or mannitol was avoided for all cases where we performed intraoperative pharmacokinetic studies. Biopsy of non-eloquent peri-tumoral brain was performed when feasible and justified as per standard neurosurgical technique as previously reported.(4-6) For these studies we decreased the FiO2 as much as tolerated up to 20% to obtain an arterial O2 pressure <100 mm/Hg. We exposed the peri-tumoral brain to be excised, positioned the SC9 device in the cranial window, flooded the field with sterile saline, and infused IV microbubbles while sonicating the brain. Immediately after, we infused fluorescein 500 mg IV, and of chemotherapy (i.e. ABX [40 mg/m^2 in patient #1, 80 mg/m^2 in subsequent patients] or carboplatin (CBDCA) [AUC 3.5]) over 30 minutes. LIPU-MB-based BBB opening was visualized and mapped using fluorescent microscopy (Zeiss™ Yellow 560 nm filter), and this was used for guiding the biopsy of peri-tumoral sonicated and non-sonicated brain for further analysis. Representative fluorescence images of the brain, and corresponding stereotaxic coordinates were obtained for each biopsy. For this, intraoperative neuronavigation was done with the Brainlab Curve system. Biopsies were performed using free-hand technique recording the localization of the biopsies using the Brainlab stereotaxic wand.

Biopsy of peri-tumoral brain:

To minimize drug contamination from enhancing tumor core, between samples, and from circulating drug in blood, samples whose stereotaxic coordinates were closer than 1 cm from enhancing tumor were excluded from analysis, non-sonicated samples were obtained first, and new scalpels and separate microsurgical instruments were used for each biopsy. In addition, every biopsy was washed in 1 liter of saline solution to minimize

residual blood in the sample surface. For drug quantification, biopsies of sonicated and non-sonicated peri-tumoral brain were collected starting 45 minutes after LIPU-MB, and the initiation of drug infusion. Samples were sectioned into approximately 30 mg aliquots and flash-frozen for subsequent quantification of drug and fluorescein levels.

Drug quantification:

PTX and CBDCA were determined in plasma and brain tissue using LC-MS/MS (5500 Triple Quad equipped with an ExionLC™ AC20, SCIEX, Framingham, MA). For PTX analysis, a 50 µL aliquot of sample was mixed with 150 µL of acetonitrile (ACN) containing 7.5 ng of PTX-d5 (Internal Standard; IS) in a 96-well deep well plate. After shaking for 5 minutes, the sample was centrifuged at 4000 rpm for 10 mins at 4°C. An aliquot of 75 µL of supernatant was transferred to another 96-well deep well plate and diluted with 75 µL of water before instrumental analysis. Chromatographic separation was achieved with a Kinetex C18, 50x2.1mm, 2.6 µm (Phenomenex, Torrance, CA) column. The mobile phase was A: 0.1% formic acid in water (v/v) and B: 0.1% formic acid in ACN (v/v). After injection, initial conditions with A at 45% were held for 0.5 min, decreased to 10% in 0.5 min and held for 3 min before returning to initial conditions within 0.5 min and re-equilibration for 1.5 min before the next sample. The flow rate was 0.3 ml/min at 25 °C. Retention times for PTX and PTX-d5 were both 1.1 min with a total run time of 4 min. A turbo ion spray interface was used as the ion source operating in positive mode. Acquisition was performed in multiple reaction monitoring mode (MRM) using m/z 854.5 → 286.0 and 859.5 → 569.2 ion transitions at low resolution for PTX, and PTX-d5, respectively.

For CBDCA analysis, a 50 µL aliquot of sample was mixed with 150 µL of ACN containing 50 ng of carboplatin-d5 (IS) in a 96-well deep well plate. After shaking for 5 minutes, the sample was centrifuged at 4000 rpm for 10 mins at 4°C. An aliquot of 100 µL of supernatant was transferred to another 96-well deep well plate and diluted with 200 µL of ACN before instrumental analysis. Chromatographic separation was achieved with a Luna HILIC, 100x2 mm, 3 µm (Phenomenex, Torrance, CA) column. The mobile phase was A: 5 mM ammonium acetate in 10% ACN and 0.1% formic acid (FA) in water (v/v) and B: 0.5 mM ammonium acetate in 90% ACN and 0.1% FA in water (v/v). After injection, initial conditions with A at 0% were held for 0.5 min, increased to 60% in 0.5 min and held for 2 min before returning to initial conditions within 0.5 min and re-equilibration for 3.5 min before the next sample. The flow rate was 0.3 ml/min at 25 °C. Retention times for both CBDCA and IS were 2.7 min with a total run time of 6 min. A turbo ion spray interface was used as the ion source operating in positive mode. Acquisition was performed in multiple reaction monitoring mode (MRM) using m/z 372.3 → 294.0 and 376.3 → 298.0 ion transitions at low resolution for CBDCA and IS, respectively.

Sodium fluorescein was determined by fluorescence spectroscopy using a Spectramax® M2 (Molecular Devices, LLC) with excitation at 460 nm and emission 540 nm. For plasma analysis, a 100 µL aliquot of plasma was mixed with 300 µL of 0.1% formic acid in acetonitrile (v/v) in a 2 mL microcentrifuge tube. After shaking for 5 min, the sample was centrifuged at 4000 rpm for 10 min at 4°C. An aliquot of 20 µL of supernatant was transferred to a 2-ml micro-centrifuge tube and mixed with 350 µL of 10mM phosphate buffer solution (pH=8.5). After shaking for 3 min, an aliquot of 200 µL sample was transferred into a 96-well black microplate for fluorescence analysis. The sample was

protected from light. Brain tissue specimens were homogenized as before, and the extracts processed as described for plasma samples.

For determination of hemoglobin in tissue, brain specimens were homogenized as described above, and the extracts were analyzed for hemoglobin by spectrophotometry using a Spectramax® M2 (Molecular Devices, LLC) with excitation at 540 and 570 nm.

Statistical analyses:

MRI-based analysis of BBB closure: For analysis of post-sonication enhancement for each of the 9 emitters between two different cycles presented in Figure 2B, the significance was calculated using student's two-tailed unpaired t-test. The analysis of BBB closure presented in Figures 2C and 2D included 2 patients with 2 datapoints, and 16 patients with single datapoints. For a simple data sample without repeated measures, the linear model fitting led to the same p-value as Pearson's correlation. Therefore, to analyze the data including the two patients with 2 datapoints, we computed and report a linear mixed-effect model to describe the relationship between time of sonication to time of gadolinium injection versus enhancement on post-sonication MRI (Figure 2B) and between time of gadolinium injection to time of MRI acquisition versus enhancement on post-sonication MRI (Figure 2C).

References

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3. Yuan Y, Hess KR, Hilsenbeck SG, Gilbert MR. Bayesian Optimal Interval Design: A Simple and Well-Performing Design for Phase I Oncology Trials. Clin Cancer Res. 2016;22(17):4291-301.
4. Heimans JJ, Vermorken JB, Wolbers JG, Eeltink CM, Meijer OW, Taphoorn MJ, et al. Paclitaxel (Taxol) concentrations in brain tumor tissue. Ann Oncol. 1994;5(10):951-3.
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Supplementary Table 1. Performance status and neurologic deficits at screening, pre-Cycle 1 and pre-cycle 2**.**

		Screening			Postop. / Pre-cycle 1			Pre-cycle 2		
ID	Location	Perf. Status		Neurol. Deficit	Perf. Status		Neurol. Deficit	Perf. Status		Neurol. Deficit
		KPS	WHO		KPS	WHO		KPS	WHO	
101	L. Parietal	100	0	R. visual field defect	100	0	R. visual field defect; dysmetria	90	1	R. visual field defect
102	Right Occipital	90	1	L. visual field defect	90	1	L. visual field defect	100	1	L. visual field defect
103	Right Parietal	90	1	L. visual field defect	90	1	L. visual field defect; dysphasia	90	1	L. visual field defect
104	Right Temporal/parietal	100	0	None	80	1	L. visual field defect, dysphasia, dysarthria, left facial droop	90	1	L. visual field defect
105	Right Temporal	90	1	L. visual field defect	90	1	L. visual field defect	90	1	L. visual field defect
106	Right Parietal	100	0	None	90	1	None	100	1	None
107	Left Parietal	90	1	R. visual field defect	80	1	R. visual field defect, dysphasia, ataxia, memory impairment	90	1	R. visual field defect
108	Left Frontal	90	1	Dysphasia	80	1	Dysphasia	100	1	Dysphasia
109	Left Parietal	80	1	R. visual field defect, R. sided muscle weakness, bilateral leg weakness	80	1	R. visual field defect, right sided muscle weakness, bilateral leg weakness, right sided decreased sensation, memory impairment	80	1	R. visual field defect, right sided decreased sensation, flat affect
110	Right Parietal	90	1	L. arm weakness	80	1	L. sided decreased sensation, arm weakness, L. visual field defect, dysmetria	90	1	L. sided decreased sensation, arm weakness
111	Left Temporal	100	0	R. visual field defect, dysphasia	100	0	R. visual field defect, dysphasia	90	1	R. visual field defect, dysphasia
112	Left Parietal	100	0	Dysphasia	90	1	Dysphasia, R. sided hyperreflexia	90	1	Dysphasia
113	Right Frontal	90	1	L. sided weakness	80	1	L. sided weakness and dysmetria	80	1	L. sided weakness, tremor

114	Right Frontal	90	1	L. sided weakness	70	1	L. sided weakness, left sided incoordination	80	1	L. sided weakness
115*	Right Frontal	90	1	L. arm weakness	70	2	L. sided weakness, L. facial droop, dysarthria, L. sided incoordination	60	2	L. sided weakness, left facial droop, dysarthria, left sided incoordination, cognitive impairment
117**	Right Occipital	80	1	L. visual field defect, left leg weakness	60	2	L. visual field defect, L. sided weakness	80	1	L. visual field defect, left sided weakness
118	Right Frontal	100	1	None	100	0	Dysphasia	90	1	None
* Subject with confirmed disease progression during Cycle 2 ** Patient had long- standing left leg weakness unrelated to disease progression										
R = right, L = left WHO, World Health Organization; KPS, Karnofsky Performance Status										

Supplementary Table 2: Tumor location and sonication-related neurological adverse events by cycle. These tended to appear immediately after sonication. Most of these resolved within minutes, and all within 2 hours of sonication.

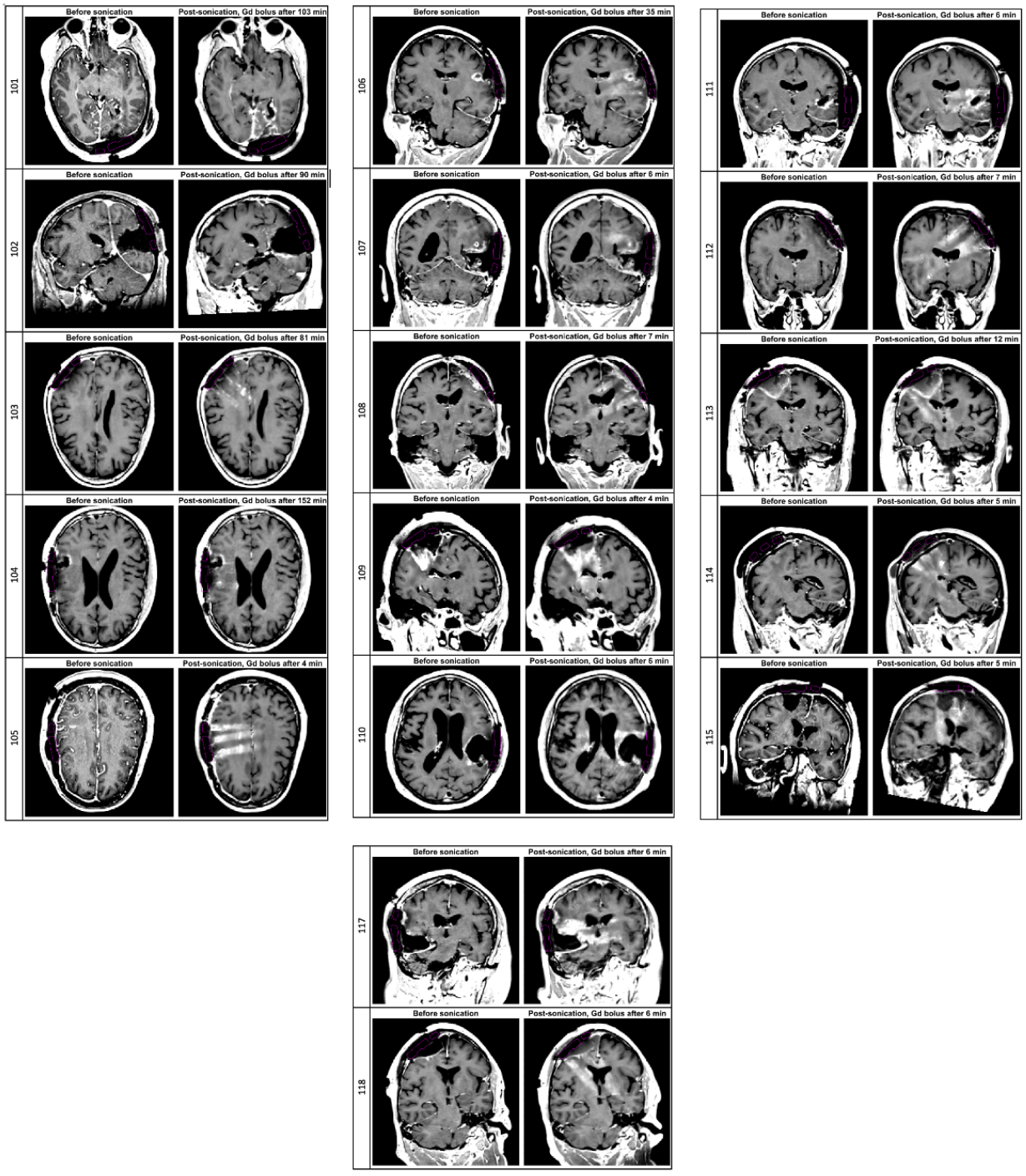
ID	Tumor location	Cycle 1	Cycle 2	Cycle 3	Cycle 4	Cycle 5	Cycle 6
101	Left parietal	Gr 1 Headache Gr 2 Seizure	Gr 1 Headache Gr 1 Blurred vision	Gr 1 Headache Gr 1 Blurred vision	NA	NA	NA
102	Right occipital	Gr 1 Headache Gr 1 Paresthesia	Gr 1 Headache Gr 1 Paresthesia	Gr 1 Headache Gr 2 Scalp pain Gr 1 Flushing	NA	NA	NA
103	Right parietal	Gr 1 Headache	Gr 1 Headache	Gr 1 Headache	NA	NA	NA
104	Right temporo-parietal	None	Gr 1 Euphoria	None	NA	NA	NA
105	Right temporal	Gr 1 Headache Gr 1 Seizure Gr 1 Dysphasia	Gr 1 Headache Gr 1 L Facial muscle weakness Gr 1 Dysphasia	Gr 1 Headache Gr 1 Blurred vision Gr 1 Dysarthria Gr 1 Dizziness	Gr 1 Somnolence	Gr 1 Somnolence	Gr 1 Somnolence
106	Right parietal	Gr 1 Blurred vision Gr 1 L Paresthesia	Gr 1 Blurred vision Gr 1 L Paresthesia	Gr 1 L Paresthesia	Gr 1 L Dysesthesia	Gr 1 L Dysesthesia	None
107	Left parietal	Gr 1 Blurred vision Gr 1 Headache	Gr 1 Headache Gr 1 Psych other (lability)	None	NA	NA	NA
108	Left frontal	Gr 1 Seizure	Gr 1 Headache Gr 2 UL R muscle weakness	Gr 1 R UL muscle weakness Gr 1 R Dysesthesia	Gr 2 R UL muscle weakness	Gr 1 Headache	Gr 1 R Dysesthesia
109	Left parietal	Gr 1 Headache	Gr 1 Headache	None	NA	NA	NA
110	Right parietal	Gr 1 Dizziness Gr 1 Dysphasia	Gr 1 Dysphasia Gr 1 Dysarthria	Gr 1 Dysphasia	Gr 1 Dysphasia	Gr 1 Dysphasia	NA
111	Left temporal	Gr 3 Encephalopathy Gr 1 Hyponatremia Gr 3 Dysphasia Gr 2 Headache	Gr 1 Headache Gr 1 R Facial muscle weakness Gr 1 R Dysesthesia	None	None	Gr 1 Headache Gr 2 Seizure	NA
112	Left parietal	Gr 1 Headache Gr 1 scalp pain	Gr 1 Headache Gr 1 Dizziness	Gr 1 Headache	NA	NA	NA
113	Right frontal	None	Gr 1 Headache	Gr 1 scalp pain	Gr 1 scalp pain	Gr 1 scalp pain	None
114	Right frontal	None	Gr 2 L UL muscle weakness	Gr 1 Blurred vision	None	None	NA
115	Right frontal	None	None	NA	NA	NA	NA
117	Right occipital	None	Gr 2 Encephalopathy Gr 2 Headache	None	NA	NA	NA
118	Right frontal	Gr 1 Headache	None	None	NA	NA	NA

Right (R), Left (L), Grade (Gr), upper limb (UL), non-applicable (NA).

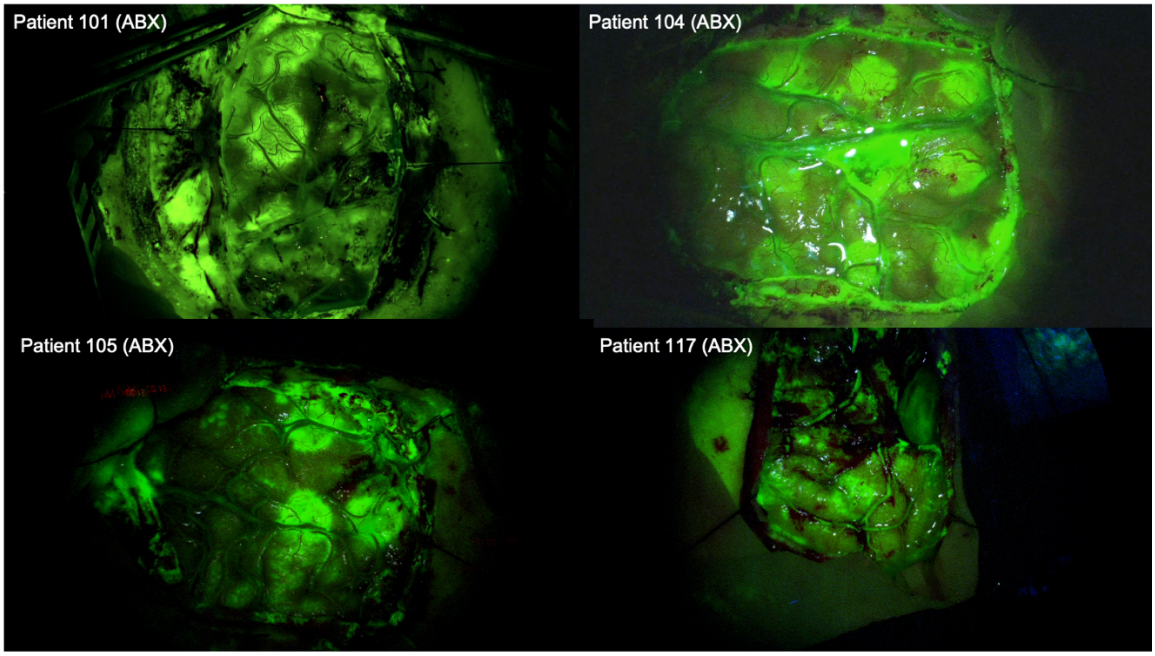
Supplementary Table 3. Treatment related-emergent toxicities grade 2-4 occurring in cycle 1, or worst grade over all cycles (n=17 patients).

	Cycle 1 (n=17 pts)						All 68 cycles, worst grade per patient (n=17)							
Grade	**2**		**3**		**4**		**2**		**3**		**4**		**All grades 2-4**	
	n	%	n	%	n	%	n	%	n	%	n	%	n	%
Hematological Toxicity														
Anemia	4	24%	-	-	-	-	7	41%	-	-	-	-	7	41%
Leucopenia	7	41%	5	29%	-	-	9	53%	5	29%	-	-	14	82%
Neutropenia	3	18%	7	41%	1	6%	4	24%	7	41%	1	6%	12	71%
Lymphocytopenia	7	41%	2	12%	-	-	8	47%	3	18%	-	-	11	65%
Thrombocytopenia	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Non-hematological Toxicity														
Encephalopathy	-	-	1	6%	-	-	1	6%	1	6%	-	-	2	12%
Peripheral sensory neuropathy	-	-	1	6%	-	-	-	-	1	6%	-	-	1	6%
Peripheral motor neuropathy	-	-	-	-	-	-	1	6%	-	-	-	-	1	6%
Peripheral neuropathy	-	-	1	6%	-	-	1	6%	1	6%	-	-	2	12%
Alopecia	1	6%	-	-	-	-	6	35%	-	-	-	-	6	35%
Arthralgia	2	12%	-	-	-	-	2	12%	-	-	-	-	2	12%
Anorexia	1	6%	-	-	-	-	1	6%	-	-	-	-	1	6%
Sonication procedure related toxicity †														
Seizure	1	6%	-	-	-	-	3	18%	3	18%	-	-	6	35%
Dysphasia			1	6%					1	6%			1	6%
Headache	1	6%	-	-	-	-	10	59%	-	-	-	-	10	59%
Muscle weakness							3	18%					3	18%
Scalp pain							1	6%					1	18%
† grade 1 transient sonication-associated toxicities over all cycles reported in Supplementary Table 2														

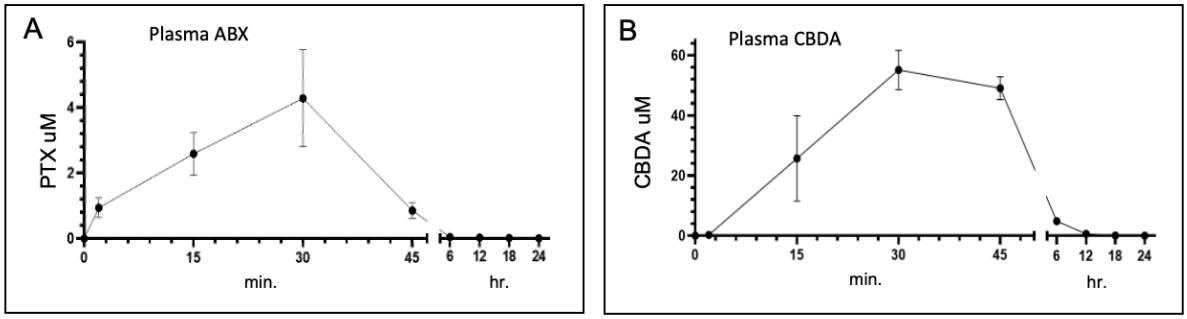
Supplementary Figure 1: Contrast-enhanced MRI images of pre and post LIPU-MB, and time between start of LIPU-MB to gadolinium injection for all subjects.



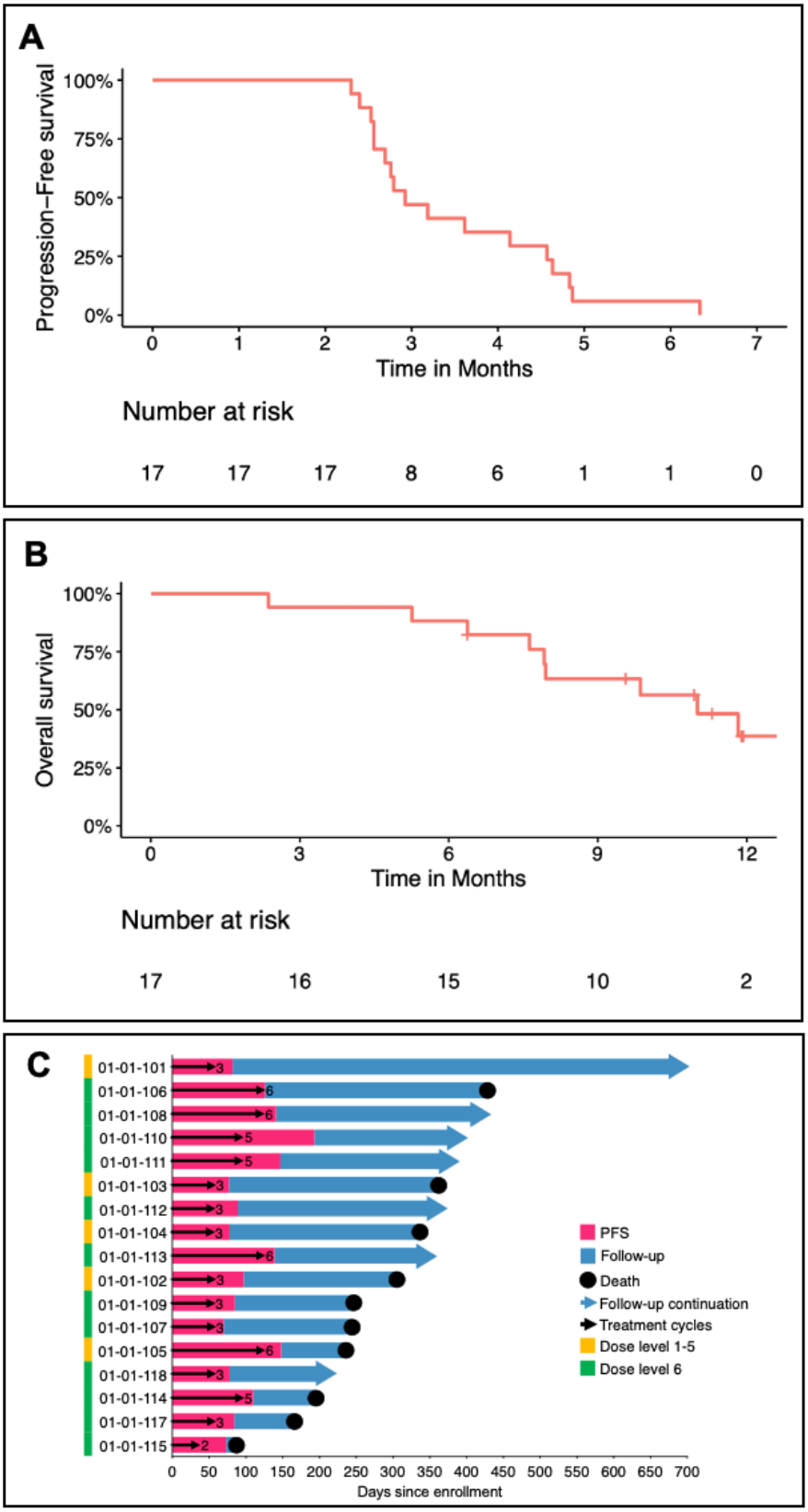
Supplementary Figure 2: Additional fluorescent images of 4 patients demonstrating BBB opening by fluorescence in the context of intraoperative LIPU-MB following by IV injection of Sodium fluorescein 500 mg, as part of the pharmacokinetic experiments performed.



Supplementary Figure 3: Plasma concentrations of paclitaxel (PTX) (n=7 patients) (B), or carboplatin (CBDCA) (n=3 patients) (C) over time in the context of 30-minute IV infusion. Error bars represent standard error of the mean.



Supplementary Figure 4: Survival curves and time on treatment



Kaplan-Meier plots representing progression-free survival (**A**) and overall survival (**B**). Swimmer's plot describing the treatment cycles for individual patients (**C**).

Summary of protocol amendments.

Modification	Modification Summary
# 1 (minor)	This modification amended study team members.
# 2 (minor)	This modification amended study team members.
# 3 (minor)	This modification amended study team members.
# 4 (minor)	Anticipated enrollment was changed from 30 to 39 participants.
# 5 (minor)	This modification amended study team members.
#6 (approved 2/13/2021)	Protocol amendment to clarify ambiguous areas in the protocol. Administrative changes of study team members and sponsor representative
# 7 (approved 7/22/2021)	The device manufacturer has provided several Updates to the Investigators Brochure by manufacturer and updated Instructions for Use. Informed consent form revisions to address IB updates.
# 8 (approved 7/29/2021)	Protocol amendment to refine tissue analyses and other minor clarifications. Changes to the ICF to reflect changes in translational analyses.
# 9	This modification amended study team members.
# 10 (approved 1/30/2022)	Clarification in ambiguous eligibility criteria (Inclusion #1, allowing inclusion of patients who had not received prior TMZ (e.g. MGMT unmethylated, treated in a clinical trial per NCCN guidelines).

Study Title: **Phase I/II trial of blood-brain barrier opening with the SonoCloud-9 implantable ultrasound device and treatment with albumin-bound paclitaxel in patients with recurrent glioblastoma**

Principal Investigators:

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Intervention(s)	Infusion of albumin-bound paclitaxel with concomitant ultrasound-based disruption of blood brain barrier
IND #	146057
IND Holder	Northwestern University
Funding Sources	Malnati Brain Tumor Institute, Lurie Cancer Center CarThera IST SC9-GBM-03 NCI application pending
CarThera IST	Carole Desseaux Ph.D., Head of Clinical Affairs (carole.desseaux@carthera.eu) Michael Canney Ph.D., Scientific Director (michael.canney@carthera.eu) Hôpital Pitié-Salpêtrière Institut du Cerveau et de la Moelle épinière - ICM iPEPS 47-83 bd de l'Hôpital - CS21414 75646 PARIS Cedex – France
Version and Date	4.0 30JAN2022

Listing of Figures

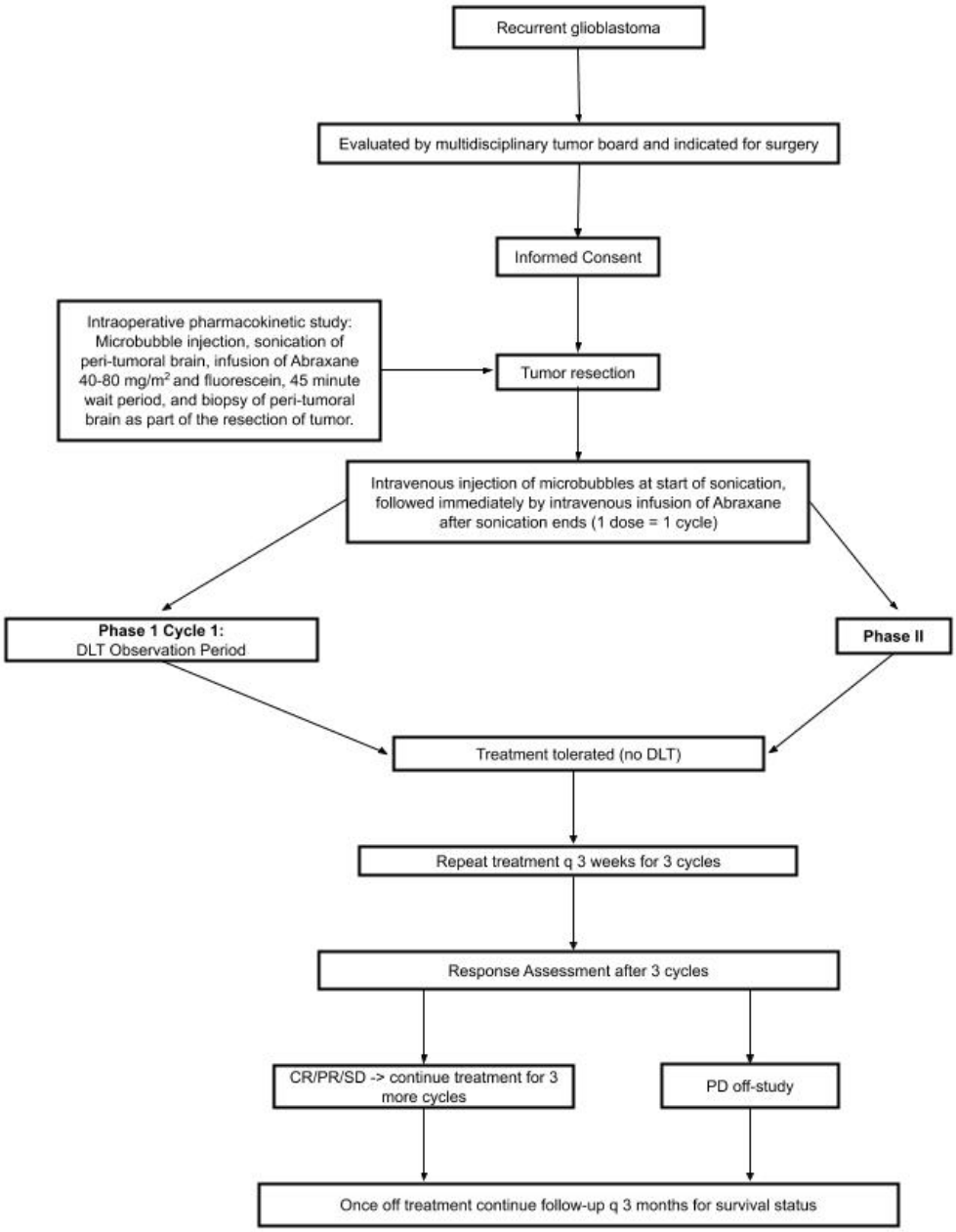
Listing of Tables

Listing of Abbreviations

ABX	Abraxane®, albumin-bound paclitaxel, nab-paclitaxel
AE	Adverse Event
BBB	Blood Brain Barrier
BOIN	Bayesian Optimal Interval design
CNS	Central Nervous System
CSF	Cerebrospinal Fluid
CTCAE	Common Terminology Criteria for Adverse Events
DMC	Data Monitoring Committee
DLT	Dose Limiting Toxicity
DSMP	Data Safety Monitoring Plan
eCRF	Electronic Case Report Form
FDA	Food and Drug Administration
FLAIR	Fluid-Attenuated Inversion Recovery (MRI sequence)
FOCBP	Female of child-bearing potential defined as: - *any woman* (regardless of sexual orientation, having undergone a tubal ligation, or remaining celibate by choice) and who has not: - undergone a hysterectomy or bilateral oophorectomy - *Has had* menses at any time in the preceding 12 consecutive months (and therefore has not been naturally postmenopausal for > 12 months)
GBM	Glioblastoma
GCP	Good Clinical Practice
IC50	Half maximal Inhibitory Concentration
IDH1	Isocitrate Dehydrogenase 1
IRB	Institutional Review Board
IV	Intravenously
KPS	Karnofsky Performance Score
MTD	Maximum Tolerated Dose
MRI	Magnetic Resonance Imaging
NCI	National Cancer Institute
OS	Overall Survival
PCR	Polymerase Chain Reaction
PTX	Paclitaxel
QAM	Quality Assurance Manager
SAE	Serious Adverse Event
SC1/SC3/SC9	SonoCloud-1 system / SonoCloud-3 / SonoCloud-9 (one, three or nine emitters)
SOP	Standard Operating Procedure
SUISE	Significant Ultrasound-Induced Signal Enhancement
UPIRSO	Unanticipated Problem Involving Risks to Subjects or Others
US	Ultrasound
WHO	World Health Organization

1. PROTOCOL SUMMARY

1.1. Study Schema



1.2. Study Synopsis

Full Title	Phase I/II trial of blood-brain barrier opening with the SonoCloud-9 implantable ultrasound device and treatment with albumin-bound paclitaxel in patients with recurrent glioblastoma
Short Title	Ultrasound-based BBB Disruption for Abraxane® Delivery in Recurrent Glioblastoma
Version	4.0 / 30. January 2022
Study Design	Bayesian Optimal Interval (BOIN) design
Study Center	Northwestern University/Northwestern Medicine
Objectives	**Primary:** • Phase I: To evaluate the safety and maximum tolerated dose of albumin-bound paclitaxel up to a dose of 260 mg/m^2 q 3 weeks after pulsed-ultrasound opening of the blood-brain barrier in patients with recurrent glioblastoma. • Phase II: 1-year survival rate (from surgery and Sonocloud-9 (SC9) implantation) • Intraoperative pharmacokinetic study: To determine the effect of ultrasound-based blood-brain barrier disruption on peritumoral brain and glioma tissue paclitaxel concentrations. **Secondary:** • Phase II: To determine safety and efficacy albumin-bound paclitaxel after pulsed-ultrasound opening of the blood-brain barrier. Efficacy will be measured as the time to progression and overall survival for patients with recurrent glioblastoma. **Exploratory:** • Phase I: To investigate the extent of tumor and peritumoral brain fields covered by pulsed-ultrasound blood-brain barrier (BBB) disruption using Magnetic Resonance Imaging (MRI) with gadolinium immediately after sonication. • Phase I and II: Investigate whether ultrasound (US)-based BBB disruption controls tumor growth and prevents local recurrence, compared to regions outside the field of sonication. • Objective response rate according to RANO criteria **Correlative**: • To investigate the level of circulating tumor DNA detected in peripheral blood before and after US-mediated BBB disruption and correlation with overall survival
Sample Size	Phase I: approx. 17 patients Phase II: an additional 18 evaluable patients Assuming possibility of 15% non-evaluable subjects, approximately 41 may be enrolled

Diagnosis & Key Eligibility Criteria	**Inclusion criteria:**
	• Confirmed diagnosis of Isocitrate Dehydrogenase 1 (IDH1) wild-type glioblastoma on pathology from initial surgery (e.g. IDH R132H neg)
	• Ability to undergo contrast-enhanced MRI
	• Radiographic evidence of tumor recurrence/progression after failure of 1 – 2 lines of prior therapy
	• Measurable or evaluable disease
	  ○ Measurable: contrast-enhancement (bidirectional diameters ≥ 1cm) on MRI
	  ○ Non-measurable/evaluable: contrast-enhancement diameters < 1 cm
	• Maximal tumor diameter pre-surgery ≤ 70 mm on T1wMRI
	• Candidate for at least partial surgical resection
	• Greater 12 weeks from completion of radiation therapy
	• Age ≥ 18 years
	• If receiving dexamethasone for mass effect, a stable daily dose of dexamethasone at ≤ 6 mg within 7 days of registration, or if dexamethasone dose is decreasing, average daily dose of ≤ 6 mg in the 7 days prior to registration. Patients on dexamethasone for reasons other than mass effect may still be enrolled. WHO performance status ≤ 2 (equivalent to Karnofsky Performance Status (KPS) of ≥70)
	• Adequate hepatic, renal and bone marrow function, documented with normal laboratory values or no more than grade 1 outside the norm performed within 14 days prior to registration
	• For patients with a childbearing potential
	  ○ Negative pregnancy test within 14 days prior to registration
	  ○ Agreement to use adequate contraception for the duration of study participation, and for 3 and 6 months after the last dose of nab-paclitaxel for men and women of childbearing potential, respectively.
	• Have the ability to understand and the willingness to sign a written informed consent prior to registration on study
	• Be willing and able to comply with the protocol for the duration of the study
	• Provide written, signed and dated informed consent prior to study registration. NOTE: no study-specific screening procedures may be performed until written consent has been obtained.
	
	Exclusion Criteria:
	• Have multifocal disease that cannot be encompassed in the ultrasound fields:
	  ○ e.g. > 70-mm apart
	  ○ tumor located in the posterior fossa
	• Patients at risk of cranial wound dehiscence
	• Have uncontrolled epilepsy or require treatment with enzyme-inducing antiepileptics
	• Have clinical evidence of peripheral neuropathy on examination
	• Have received any other investigational agents within 4 weeks of registration

	• Have received prior therapy with or have history of allergic reactions attributed to compounds of similar chemical or biologic composition to paclitaxel • Medical contraindications to Abraxane® • Have an uncontrolled intercurrent illness • Are pregnant or nursing • Have a history of active malignancy within 3 years prior to registration. • Have a known history of hypersensitivity reactions to perflutren lipid microsphere components or to any of the inactive ingredients in Definity® (the FDA-approved ultrasound contrast agent to be used in this study) • Patients with coils, clips, shunts, intravascular stents, and/or non-removable wafer, non-resorbable dura substitute, or reservoirs. • Patients with medical need to continue antiplatelet therapy. • Patients with known significant cardiac disease, known to have right-to-left shunts, severe pulmonary hypertension (pulmonary artery pressure > 90 mmHg), uncontrolled systemic hypertension, or adult respiratory distress syndrome (patient at risk for microbubble reaction) • Patients with impaired thermo-regulation or temperature sensation (due to device)
Treatment Plan	Patients will be screened and consented prior to any study related procedure and prior to surgery. At the planned surgery for recurrent tumor debulking and after tumor resection, the sonication device SC9 will be implanted on a cranial window. The removed bone flap will be stored in the bone bank for possible later re-implantation, if required. Routine postoperative MRI for disease assessment will take place within 48 hours after surgery and patient will be discharged. Approximately two weeks after surgery, patients will undergo a sonication baseline MRI for evaluation of residual tumor burden and blood-brain barrier disruption, and 48 hours later (± 24 hours), the first therapy cycle will take place. The sonication device will be connected to the radiofrequency generator using the transdermal needle and sonication will commence (activation of 9 emitters) with simultaneous IV microbubble injection followed by IV injection of Abraxane ® (albumin-bound paclitaxel, ABX) over approximately 30 minutes and when planned, a post-sonication MRI. Immediately after, patients remain in observation for 2-6 hrs. Patients will be closely monitored for signs of toxicity for the whole first cycle (3 weeks) as outpatients. Microbubble injection simultaneously to sonication followed by ABX will be repeated every 3 weeks. Surveillance MRI will be performed after every 3 cycles of treatment or as clinically indicated. Therapy will continue until signs of toxicity or evidence of recurrence.

Endpoints	Phase I: Determination of dose limiting toxicity (DLT) related to sonication and/or ABX. Clinically significant, dose-limiting toxicity is defined as (for details, please refer to protocol section 8.2): Any related toxicity ≥ grade 3 that does not respond to optimal medical management (including steroids) within 10 days, exceptions are enumerated here below. ○ CNS toxicity of ≥ grade 2 that does not revert to grade ≤ 1 within 21 days, i.e. time for next treatment cycle ○ Grade 4 CNS toxicity ○ Any treatment-emergent and related toxicity (except hematotoxicity, nausea/vomiting, fatigue and hypersensitivity to Abraxane® or Definity® microbubble injections) > grade 2 that has not reverted to a grade ≤ 2 by day 22 of the first cycle. Treatment-emergent toxicity/events that are unequivocally not related to the sonication or ABX (e.g. attributed to disease progression) will not be considered as dose-limiting toxicity (DLT). Toxicity will be scored according to the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 5. Patients will be monitored closely throughout the study and all treatment-emergent toxicity will be recorded. We propose to test up to 6 dose levels of IV ABX, 40 mg/m^2, 80 mg/m^2, 135 mg/m^2, 175 mg/m^2, 215 mg/m^2 and 260 mg/m^2. Phase II: The primary endpoint for the Phase II study will be overall survival (OS) at 1 year from ultrasound implantation. Secondary endpoints are overall and progression-free survival, tolerance, feasibility and side effect profile. Exploratory endpoints: Extent of BBB disruption, pattern of tumor recurrence, objective response rate (RANO) and PTX concentration in peritumoral brain tissue after intraoperative ABX test dose administration
Statistical Methodology	*Bayesian adaptive clinical trial statistical approach:* The proposed Phase I trial uses a Bayesian Optimal Interval (BOIN) design. The Phase II component of the proposed clinical trial will build on the Bayesian analysis paradigm established for the Phase I trial and apply a 'BOP2' Bayesian optimal design.

2. BACKGROUND

2.1. Disease Background

Glioblastoma (GBM) remains an incurable disease with a poor prognosis. Two and five-year survival rates are 15-20% and less than 5% respectively.[1] Despite state of the art treatment with surgery, radiation, temozolomide chemotherapy and tumor treating fields therapy at initial diagnosis, these tumors almost always recur or progress, and there are only few treatment options with limited efficacy available for recurrent or progressive disease.[2] The effectiveness of temozolomide in prolonging survival is attributed to some extent to its ability to readily cross the blood-brain barrier (BBB). This is in sharp contrast to other agents that have shown impressive activity in preclinical models and in many solid tumors but have been found to be ineffective in GBM because of their inability to penetrate the BBB. Therapeutic opening of the BBB, using pulsed ultrasound, may provide a greater range of treatment options for GBM, incorporating drugs that may have otherwise been deemed ineffective in the treatment of recurrent GBM.

2.2. Pulsed-Ultrasound for Blood-Brain Barrier Disruption

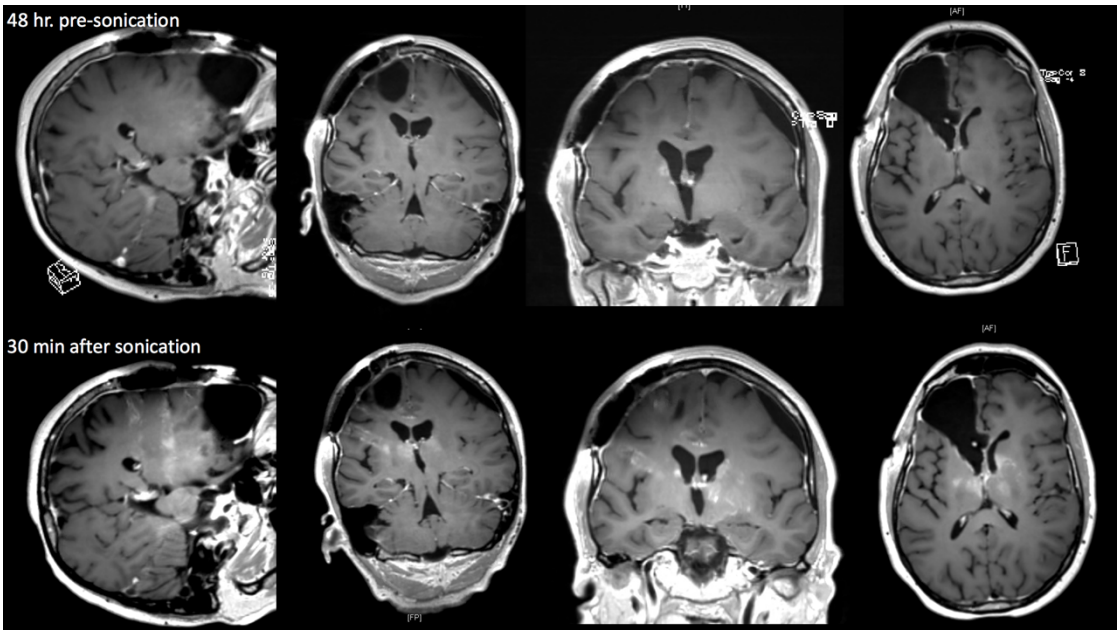
The BBB is a major impediment to drug therapy of brain tumors. Pulsed-ultrasound (sonication), when used in combination with intravenous injection of microbubbles, transiently and reversibly disrupts the BBB. This strategy has been shown to enhance delivery of anti-tumoral agents to the brain in a multitude of pre-clinical models.[3–7]

Translation of this approach into patients requires the ultrasonic waves to bypass the relatively thick human skull. To overcome this, an implantable cranial sonication device, SonoCloud-1 (SC1) was developed.[8,9] Through percutaneous connection of the ultrasound device to an external radio-frequency generator, low-intensity pulsed ultrasound can be delivered sequentially in a controlled manner and for a very short period of time. This regimen allows for local and transient increase in the permeability of the BBB, leading to enhanced penetration of chemotherapy, into the cerebral parenchyma of patients with malignant gliomas.

The SC1 device was tested with carboplatin in a pre-clinical study by our collaborators. In primates, the use of the SC1 device with BBB disruption led to a 7-fold increase in brain carboplatin concentrations.[10] A Phase I clinical trial (NCT02253212) using sonication and microbubble injections with concomitant carboplatin demonstrated feasibility of this approach for opening the BBB and outlined a treatment schedule that was well tolerated. BBB disruption was demonstrated with contrast-enhanced Magnetic Resonance Imaging (MRI) and BBB opening was associated with prolonged progression-free survival.[8,9,11] The sonication regimen was well-tolerated in the context of sonication/microbubble injection with concomitant carboplatin and an optimal acoustic pressure was determined. More recently, a second-generation implantable sonication device that includes three emitters, SonoCloud-3 (SC3) has been tested and deemed safe in a clinical trial, and a third-generation device of nine ultrasound emitters, Sonocloud-9 (SC9) has entered clinical testing in early 2019 (NCT03744026). Sonication with 9 emitters using the SonoCloud-9 System has been demonstrated as safe with no procedure related toxicity identified (7 patients treated, April 2020). Further recruitment of up to 15 patients is ongoing.

Yet, irrespective of the chemotherapeutic agent, the effect of ultrasound-based BBB disruption on drug concentrations in peritumoral human brain, which is key for targeting GBM infiltration beyond surgical margins, remains unexplored.

Figure 1. BBB Disruption with the SonoCloud-9 Device



Representative MRI shows T1w gadolinium contrast enhancement in the brain following BBB disruption with an implantable Sonocloud-9 (SC9) ultrasound *device.*

2.3. Study Rationale

In this study, we hypothesize that BBB disruption using low-intensity pulsed ultrasound will enhance the delivery of paclitaxel (PTX) to glioblastoma tissue and surrounding brain parenchyma, with drug concentrations reaching therapeutic levels for human GBM. To maximize the volume of BBB disruption, we will use the implantable sonication device, SonoCloud-9, that is designed to cover the tumor and surrounding infiltrative tissue. Moreover, we hypothesize that low-intensity pulsed ultrasound augmented delivery of PTX will be safe and effective against recurrent GBM.

In the intraoperative portion of the study, we aim to demonstrate that BBB opening will lead to increased drug concentrations in the tumor and its surrounding tissue. We will measure PTX concentrations in the tumor and in the surrounding infiltrated brain tissue by applying a local sonication using a single emitter at surgery before tumor removal. For this, patients will receive one test dose of Abraxane® (albumin-bound paclitaxel, ABX) perioperatively, followed by sonication/microbubbles immediately preceding resection. Based on our preclinical studies, we anticipate that ultrasound-based BBB disruption will increase PTX concentrations in the brain by at least 5-fold and achieve a brain/plasma concentration ratio of >0.3-0.5.

After device implantation, patients will receive repeated BBB disruption and Abraxane administration every 3 weeks. We will map the field of sonication-induced BBB disruption accomplished by the SC9 device by a post-sonication contrast-enhanced MRI. By overlaying this map with areas of residual tumor from postoperative period and at recurrence, we will determine

whether sonication-induced BBB disruption controls tumor growth and prevents loco-regional recurrence, compared to regions outside the field of sonication.

Our work will address a significant gap of knowledge in the field/technology of ultrasound-based BBB disruption for drug delivery to brain tumors, which is to understand its effects on drug levels in the peritumoral human brain. Our intraoperative study design/approach offers a unique opportunity to address important and fundamental questions relevant to the whole field of ultrasound-based technology for increased drug delivery. Moreover, the use of a wide BBB disruption field with the implantable US array, along with delivery of PTX, one of the most potent chemotherapies for GBM might provide the best chances of achieving a therapeutic benefit from this novel approach.

3. INVESTIGATIONAL TREATMENTS

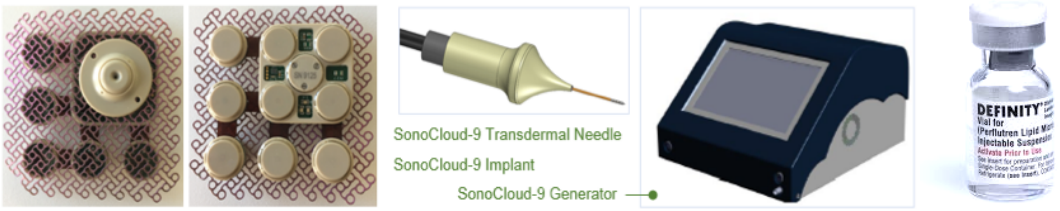
3.1. SonoCloud-9 (SC9) system

For our Phase I/II study of ultrasound-enhanced delivery of ABX for recurrent GBM, we propose to use the novel implantable sonication device referred to as SonoCloud-9 System (SC9). The system is composed of nine ultrasound emitters. The SC9 is designed to provide a broad BBB-disruption field and currently is being studied in combination with carboplatin in an FDA-approved clinical study under IND139771 (NCT03744026).

The SC9 system (Figure 2) consists of four principal components:

- a sterile and implantable component: the ultrasound emitter;
- a sterile transdermal needle connection device;
- a non-sterile and external component: the external radiofrequency generator; and,
- a sterile injectable component (ultrasound resonator).

Figure 2. SonoCloud-9 System



The SC9 Device is a Class III medical device falling under the Neurological Therapeutic Device sub-group governed by Code of Federal Regulations (CFR) Part 882 - Neurological Devices - Subpart F - Neurological Therapeutic Device in United States, for investigational use only. The SC9 implant, transdermal needle, and generator are manufactured by or under the strict supervision of CarThera according to the Essential Requirements of the Directive 90/385. The Ultrasound resonator is manufactured by Lantheus Medical Imaging, N. Billerica, MA according to current GMP (cGMP).

The SC9 implant is fixed to a bone window in the skull in place of a bone flap during planned surgery. It contains no internal energy source and is MRI-compatible. When implanted, the device can be activated by connecting the implant to the external generator system using the transdermal

needle. Once connected to the external generator, the SC9 device can generate a reproducible low intensity ultrasound field directly on the targeted brain tissue without the need for correction or feedback mechanisms. In particular, the variability that occurs due to acoustic transmission through the skull bone with external focused ultrasound does not exist with the SC9 device, which allows for a known pressure output of the device. This device is therefore adapted to treat sub-cortical and cortical areas and does not require MRI monitoring during the treatment.

The ultrasound resonator that is used with the SC9 device is a phospholipid microbubble contrast agent commercialized by Lantheus under the Definity® brand in the United States. The ultrasound resonator is supplied as a single use clear glass vial containing 1.5 mL of clear liquid. Upon activation by agitation with a VialMix® (specialized mixer), the clear colorless liquid becomes a homogenous, opaque, milky white injectable suspension of perfluoropropane (PFP) lipid microspheres of consistent number and size distribution.

The ultrasound resonator will be used at the dose of 10 µL/kg by slow bolus intravenous injection, followed by a 10 mL bolus of sodium chloride 9 mg/mL (0.9%) solution for injection. This is the same dose as the one used for echocardiography.

The total dose of ultrasound resonator will be injected in a 20 to 30 second bolus via a peripheral venous catheter that will be used further for MRI contrast agent injection and chemotherapy infusion. The ultrasound resonator is not detectable after 10 minutes in most subjects either in the blood or in expired air.

The Definity® microbubbles will be provided by the hospital pharmacy for the study.

Further details about the SonoCloud-9 System are provided in the Investigator's Brochure and Instructions for Use.

3.2. Paclitaxel and nab-Paclitaxel

Paclitaxel (PTX), a microtubule-stabilizing drug, is exquisitely potent against GBM, at an IC50 concentration 1400-fold lower than that exhibited by temozolomide and 120-fold lower than carboplatin (Figure 3). Nevertheless, studies exploring PTX's role in human gliomas showed that tumor tissue concentrations of PTX were minimal, and, in the surrounding brain parenchyma – an essential compartment for effective treatment of infiltrative disease – they were undetectable.[1]

Heimans, et al.[1] examined tumor samples from three patients that had received a preoperative PTX infusion and found that concentrations in tumor tissue 3-4 hours after PTX infusion were 464-2000 ng per gram of tumor tissue. They also showed that PTX was below the detection limit levels in peritumoral brain tissue. These results were in accordance with a distribution experiment performed in rats using ^{3}H-paclitaxel.[12] The findings suggest that while PTX can penetrate into the bulk of brain tumors tissue due its deficient BBB, it is unable to cross the intact BBB of healthy brain and peritumoral tissue, thus not affecting infiltrative tumor cells at the margin of the visible (contrast-enhancing) tumor. Furthermore, Fellner et al.[13] showed that PTX is a substrate for multidrug resistance protein, P-glycoprotein (P-gp), an efflux pump highly expressed in brain capillary endothelial cells. When using the P-gp blocker valspodar, PTX showed significant increased effectiveness in an orthotopic glioma xenograft model in nude mice.[13]

Local delivery of PTX through convection-enhanced delivery (CED) showed 73% response rate, but was associated with toxicity attributed to the delivery method.[2] Complications in this study included transient chemical meningitis (*n*=6), infectious complications (*n*=3), and transient

neurological deterioration (*n*=4, due to increased peritumoral edema). Moreover, several studies showed that Cremophor EL (CrEL), the solvent used in conventional PTX formulations, accumulates in and is toxic for peripheral nerves, whereas PTX per se was not detectable in this tissue.[14,15]

Our study differs in several aspects from prior clinical investigations with PTX in GBM patients. First, Lidar et al.[2] used commercial paclitaxel (Taxol®) that required dilution with Cremophor™ that has inherent neurological toxicity.[14,15] Chemical meningitis was likely a result from fluid back-flow into the subarachnoid space, a common complication of many CED reports. Similarly, risk of infection due to catheter placement is a major limitation of CED.[2]

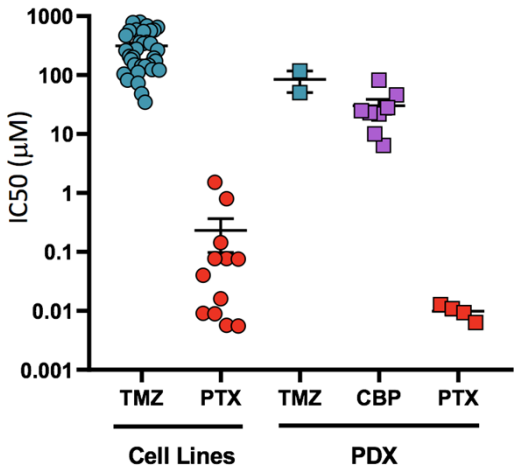
Thus, whereas PTX remains one of the most potent drugs against GBM, to date it could not be exploited due to insufficient or inadequate BBB penetration and vehicle-related toxicity. A novel clinical formulation of albumin-bound PTX, Abraxane (ABX) has improved solubility, a better distribution profile and does not require the toxic solvent CrEL. ABX has been established as the preferred paclitaxel formulation and this formulation is FDA approved for breast, lung and pancreatic cancer. In mouse glioma models, we found that ABX is well-tolerated and exhibits better brain penetration than CrEL-PTX. Following systemic ABX administration, ultrasound-based BBB disruption increased PTX brain tissue concentrations by 5-fold, achieving higher drug levels than IC50 concentrations for most human glioma cell lines.

Our premise is that PTX will be effective against human GBM if sufficient tumor and brain concentrations are achieved in the tumor and the surrounding infiltrated “normal” brain tissue. We hypothesize that ultrasound-based enhanced delivery of ABX in humans will be well-tolerated and that increased PTX concentrations in peritumoral brain will provide a substantial survival benefit for glioma patients.

ABX will be provided by the hospital pharmacy for the study. For further preparation, method of administration and conditions of use, please refer to full prescribing information in Section 0.

Figure 3. IC50 values for glioma cell lines

Comparison of IC50 drug concentrations across glioma cell lines from the Sanger/CCLE database and patient-derived glioma xenografts (PDX) from our lab shows PTX (red) is approximately 1400-fold more potent than temozolomide (TMZ) and 120-fold more potent than carboplatin (CBP).



3.3. Benefit/Risk Assessment

To date the effect of ultrasound (US)-based BBB disruption on drug concentrations in peritumoral human brain and glioma tissue remains unknown. This question is key for the proposed mechanism of action of this technology (implantable ultrasound), and the entire field of US-based BBB disruption for drug delivery in general. The implantable sonication device and the intraoperative study design offers a unique opportunity to address this question. By evaluating the effects of US-based BBB disruption on ABX penetration into tumor and the infiltrated peritumoral brain tissue we will be able to gain an understanding of this technology and its effect in the human brain. Given that BBB penetration is one of the main limitations for effective therapies for gliomas and brain tumors, this work has tremendous relevance for advancing therapies for this disease in general. Independent of the chemotherapeutic agent used, the results of the proposed studies will be highly informative and potentially impact the design of other clinical studies evaluating brain tumor therapies with poor brain penetration.

Another important objective is to determine the safety of delivery of ABX in the context of ultrasound-based BBB opening. PTX in its cremophor-based formulation (Taxol®) as well as ABX are known to cause peripheral neuropathy.[16] On the other hand, most pre-clinical studies describing neurotoxic effects of PTX have used cremophor as a vehicle for this drug.[12,15] We recently tested the safety of systemic delivery of ABX, Taxol®, and cremophor alone in the context of concomitant ultrasound-based BBB opening in mice. This study revealed that ABX is well tolerated and there are no clinical or histological signs of toxicity that can be attributed to this formulation of PTX following a single dose, whereas Taxol® and cremophor are neuro-toxic and can be lethal when combined with ultrasound-based BBB opening.[17] Thus, while the pre-clinical data suggests that delivery of ABX through ultrasound-based BBB opening, will be safe and well tolerated, neurotoxicity from this agent is a risk, and safety of this treatment will be one of the main objectives of this study.

Results obtained in previous trials with SC1, SC3 and SC9 show clear evidence of BBB opening and the safety profile appears tolerable when BBB disruption is performed prior to IV carboplatin chemotherapy. Detailed information about the known and expected benefits and risks and reasonably expected Adverse Events (AE) of the SC9 system may be found in the Investigator Brochure which will be considered as the Safety Reference Document on which the evaluation of AEs will be based, particularly with regard to their expectedness, severity, and outcome. The approved labeling for Abraxane and Definity will be the basis for the evaluation of AEs related to the drugs.

4. OBJECTIVES

4.1. Primary Objectives & Endpoints (Phase I and II)

Phase I: To evaluate the safety and maximum tolerated dose of albumin-bound paclitaxel up to a dose of 260 mg/m^2 q 3 weeks (260 mg/m^2 is the recommended and approved dose for other cancers) after pulsed-ultrasound opening of the blood-brain barrier in patients with recurrent glioblastoma.

Phase II: 1-year overall survival rate (calculated from day of surgery for recurrent disease and device implantation). Patients treated in the phase 1 part at the recommended Phase II dose will be included in the Phase II population.

Intraoperative pharmacokinetic study: To determine the effect of ultrasound-based blood-brain barrier disruption on peritumoral brain and glioma tissue paclitaxel concentrations.

4.2. Secondary Objectives & Endpoints (Phase II)

Phase II: Feasibility, toxicity and progression-free survival from device implantation

4.3. Exploratory Objectives & Endpoints (Phase I and II)

Phase I: To investigate the extent of tumor and peritumoral brain fields covered by pulsed ultrasound BBB disruption by performing gadolinium-enhanced MRI immediately after sonication. This will be determined by the volumetric measurement of the extent of gadolinium enhancement measured after sonication, compared to the sonication baseline MRI obtained ≤ 48 hours prior to sonication.

Phase I and II: Pattern of failure and investigation of whether sonication/microbubbles-based BBB disruption controls tumor growth and prevents local recurrence in field, compared to regions outside the field of sonication.

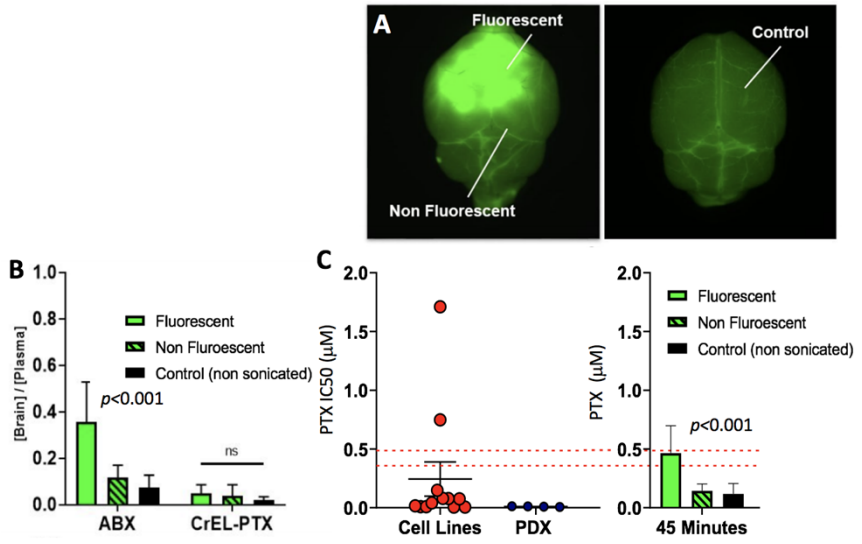
Objective response rate according to RANO criteria.[18]

Measurement of PTX concentrations in GBM tissue and peritumoral human brain following US-based BBB disruption

Rationale: Several studies in animal models have demonstrated that US-based BBB disruption enhances delivery of multiple drugs into the brain.[4,6,7,10,19,20] In primates, implantable US-based BBB led to 7-fold increase in carboplatin concentrations in the brain.[10] Yet differences in acoustic pressures, technique, and unknown factors that might be species-specific, limit the scientific rigor of extrapolating these findings to the human scenario. It is thus of utmost importance to measure actual drug concentrations in areas of the brain where the BBB is intact but has been disrupted by sonication. Demonstration of adequate clinically effective drug levels is needed to make BBB opening a clinically useful strategy. Our pre-clinical studies have demonstrated that following systemic administration of ABX, PTX brain tissue concentrations are enhanced by US-based BBB disruption. The effect of the BBB disruption by sonication on PTX brain concentrations can be detected as early as 45 minutes, a timeframe we will utilize for the intraoperative study proposed (Figure 4).

Intervention: Before the planned tumor resection, we will perform targeted biopsies of the planned resection zone after sonication of the targeted areas. The regions to be biopsied for this purpose are areas of peritumoral brain that get resected in the process of removing the tumor routinely. The tissue will be processed immediately in order to measure drug concentration (here ABX or its active compound PTX). Mock sonication will be performed in some cases and consist of placement of the device over the brain surface without activation of the ultrasound. This will allow us to control for BBB disruption related to physical contact between the device and the brain.

Figure 4. PTX concentrations in mouse brain after US BBB Disruption



*(**A**) The effect of brain sonication on PTX levels in healthy mouse brain was determined using fluorescein to map BBB-disruption injected with either ABX or CrEL-PTX 12 mg/kg IV and compared to non-fluorescent brain regions and non-sonicated mouse brains as controls.*
***(B)** Effect of US-based BBB disruption on brain/plasma PTX ratios for ABX and PTX.*
*(**C**) IC50 concentration for glioma cell lines from Sanger /CCLE database and glioma PDX is compared to the PTX brain concentrations achieved following IV injection of 12 mg/kg of IV ABX in regions of the brain that had BBB opening or not.*

Peritumoral brain will be defined intraoperatively as an area of the brain that does not exhibit contrast enhancement, that contains FLAIR abnormal signal (determined by the MRI used for stereotaxic navigation), in particular, the 2 cm of brain surrounding the enhancing core is the region that gives rise to 80-90% of recurrence in GBM.[21–23] Yet, histological analysis for tumor density will be performed on these samples to confirm this is brain parenchyma.

To investigate the effect of BBB disruption on PTX concentrations in the brain, we will map and sample fluorescent and non-fluorescent areas, as well as gross tumor tissue. This will be done using fluorescent microscopy (Zeiss® Yellow 560 nm filter) as we have previously done in the operating room.

Fluorescein will allow intraoperative identification of BBB disruption and serve to identify tissue with highest PTX concentrations as we have established in mice (Figure 5). Representative fluorescent pictures of the cortex will be obtained using this microscope.

Statistical analysis and power considerations: Table 1 provides a summary of the number of patients and conditions, and approximate number of samples that will be collected and undergo quantification of fluorescein and PTX concentrations for these studies. We will use mixed-model regression methods to evaluate differences in drug concentrations for the comparisons described above: within-patient comparisons of fluorescent vs. non-fluorescent brain tissue, fluorescent (patients that underwent sonication) vs. non-fluorescent brain tissue (patients that underwent mock sonication), glioma tissue for patients that underwent sonication v. mock sonication. Fixed main effects will be specified for tissue type (fluorescent, non-fluorescent, gross tumor tissue) and treatment (sonication, mock sonication).

Additional analysis of sonicated and non-sonicated peri-tumoral brain and tumor tissue: In addition to quantification of paclitaxel levels, the samples collected will undergo other analyses to characterize the effect of ultrasound-based BBB opening on the brain. These analyses include single-cell RNA-seq, and different microscopy techniques among other approaches.

Table 1. Summary of approximate number of samples that will be obtained in intraoperative PTX quantification studies

	Treatment category			
	Sonication		**Mock sonication**	
Number of patients	8		8	
	n per patient	*n* per condition	*n* per patient	*n* per condition
Fluorescent peritumoral brain	3	24	0	0
Non-fluorescent peritumoral brain	3	24	3	24
Glioma Tissue	3	24	3	24
Plasma	3	24	3	24
Total number of samples (approximate)	12	96	9	72

Note: If we do not detect differences in PTX concentration in regions that are sonicated (and fluorescent) vs those that are not on an initial analysis, in subsequent patients we will obtain samples of brain that has undergone mock sonication. Mock sonication procedure is designed to rule out the possibility that the transient ultrasound placement over the brain surface might cause BBB disruption. To control for this, we will obtain biopsies of regions that undergo placement of the device without microbubble injection or activation in a subset of patients.

4.4. Correlative Objectives & Endpoints (Phase I and II)

To investigate the level of circulating tumor DNA detected in peripheral blood after US-mediated BBB and correlation with overall survival.

Rationale: Current methods to diagnose glioma and monitor its response to treatment rely on a combination of neuroimaging (MRI) and tissue biopsy. Because MRI often fails to distinguish between treatment-related changes within the brain and true tumor progression, tissue biopsies are considered the only definitive way to establish disease progression. However, repeated tissue biopsy is not feasible due to its highly invasive nature. Determining progression is instrumental to evaluate efficacy of novel therapies, and to guide clinical management for these patients. Thus, there is a pressing need for a non-invasive method to monitor treatment response and tumor growth for glioma patients.

Detection of circulating tumor nucleic acids is an emerging diagnostic tool with great potential in its ability to genotype and detect tumors non-invasively. Prior attempts at detecting circulating biomarkers derived from glioblastoma within the blood have exhibited limited sensitivity, in part due to the blood-brain barrier (BBB) preventing tumor material from escaping the intracranial compartment.

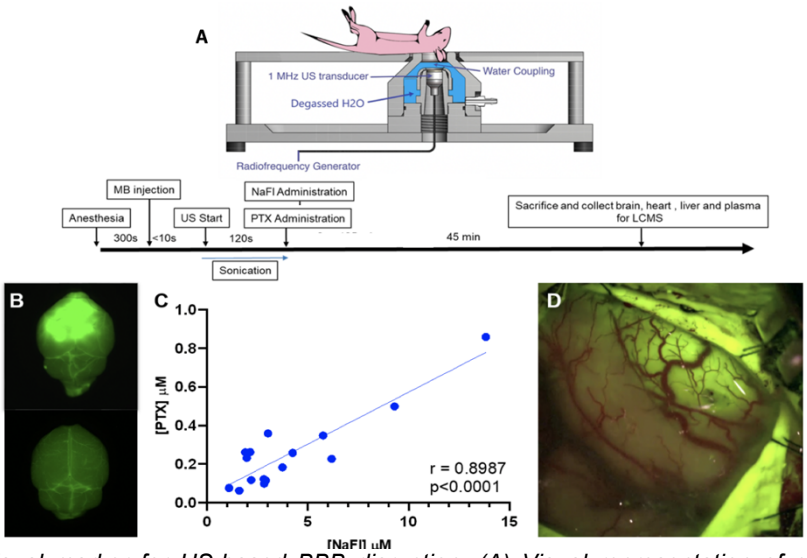
Many solid tumors have been shown to release proteins, metabolites and genetic material into the bloodstream of patients. These circulating tumor biomarkers can by isolated and studied to provide information regarding a tumor's molecular makeup and response to certain targeted molecular therapies in a process termed "liquid biopsy." The source of circulating tumor nucleic acids can be DNA shed from apoptotic tumor cells, called circulating tumor DNA, or mRNA and miRNA from circulating tumor exosomes. Several preclinical and clinical studies have

demonstrated the utility liquid biopsies have in predicting survival and detecting minimal residual disease.

In glioma, various studies have shown liquid biopsy to be successful in detecting circulating tumor DNA within the cerebrospinal fluid (CSF) with moderate sensitivity (49.4-62%). However, circulating tumor DNA was difficult to detect in patients with tumors not in contact with a CSF compartment or ventricular space. Moreover, circulating biomarkers derived from gliomas are difficult to detect within the peripheral blood of patients, in part, we believe due to the protective blood brain barrier (BBB) preventing genetic material shed from tumor cells to enter the circulatory system.

We hypothesize that ultrasound-mediated BBB opening will allow increased detection of circulating GBM nucleic acids within the peripheral blood of patients, and that these will serve as biomarkers for treatment response and disease progression in persons undergoing ultrasound-mediated BBB opening.

Figure 5. Fluorescence and PTX concentration after US-BBB Disruption



Fluorescein is a visual marker for US-based BBB disruption. (A) Visual representation of experiment for BBB disruption using microbubbles and pulsed ultrasound. PTX concentrations were measured 45 minutes after sonication. (B) Mouse brains following injection with both fluorescein (20mg/kg) and ABX (12mg/kg) +/- sonication for BBB disruption. (C) The scatter plot shows strong correlation between fluorescein and PTX concentrations in the brain.[17] *(D) Human GBM that fluoresces following fluorescein injection given defective BBB in the lesion, we would expect similar fluorescence in peritumoral brain following intraoperative US-based BBB disruption as shown in mouse brain on the left.*

5. PATIENT ELIGIBILITY

Patients suffering from recurrent glioblastoma and who require a surgical tumor debulking are eligible for participation in this study. This will be a single-center Phase I trial conducted at the Lou and Jean Malnati Brain Tumor Institute of Northwestern University and the Lurie Comprehensive Cancer Center, and Northwestern Medicine, Departments of Neurological

Surgery and Neurology. For the Phase II part, participation of other experienced centers may be considered.

A total of up to 41 evaluable subjects will be treated within this trial, up to 17 subjects in the Phase I component, and an additional 18 subjects in the Phase II expansion cohort (with up to an additional 5 subjects enrolled to provide for 15% of non-evaluable subjects). All surgery will be carried out by or under direct guidance of Dr. Adam Sonabend.

Indication for surgery will be reviewed at the multidisciplinary weekly tumor board, and patient eligibility will be carefully evaluated by the study team. Study treatment may not begin until a subject is registered, and all protocol related investigations shall not be carried out before formal and duly signed informed consent has been obtained. Please refer to Section 13.4 for complete instructions regarding registration procedures.

5.1. Inclusion Criteria

All of the following inclusion criteria must be met:

1. Have a histologically confirmed diagnosis of IDH1 (R132H) wild-type glioblastoma per 2016 WHO criteria from the pathology report from surgery that led to diagnosis, that has progressed after standard radiotherapy (RT) and temozolomide with or without TTFields (*Note: pathology of the resected tumor will be reviewed by our expert reference neuropathologist, for trial inclusion we will rely on the previously established local diagnosis of a GBM*.) Patients who have received fractionated first-line radiation therapy and no prior chemotherapy (e.g. as common practice for MGMT unmethylated tumors), or who have participated in an investigational protocol substituting TMZ for a novel agent are eligible.
2. Be able to undergo contrast-enhanced magnetic resonance imaging (MRI)
3. Have radiographic evidence of tumor progression with measurable (≥ 1 cm) or evaluable disease by contrast-enhancement on MRI, according to RANO criteria [Wen, Macdonald, Reardon et al. JCO 2010]
    a. Measurable: contrast-enhancement of bidirectional diameters ≥ 1 cm.
    b. Evaluable disease is defined as tumor that can be seen on MRI, that due to small size or irregular shape cannot be measured in a reproducible way.
4. Maximal tumor diameter at inclusion (pre-surgery) ≤ 70 mm on T1wMRI, or expected residual peri-tumoral brain (after resection) of ≤ 70 mm.
5. Candidate for surgical resection for disease recurrence or progression
6. Interval since completion of radiotherapy > 12 weeks, unless there is tissue confirmation of tumor recurrence or progression outside the radiation treatment field. Prior treatment with radiosurgery or other high-dose focused radiation is acceptable, but the patient must have subsequent histologic documentation of recurrence, unless the recurrence occurs remote to the treated site
7. Interval since last cytotoxic therapy until presumed date of surgery ≥ 1 cycle or ≥ 2 biological half-lives, i.e.
    a. ≥ 4 weeks since start of last cycle of temozolomide
    b. ≥ 6 weeks since start of last cycle of lomustine or other nitrosourea
    c. ≥ 3 weeks since start of last cycle of a small molecule targeted agent
    d. ≥ 12 weeks from last bevacizumab infusion
8. Ability to understand patient information and written informed consent and has provided signed and dated informed consent prior to study registration and study related procedures
9. Age ≥ 18 years

10. If receiving dexamethasone for mass effect, a stable daily dose of dexamethasone at ≤ 6 mg within 7 days of registration, or if dexamethasone dose is decreasing, average daily dose of ≤ 6 mg in the 7 days prior to registration. Patients on dexamethasone for reasons other than mass effect may still be enrolled.
11. WHO performance status ≤ 2 (corresponding to a Karnofsky Performance Status (KPS) of ≥70)
12. No signs of peripheral neuropathy or neuropathy ≤ grade 1.
13. Have adequate organ and bone marrow function within 14 days prior to registration, as defined below:
    a. Hemoglobin ≥ 9.0 g/dL
    b. Leukocytes ≥ 3,000/μL
    c. Absolute neutrophil count ≥ 1,500/μL
    d. Platelets ≥ 100,000/μl
    e. Total bilirubin < 1.5 mg/dL
    f. AST(SGOT)/ALT(SPGT) ≤ 3 X institutional ULN[1]
    g. Creatinine ≤1.5 mg/dL
14. Have a negative pregnancy test within 14 days prior to registration on study (for FOCBP[2])
15. Agree to use adequate contraception:
    a. (e.g. hormonal or barrier method of birth control; abstinence) if a female of child-bearing potential (FOCBP) or a male, prior to study entry, for the duration of study participation, and for 3-6 months after the last dose of ABX; for men: 3 months after the last nab-paclitaxel dose, for FOCBP for 6 months after the last nab-paclitaxel dose. Should a female patient become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.
    b. NOTE: A FOCBP is *any woman* (regardless of sexual orientation, having undergone a tubal ligation, or remaining celibate by choice) who meets the following criteria:
    c. *Has not* undergone a hysterectomy or bilateral oophorectomy
    d. *Has had* menses at any time in the preceding 12 consecutive months (and therefore has not been naturally postmenopausal for > 12 months)

5.2. Exclusion Criteria

None of the following exclusion criteria shall apply:

1. Have multifocal tumor (unless all localized in a 70-mm diameter area accessible to ultrasound field) or tumor located in the posterior fossa
2. Are at increased risk of wound dehiscence (e.g. 3 or more previous craniotomies/brain surgery within the last 3 months, poor skin condition, and/or previously infected surgical field) or any other condition that is of **increased** infectious **risk in the opinion of the neurosurgeon**). The examples are of illustrative intent only, neither comprehensive nor exclusive or definitive.
3. Have uncontrolled epilepsy
4. Require treatment with enzyme-inducing antiepileptic drugs
5. Have clinical evidence of peripheral neuropathy on examination > grade 1
6. Have received any other investigational agents within 4 weeks of registration

[1] ULN, upper limit of normal

[2] FOCBP, female of child-bearing potential

7. Have received prior therapy with or have history of allergic reactions attributed to compounds of similar chemical or biologic composition to paclitaxel
8. Hypertension grade 3 or higher without adequate control on medications
9. Ongoing or active infection requiring systemic treatment
10. Symptomatic congestive heart failure
11. Unstable angina pectoris
12. Unstable cardiac arrhythmia
13. Pneumonitis
14. Psychiatric illness/social situations that would limit compliance with study requirements
15. Any other illness or condition that the treating investigator feels would interfere with study compliance or would compromise the patient's safety or study endpoints
16. Patients with coils, clips, shunts, intravascular stents, and/or non-removable wafer, non-resorbable dura substitute, or reservoirs.
17. Patients with medical need to continue antiplatelet therapy.
18. Patients with known significant cardiac disease, known to have right-to-left shunts, severe pulmonary hypertension (pulmonary artery pressure > 90 mmHg), uncontrolled systemic hypertension, or adult respiratory distress syndrome. (patient at risk for microbubble reaction)
19. Patients with impaired thermo-regulation or temperature sensation
20. Are pregnant or nursing
21. Patients with a history of active malignancy within 3 years prior to registration. Note: Exceptions to this requirement include adequately treated non-melanoma skin cancer or lentigo maligna or carcinoma in situ without evidence of disease, or intraoperative proof that lesion to be operated is indeed recurrent glioblastoma
22. Known history of hypersensitivity reactions to:
    i. perflutren lipid microsphere components or to any of the inactive ingredients in Definity® (European Brand name: Luminity®) microbubbles products.
    ii. Fluorescein (Fluorescite®) and other ingredients in this product.

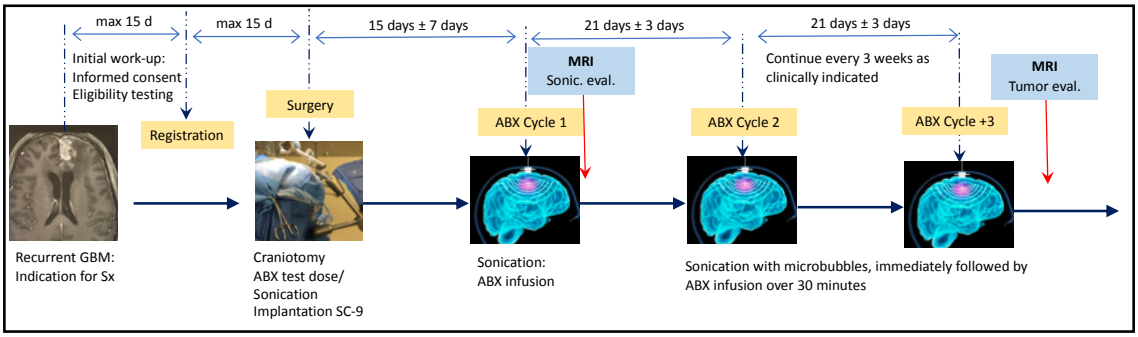
6. PROCEDURES AND TREATMENTS

6.1. Procedure Overview

Patients will undergo routine craniotomy, with the sonication device implanted in the bone window upon completion of resection (Figure 6). Patients will receive routine peri- and postoperative care, including a new baseline postoperative MRI for disease assessment obtained within 48 hours of surgery. Patients will be discharged home or for rehabilitation as per routine. Before tumor resection a "low test dose" of ABX will be delivered in the operating room aiming at determination of the effects of blood-brain barrier disruption on ABX concentration in the peritumoral brain tissue, and at tumor tissue (see details under Section 6.3.1).

Approximately two weeks from surgery, patients will undergo sonication/microbubbles infusion immediately followed by the first dose of ABX infusion (Figure 6). This treatment will be repeated every 3 weeks until disease progression or as clinically indicated, provided there is no relevant toxicity observed. Patients will be monitored closely, with a full clinical and laboratory evaluation at least every 3 weeks. The interval from start until the end of cycle 1 of ABX (ABX infusion/sonication + 3 weeks) will determine the maximum tolerated dose (MTD) defining observation period.

Figure 6. Schematic of trial workflow (approximate)



6.2. Chemotherapy (albumin-bound paclitaxel)

Intravenous injection of microbubbles and activation of the SC9 system using the percutaneous needle will be performed first. This will be followed by an intravenous ABX dose. For the first cycle, the patient will undergo MRI with contrast immediately after sonication/ABX is completed to map regions of BBB disruption using gadolinium. The Phase I study will involve ABX dose escalation over up to six dose levels (40 mg/m^2, 80 mg/m^2, 135 mg/m^2, 175 mg/m^2, 215 mg/m^2, 260 mg/m^2). In cycle 1, patients will be monitored for at least 6 hours post-sonication before being discharged. For cycles 2-6, ABX infusion will commence immediately after microbubble SC9 sonication treatment and be repeated every 3 weeks for a total of 6 cycles, with each 3-week period comprising one cycle. MRI with/without contrast will be obtained after every 3 cycles. Surveillance post-sonication may be shortened to 2 hours depending on prior tolerance, per investigators discretion.

Peripheral blood (up to 20 ml), samples will be obtained before and after sonication for cycles 1-6 of ABX infusion for analysis of circulating tumor nucleic acids. A buccal swab will also be obtained once to isolate non-tumor DNA.

6.2.1. Monitoring of toxicity

Patients will be carefully monitored during and after sonication and ABX therapy. For cycle 1, patients will be monitored for at least 6 hours post sonication, and in the absence of significant acute toxicity may then return to home. In case of toxicity or concerns, management including hospitalization and surveillance will be as per routine practice, using established practice guidelines and investigator's clinical expertise. In subsequent cycles monitoring may be shortened to 2-4 hours, at the investigator's discretion.

During cycle 1, patients will be evaluated (in person or telehealth) by the study team and have a CBC on a weekly basis. For subsequent cycles, monitoring will be performed as per standard oncological routine. Monitoring will include interim history, physical and neurological exam with particular attention to neurological toxicities. Central neurological condition and cognition, as well as absence or presence of sensory neuropathy will be specifically recorded.

Expected toxicities due to Abraxane® are: myelosuppression (including grade 3 / 4 temporary neutropenia), neutropenic fever and infection, alopecia and cumulative peripheral sensory

neuropathy. Furthermore, myalgia and arthralgia, asthenia, fatigue, nausea and vomiting, diarrhea and fluid retention may occur with Abraxane® therapy.

6.2.2. Dose reductions and delays

For subsequent cycles of treatment, Abraxane®-associated toxicities have to have resolved or improved to grade ≤ 2. No dose reduction is required for grade 1 or 2 alterations of liver enzymes (AST or ALT up to 5x ULN or bilirubin up to 3x ULN).

Table 2. Abraxane dose reductions and delays

Toxicity	Action	Remarks
Hematological: Febrile neutropenia (ANC< 1000/mm^3)	reduce ABX by 1 dose level	Consider* adding G-CSF
Neutropenia grade 4 (> 10 days)	reduce by 1 dose level	and/or consider* adding G-CSF at investigator discretion
Neutropenia grade 3 (> 10days)		Consider* adding G-CSF at investigator discretion
Thrombocytopenia grade 4	reduce by 1 dose level	
Thrombocytopenia grade 3	increase monitoring at least twice a week until platelet count resolves at least to grade 2 (≥ 50,000)	Consider* dose reduction for subsequent cycle at investigator discretion
Non-hematological: Peripheral neuropathy grade 2 with pain or peripheral neuropathy ≥ grade 3 for > 2 weeks	Hold treatment until resolved to grade ≤ 2 and reduce 1 DL	Initial dose may be maintained if lasting < 2 weeks; per investigator discretion dose may be reduced one level for concerns about cumulative peripheral neuropathy
Central neurotoxicity ≥ grade 3 Exceptions see DLT exceptions (8.2.2)	Hold until ≤ grade 1 and reduce by 1 DL	Discontinue if grade 4 toxicity
Central neurotoxicity ≤ grade 2	Hold treatment until resolved ≤ grade 1	Consideration of dose reduction at the investigator's discretion
Myalgia, arthralgia, asthenia, fatigue	Keep same dose	and increase symptomatic or prophylactic treatment according to local practice
Nausea/vomiting		
Delay of next cycle by > 7 days	reduce by 1 dose level if due to toxicity	

*Definition of "consider": Allows investigators to make a final assessment on the clinical severity of laboratory finding, adapted to patient's general condition and frailty; example: Thrombocytopenia of 40.000 without bleeding may be fully manageable and does not necessarily require a dose reduction, while thrombocytopenia of 30.000 (also grade 3) may be in the subsequent cycle 20.000 and thus grade 4 with an increased bleeding risk. Similarly, slowly recovering neutropenia of 0.9 (grade 3) may be acceptable, while prolonged neutropenia of 0.6 may warrant an intervention upon subsequent treatment.

6.2.3. Phase I dose escalation scheme

The planned ABX starting dose is 40 mg/m^2 of ABX, to be escalated in the absence of significant toxicity up to 260 mg/m^2 (a stepwise increase of 20 – 30%), as shown in Table 3.

Table 3 delineates the dose escalation scheme. In order to make determinations regarding escalating or de-escalating the dose, the evaluable patients must have been observed until at

least the completion of cycle 1 of ABX. Additional patients requiring treatment before this interval shall be treated at the current dose level. No more than 2 patients shall be simultaneously treated in cycle 1 at a given dose level during the escalation phase. This is to allow for adequate safety assessments before moving to the next dose level.

Data and Safety Monitoring Committee (DSMC) approval is required before escalating to the next dose level (for DSMC details, please refer to Section 13.8, for definition of DLT, please refer to Section 8.2). No intra-patient dose escalation shall occur, as all dose levels are corresponding to systemically active doses. DSMC and PI may decide to treat additional patients at a given dose level if in the interest of safety and doubtful attribution of emerging toxicity.

Table 3. Phase I Dose Escalation Scheme

Dose level (DL)	ABX dose	No of pts
Intraoperative test dose	40-80 mg/m^2 †	All pts †. In DL1 + 2, 40 mg/m^2.
DL 1	40 mg/m^2	1 – 12 pts per DL, max 15 total, depending on occurrence of DLT. Escalation according to Bayesian Optimal Interval (BOIN) design
DL 2	80 mg/m^2	
DL 3	135 mg/m^2	
DL 4	175 mg/m^2	
DL 5	215 mg/m^2	
DL 6	260 mg/m^2	
† test dose is in conjunction with sonication		

6.2.4. Dose Limiting Toxicity (DLT)

Patients will be carefully followed and assessed throughout all interventions and treatments. Treatment-emergent symptoms and toxicity will be recorded and scored according to the Common Toxicity Criteria, Version 5 (see Appendix 4: Common Toxicity Criteria Adverse Events). Relationship to the treatment will be attributed by the investigators as unlikely, possible, or probable. Definition of DLT is detailed in Section 8.2.

6.2.5. Justification of the Abraxane® Starting Dose

Up to six dose levels (DL) will be explored (see Section 6.2.3). The preselected DL are based on established dosing regimens for paclitaxel and albumin-bound paclitaxel. The approved dosing for paclitaxel for solid tumors alone or in combination with other cytotoxic agents is 175 mg/m^2 infused over 3 hours every 21 days (there are also weekly schedules used, however this does not apply to the proposed clinical study). The approved dosing for albumin-bound paclitaxel is 260 mg/m^2 intravenous infusion over approximately 30 minutes every 3 weeks. Our proposed ABX starting dose is 40 mg/m^2 of ABX, to be escalated in the absence of significant toxicity up to 260 mg/m^2 (a stepwise increase of 20 – 30%).

1) Prior preclinical studies have administered nab-paclitaxel in rats up to a dose of 120 mg/kg without CNS toxicity. This translates to a human equivalent dose of 720 mg/m^2 [*Ref Celgene IB ed. 21 (11. Dec 2018), page 21*] based on a conversion factor rat → human of 6.[24]

2) In our studies in mice we have demonstrated meaningful plasma levels at a dose of 12 mg/kg (Figure 5), and we demonstrated prolonged survival at a dose of 24 mg/kg (corresponding with conversion factor of 3 to a human dose 72 mg/m^2).[24] In conjunction with ultrasound-based blood-brain barrier opening led to a 4-5x increase in brain concentration of paclitaxel that was well tolerated and did not lead to significant neurotoxicity following a single administration and following repeated dosing M/W/F for 8 doses, a dosing regimen that is approximately 7 times more frequent than the planed administration on the trial. At this dosing, ultrasound-based blood-brain barrier opening led to an increase in brain concentration of paclitaxel that was well-tolerated and did not lead to significant neurotoxicity following a single administration and following repeated dosing M/W/F for eight doses. This pre-clinical dosing regimen was approximately 7 times more frequent than the planed administration in this trial. A detailed description of these studies is available in our recent article.[17]

3) Dose of 40 mg/m^2 of Abraxane® is likely to be safe (the approved Abraxane dose is 260 mg/m^2, over 6-fold higher than our starting dose). We anticipate intraparenchymal dose increase of 4-5 times after ultrasound-based opening of the blood brain barrier, thus the first 2 dose levels will be below the threshold of what is achieved in daily practice in treatment of many solid tumors (Figure 5).

4) Our initial dose levels of Abraxane® are considerably lower than the doses determined to be safe in clinical trials evaluating other formulations of paclitaxel for treatment of malignant gliomas.[2,25–31] To the best of our knowledge, Abraxane® has not been investigated in the treatment of malignant gliomas, and thus, comparisons can only be made with other paclitaxel formulations. Several studies reported dose-escalation for systemic administration of Taxol® (Cremophor-based paclitaxel).[25–29] These studies tested the efficacy of PTX administered every 21 days and the doses used ranged from 140-390 mg/m^2. 25% of patients receiving PTX therapy at 240 mg/m^2 experienced mild CNS toxicity (<Grade II) in the form of headaches. Incidence and severity of CNS toxicity increased when the dose of PTX increased to 360 mg/m^2 and above. One study[27] reported that in the context of hepatic enzyme inducing anticonvulsive therapy: 360 mg/m^2 of Taxol® led to Grade III CNS toxicity (somnolence/encephalopathy) in 2 of 6 patients, whereas 390mg/m^2 Taxol® led to Grade IV CNS toxicity (seizures/coma) in 2 of 3 patients. Based on this, the authors defined the maximally tolerated dose as 360 mg/m^2, and 330 mg/m^2 for patients on anticonvulsive drugs. Nevertheless, much of the CNS toxicity observed is likely attributable to the solvent Cremophor, that is of a smaller size and has chemical properties to pass through the blood-brain barrier.

5) ANG1005, a peptide-drug conjugate of paclitaxel designed to penetrate across the blood-brain barrier, was investigated in a Phase 1 trial for malignant gliomas.[31] The MTD was 650 mg/m^2 every 3 weeks, corresponding to 325 mg/m^2 of paclitaxel [personal communication Angiochem]. Dose-limiting toxicities were grade 3 mucositis and grade 4 neutropenia. There was no evidence of central nervous system toxicity. In a subsequent phase 2 study, ANG1005 was administered at a dose of 600 mg/m^2 every 3 weeks to 72 breast cancer patients with brain or leptomeningeal metastases.[32] Patients were heavily pre-treated including prior radiotherapy to the brain in 85% of patients. Toxicity was mainly myelosuppression, with fatigue, nausea and peripheral neuropathy in a minority of patients. No case of central neuropathy was noted.[32]

6) In general, Abraxane® is much better tolerated than Taxol® both with regards to systemic toxicity[33–35] as well as with regards to CNS toxicity in the context of ultrasound-based blood-brain barrier disruption.[17] This has led to considerably higher MTD established for Abraxane® than for Taxol®. Thus, a starting dose of 40 mg/m^2 of Abraxane® is likely to be

safe as significantly higher doses of Taxol® and ANG1005 were deemed tolerable in the context of treatment of glioma patients.

7) Abraxane® 40 mg/m^2 as a starting dose, which accounts for 40% of the weekly dose that has been shown effective and safe in combination with carboplatin in lung cancer patients. This should allow for a large safety margin while using a single dose that may still be high enough to exert antitumor activity. Abraxane has been used and is approved in a range of dosages and schedules as follows (see Abraxane FDA Labeling):
   a. Breast cancer: after failure of at least 1 line of prior chemotherapy: 260 mg/m^2 IV over approximately 30 minutes every 3 weeks.
   b. NSCLC: 100 mg/m^2 weekly (i.e. day 1, 8, 15 every 21-day cycle in combination with carboplatin AUC 6 on day 1.
   c. Pancreas carcinoma: 125 mg/m^2 weekly day 1, 8, 15 every 28-day cycle in combination with gemcitabine 1000 mg/m^2 days 1, 8, 15 every 4 weeks.
8) Our pre-clinical studies showed a 4-5 fold increase in parenchymal concentration of Abraxane® when the ultrasound-based BBB opening was used. Thus, a low dose of Abraxane® with ultrasound-based BBB opening might lead to similar parenchymal concentrations as a high-dose of Abraxane® used in the absence of concomitant ultrasound-based BBB opening (as already routinely done in the clinical setting). Abraxane®, when given on a q 3-week schedule has a maximal tolerated dose of 375 mg/m^2 every 3-weeks. It is important to consider than even at this dose of 375 mg/m^2, the DLT observed was peripheral and not central neuropathy, stomatitis and keratopathy, but not CNS-related toxicity[36] (Abraxane Investigator's Brochure, Celgene). A dose level of 40 mg/m^2 is approximately 1/10 of 375 mg/m^2. Thus, we do not anticipate that this starting dose will lead to CNS toxicity as it likely to achieve concentrations similar to those that might already take place when patients get high-dose Abraxane® infusions.
9) Nevertheless, we aim to ultimately administer doses ≥ 80 mg/m^2, corresponding to the dose of 24 mg/kg explored in mice and demonstrating increased survival. Concentrations achieved with lower doses might not be efficacious, and thus this treatment might not be a substantial benefit for the patient.

6.3. Surgery

All patients will undergo a craniotomy for resection of recurrent glioblastoma, as part of their standard clinical management. The usual surgical precautions will apply, and patients will be informed, hospitalized and receive a minimum of 24 hours of postoperative antibiotics and regular surgical care as per institutions standard operating procedures.

All patients shall agree to tumor resection, but also test dose of ABX and determination of drug levels in tumor and adjacent infiltrated brain, if technically feasible. Figure 6 illustrates the intraoperative workflow and goals of surgery within the context of our studies.

6.3.1. Intraoperative mapping of ultrasound-based BBB disruption

To date, BBB-disruption by US has been demonstrated using Evan's blue injection in animal models[3], or by gadolinium contrast-enhancement seen on MRI in patients.[9] Direct intraoperative visualization of BBB disruption is essential to investigate whether US/microbubble treatment leads to elevation of chemotherapeutic drug concentrations up to therapeutic ranges in peritumoral brain in humans, as this will require obtaining biopsies from these areas. As opposed to Evan's blue, fluorescein (Fluorescite®) is an FDA-approved angiography agent that leaks into glioma

tissue through vessels with deficient BBB (Figure 5). Thus, fluorescein has been recently introduced as an aid for intraoperative tumor visualization.[37–41] We routinely use it in this context at our institution.

In order to determine the effect of US on PTX concentrations intraoperatively, we have established fluorescein as a tool to map BBB disruption by this technology. We showed that areas of the mouse brain that have been treated with US-microbubbles accumulate fluorescein, and US-based disruption of BBB identified by fluorescein shows elevated PTX levels compared to non-sonicated brain (Figure 5). For these experiments, we harvested tissue 45 minutes after sonication, a timeframe that is compatible with an intraoperative study.

Glioma resection often requires accessing tumor through non-eloquent brain tissue and resecting non-eloquent brain. This offers the opportunity for sampling of peritumoral brain for scientific investigation without additional risk to the patient, as previously demonstrated.[40] To investigate the effects of US-based BBB disruption on the concentrations of PTX in peritumoral brain and glioma tissue, we will infuse ABX with concomitant administration of microbubbles and sonication of peritumoral brain. Intravenous fluorescein injection will be performed after sonication so that the fluorescence can be used to map blood-brain barrier disruption. We will then perform targeted biopsies of non-eloquent peritumoral brain before resecting the tumor and the peritumoral brain tissue. With fluorescein we will visualize and map areas of BBB disruption, a strategy we have demonstrated a powerful tool in preclinical models (Figure 5). Peritumoral brain parenchyma will be distinguished from gross tumor based on preoperative MR imaging, stereotaxic navigation and tissue texture (part of conventional surgical tumor resection). This part of the investigation may not feasible in all patients as it depends on tumor location and anatomical necessity to resect non-enhancing brain tissue.

6.3.2. Practical Aspects and Timing of Intervention

1. Perform a craniotomy and expose the tumor and the surrounding peritumoral brain as per standard surgical practice.

2. Adjust the FIO_2 so that the PAO_2 pressure is as close as possible to <100 mm/Hg to obtain similar microbubble half-life as an awake patient in a regular sonication.[42]

3. Infuse intravenous microbubbles immediately followed by brain sonication using a manually placed SonoCloud-9 device positioned in non-eloquent peritumoral brain within the surgical corridor necessary to access and resect the tumor. Of note, the sonication parameters are comparable to those used routinely used during the therapeutic sonication cycles.
4. Immediately thereafter, we will inject intravenous fluorescein[3] (3-10 mg/kg, up to a maximum total dose of 500 mg), simultaneous with
5. Intravenous ABX 40-80 mg/m^2 infusion[4]. To investigate the effects of the sequence of ABX infusion vs sonication on the concentrations of paclitaxel on peritumoral brain in some patients we will alter the sequence described above and first infuse ABX followed by microbubble infusion and sonication.

[3] Fluorescite®, approved standard dosing in adults: 500 mg i.v.

[4] For DL1+first patient on DL2 we will use a 40 mg/m^2 test dose.

6. Wait for 45 minutes (from initiation of sonication) to allow drug to accumulate in the brain with disrupted BBB (time may be adjusted per surgeon discretion).

7. To investigate the effect of BBB disruption on PTX concentrations in the brain, we will map and sample fluorescent and non-fluorescent areas, as well as gross tumor tissue. This will be done using fluorescent microscopy (Zeiss™ Yellow 560 nm filter) as is part of the surgical routine (Figure 5).To characterize the effects of the ultrasound over the peri-tumoral brain, some patients have peri-tumoral brain sampled/biopsied prior to 45 minutes post-sonication.

8. Representative fluorescent pictures and video of the cortex will be obtained using this microscope. In addition, a snapshot of the location of peritumoral brain to be biopsied will be recorded using the stereotaxic navigation device to document the MRI appearance of these regions.

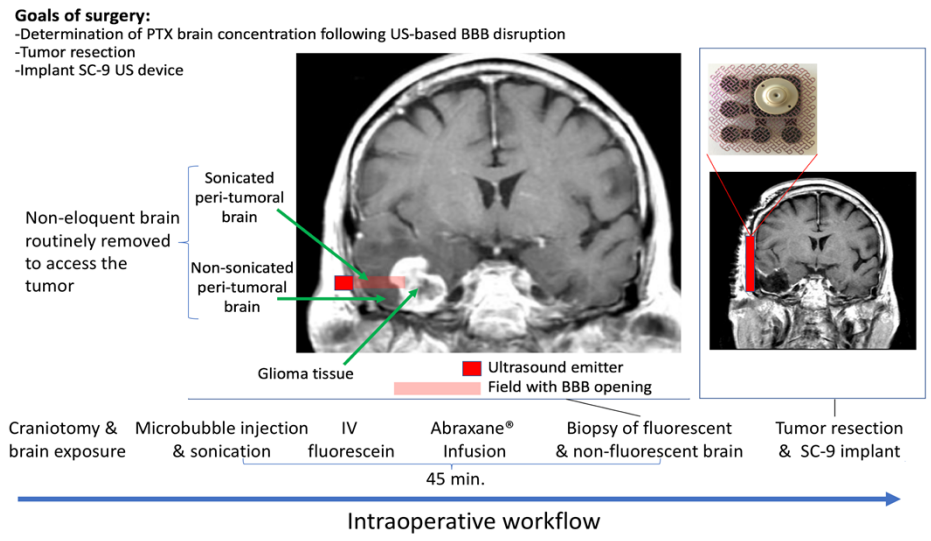
9. Standard tumor debulking. Of note, given that fluorescein will be used for mapping of blood-brain barrier opening, we will not use fluorescein to identify tumor or maximize resection on these cases.

10. Implantation of the SC9 device.

11. Wound closure and completion of procedure.

12. Draw blood samples (10 ml per time point) for 6 time points during surgery and up to 24 hr. after surgery for pharmacokinetics and isolation of circulating tumor DNA.

Figure 7. Intraoperative Workflow



Scheme of goals of surgery and intraoperative workflow. Illustrative example of a case that requires excision of peritumoral brain to access a GBM lesion for resection, conducive to biopsy of brain tissue that has been subject to US-based BBB disruption (fluorescent) or not (non-fluorescent) as shown on Figure 5. *PTX: paclitaxel. ABX: albumin-bound PTX.*

6.3.3. Processing of the tumor tissue

Samples will be labelled and categorized with regards to location and extent of fluorescein activity, and prior MRI contrast enhancement.

A portion of the sample will be fixed in formalin for histological analysis of tumor density, and a separate section will immediately (within 15 minutes) be flash frozen for subsequent quantification of drug and fluorescein levels. Additional ex-vivo analyses will be performed on the tissue collected to characterize the effect of the ultrasound on the brain.

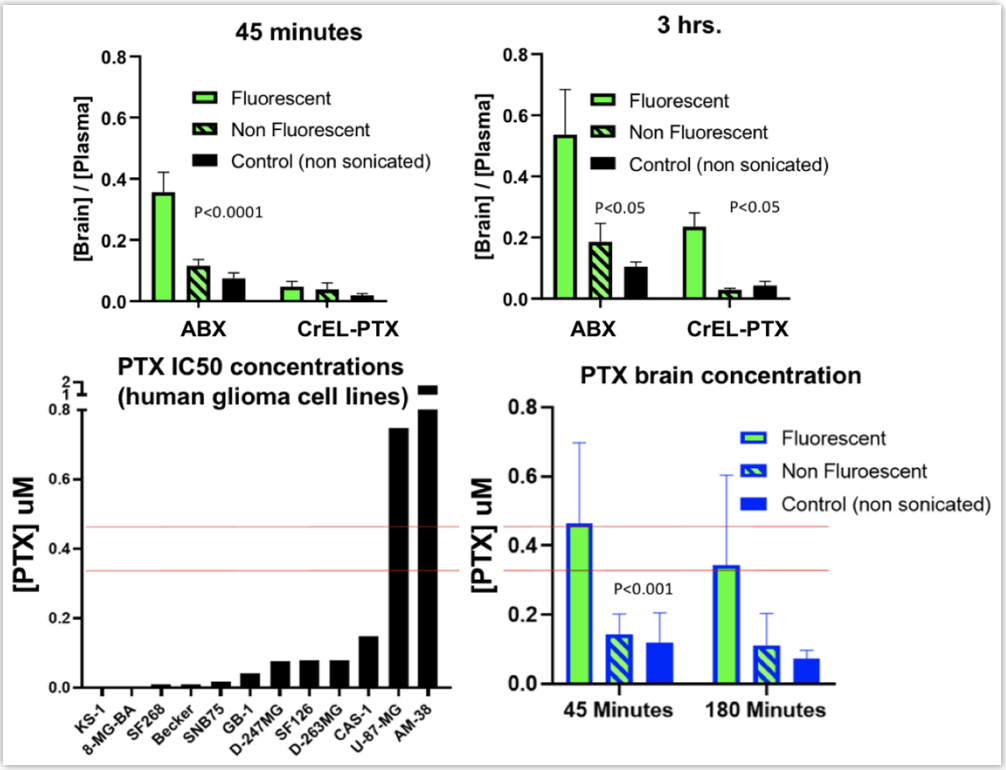
Subsequent analyses using LC-MS will be performed by our collaborators at IITRI (Illinois Institute of Technology Research Institute). Feasibility and techniques have already been established as part of our preclinical experiments.[17] Quantification of drug concentrations requires only 20 mg of tissue, thus in most cases we will obtain 3 representative biopsies for each category of sample. This will allow analysis of individual patients, and a robust dataset for analysis of pulled samples.

6.3.4. Drug level analytics in tissue and serum

PTX tissue and plasma concentrations will be determined using LC-MS/MS instrumentation (5500; AB SCIEX, Foster City, CA and 1200 System with PAL HTC Autosampler; Agilent Technologies, Wilmington, DE or equivalent). Analytical methods are validated following the most recent FDA Bioanalytical Method Validation Guidance for Industry (May 2018). Fluorescein tissue concentration will be determined using a microplate fluorometer after extracting the study samples with water-acetonitrile. We designed our studies to gain a thorough understanding of the effects of US-based BBB disruption on the concentration of PTX in the brain and tumor tissue. First, in the early phases of this study, we will confirm that the correlation between fluorescein and PTX concentration that we established in mice (Figure 4) is maintained in peritumoral human brain tissue. To evaluate whether PTX levels reach therapeutic ranges, we propose the following comparisons, similar to the studies performed in mice (Figure 8):

- Fluorescent vs. non-fluorescent brain tissue (within patients that underwent sonication).
- Fluorescent (patients that underwent sonication) vs. non-fluorescent brain tissue (patients that underwent mock sonication).
- Glioma tissue of patients that underwent sonication vs. that of patients that underwent mock sonication.

Figure 8. PTX brain levels after US-BBB disruption

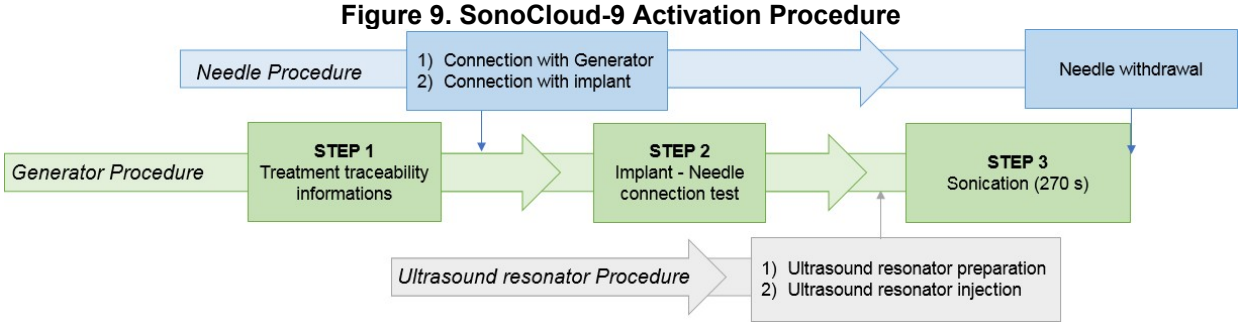


The effect of brain sonication on PTX levels in the brain was determined using fluorescein to map BBB-disruption in mice injected with either ABX or CrEL-PTX 12 mg/kg IV and compared to non-fluorescent brain regions and non-sonicated mouse brains as controls. Brain/plasma PTX concentration ratios were determined at 45 minutes (top left) or 3 hrs (top right). Susceptibility of human glioma cell lines to PTX illustrated by IC50 concentration from Sanger/CCLE database (n=12) (bottom left) is shown in the context of PTX brain concentrations achieved following IV injection of 12 mg/kg of IV ABX. Significance was determined One-way ANOVA test.

6.4. Sonication procedure

The subsequent activation of the SC9 implant is performed as an outpatient procedure and shall be performed by specifically trained personnel. The SC9 implant is activated before each planned ABX administration. The SC9 device uses low intensity pulsed ultrasound energy to open the BBB (Figure 9). Each sonication step consists of the generation of pulsed ultrasound at constant acoustic pressure equal to 1.03 MPa, in combination with the administration of the ultrasound resonator. During the procedure, the operator is guided by an interactive software interface through a series of steps in the generator software in which the operator enters the patient information (touchscreen), the physician information (STEP 1), verifies that the needle is properly connected to the implant (STEP 2), and then begins the sonication (STEP 3, see the instructions for use for further details).

Figure 9. SonoCloud-9 Activation Procedure



6.4.1. Justification of the Acoustic Pressure Dose

In the SC1 and SC3 trial, an acoustic pressure of 1.03 MPa was identified to be the optimal dose of ultrasound using the SonoCloud acoustic pulsing parameters. This corresponds to a Mechanical Index (MI) = 1.03, a peak positive pressure = 1.03 MPa, and a peak negative pressure = 1.03 MPa. At this level of acoustic pressure, systematic opening of the gray and white matter was observed without toxicity. As of today, this dose of acoustic pressure is confirmed to be safe and effective to disrupt the BBB in the ongoing SC9 trial and no dose limiting toxicity or related serious adverse events have been reported thus far using nine activated emitters.

7. TOXICITY MANAGEMENT & DOSE DELAYS/MODIFICATIONS

Any patient who completes at least cycle one of study treatment (i.e. ABX preceded by SC9/microbubbles sonication [ABX/SC9] will be evaluable for toxicity endpoints.

All treatment-emergent toxicity from surgery until end of study will be recorded including surgical complications, infusion-related reactions and any other form of toxicity. Grading and resolution of the event will be reported using Common Terminology Criteria for Adverse Events (CTCAE) v5.0.

7.1. Toxicity during Sonication

During a sonication session, the Investigator may stop the procedure at any time in case of any serious symptoms, pain or neurological deficits; or when suspecting a deficiency of the investigational device. The ultrasound session must not be resumed at that same visit, even when the signs or symptoms that justified discontinuation of the procedure resolve. The duration of the sonication and the reason for suspending the sonication should be adequately documented in the participant's source file and in the CRF. The decision to resume SC9 treatment at the subsequent treatment cycle requires discussion with the principal investigator, the device manufacturer if malfunction is suspected, and the DSMC.

The Investigator may also decide to temporarily suspend a SC9 treatment for any safety reason (*e.g.*, ongoing infections, compromised wound healing, ABX side-effects requiring postponement of chemotherapy). The reason for suspending and resuming the treatment should be documented in the participant's source file and in the electronic case report form (eCRF).

7.2. Toxicity during chemotherapy infusion

Infusion reactions, local and systemic toxicities will be managed per institutional practice and clinical guidelines. Abraxane®-related toxicities, dose delays or modifications will be managed per local practice and as directed by registration label. For temporary/reversible grade 2 peripheral neuropathy with pain or grade 3 peripheral neuropathy, the initial dose level may be maintained unless symptoms and grade persist for >2 weeks, in this case hold dose until resolved to grade ≤ 2 and then resume at 1 dose level lower. However, per investigator discretion dose may be reduced one level for concerns about cumulative peripheral neuropathy.

7.2.1. Treatment-emergent toxicity and dose reductions/delays

Similarly, for severe and prolonged grade IV hematological toxicity or grade III/IV non-hematological toxicity (except nausea/vomiting, and fatigue) subsequent doses should be reduced by 1 dose level. For details on monitoring and dose modifications, please refer to Section 6.2.2.

Concomitant Medications/Treatments:

All concomitant therapies must be recorded in the appropriate eCRF and source documents throughout the study, beginning with the time of written informed consent through end of treatment.

Standard supportive care therapies needed for the management of treatment-related symptoms are permitted, as clinically indicated. Non-enzyme-inducing anti-epileptic drugs should be used for seizure prophylaxis and treatment.

The use of the <u>following medications is prohibited</u> during the study:

- Any non-study cytotoxic chemotherapy or other anticancer therapy
- Anticancer immunotherapy
- Experimental therapeutics: any investigational medicinal product within 30 days prior to registration and during the study
- Platelet aggregation inhibitors
- Antibiotics with known neurotoxicity (e.g., aminoglycosides, cephalosporin, quinolones), unless substitution is not possible or given as perioperative prophylaxis per standard of care; and,
- Non-absorbable material (dura matter substitute, hemostatic agent…)

Treatment temporarily prohibited:

Anticoagulants should be discontinued before any surgery and sonication. The timing of interruption, defined as per standard pre-surgery procedures, will be documented in the medical record and eCRF.

Patients who are stable on benzodiazepines or antidepressant at the time of inclusion may continue their treatment at the same or lower dose.

Treatments of potential toxicity to the CNS (e.g. with antipsychotics/neuroleptics), older antiepileptics or antidepressants (especially tricyclics or monoamine oxidase inhibitors) should be avoided whenever possible.

The metabolism of paclitaxel is catalyzed by CYP2C8 and CYP3A4. In the absence of formal clinical drug interaction studies, caution should be exercised when administering ABRAXANE concomitantly with medicines known to inhibit (e.g., ketoconazole and other imidazole antifungals, erythromycin, fluoxetine, gemfibrozil, cimetidine, ritonavir, saquinavir, indinavir, and nelfinavir) or induce (e.g., rifampicin, carbamazepine, phenytoin, efavirenz, and nevirapine) either CYP2C8 or CYP3A4.

7.3. Other Modalities or Procedures

Tumor resection surgery is a standard of care procedure that will follow institutional guidelines. The tissue collected during surgery will undergo clinical evaluation as well as testing for research purposes. Any other procedures related to surgery are standard of care.

7.4. Duration of Therapy

Within 30 days of screening (and 15 days of registration), patients will undergo surgical resection. Patient will receive an ABX test dose and sonication during initial surgery, the first therapeutic dose treatment cycle will be delivered about 2 weeks after surgery, subsequent cycles are to be given at 21 days interval (± 3 days), depending on toxicity and blood counts. The treatment will be continued for up to 6 cycles as long as clinically indicated, and discontinued in case of significant toxicity, patient refusal or at the investigator's discretion in the patient's best interest. Treatment duration may be prolonged if judged beneficial for a specific patient.

Patients may continue to receive cycles of treatment as specified in the protocol until any of the following occur:

- 6 cycles are completed
- Unequivocal disease progression
- Development of an inter-current illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Patient decides to withdraw from either study treatment or from the study
- The treating investigator determines that the patient should be taken off treatment for any reason (i.e. changes in condition, inability to comply with study treatment or procedures)
- Non-compliance with the study-specific procedures and follow-up

7.5. Duration of Follow Up

Once treatment is completed, patients will undergo an end of treatment visit 4 weeks after the start of the last cycle (+/- 14 days) or earlier, if a subsequent treatment regimen is to start. During follow-up if no other tumor-specific treatment has been initiated, a clinic visit including a full physical exam is planned every 2 months (+/- 28 days) until disease progression or for up to 1 year from last treatment administration. If the patient decides not to return to the clinic, follow-up data will be collected via phone. Patients will be monitored with brain MRI every 2 - 3 months (+/- 28 days) per local practice. Survival information will be recorded every 3 months. Patients removed from treatment for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

7.6. Duration of trial

The duration of this study is expected to be 36 months from the first patient's first visit to the last patient's last visit. The recruitment period will be 24 months; treatment and short-term follow-up will be 6 months. The survival status of the last enrolled patient will be collected 1 year after the last patient registration visit.

7.7. Removal of Subjects from Study Treatment and/or Study as a Whole

Patients can be taken off the study treatment and/or study as a whole at any time at their own request, or they may be withdrawn at the discretion of the investigator for safety, behavioral or administrative reasons. The reason(s) for discontinuation must be clearly documented in the medical record and appropriate eCRF and may include:

- Patient voluntarily withdraws from treatment (follow-up permitted)
- Patient withdraws consent (no follow-up permitted)
- Patient is unable to comply with protocol requirements
- Patient demonstrates disease progression
- Patient experiences unacceptable toxicity
- Treating physician determines that continuation on the study would not be in the patient's best interest
- Patient becomes pregnant
- Patient develops a second malignancy that requires treatment which would interfere with this study
- Patient becomes lost to follow-up

7.8. Patient Replacement

If a patient withdraws for any reason after consent or registration but before receiving the investigational device and ABX, he or she will be replaced. If a patient drops out of the study before the end of the first cycle (DLT evaluation period) for any reason other than treatment related toxicity, he/she shall be replaced.

7.9. Early Trial Stopping Rules

If any of the following scenarios occur with a reasonable possibility of a causal relationship with the study treatment, the trial will be stopped:

If any of the following scenarios occur with a reasonable possibility of a causal relationship with the study treatment, the trial will be stopped:

- If 1 or more patients experience an intervention-related (intervention defined as sonication in association with ABX chemotherapy) death
- If ≥ 2 patients experience partially irreversible life-threatening CNS toxicity

7.10. Study Procedures

A list of procedures and assessments is presented in the Schedule of Assessments (Appendix 1; Table 9).

7.10.1. Screening Visit

Written informed consent must be obtained prior to any protocol-specified assessments or procedures are performed. The following procedures should be performed as outlined below. See also additional details in Section 13.4.

- Collection of Demographic and Medical Information:
  - Date of birth
  - Gender
  - Clinical characteristics: date of diagnosis, disease status at inclusion (including clinical assessment), previous treatments, etc.
  - Tumor characteristics (location and size) on MRI performed within 28 days of surgical visit
  - Relevant medical history other than the studied disease
  - Prior and concomitant medication
- Complete physical and neurological examination including
  - vital signs,
  - Karnofsky performance status (KPS) and ECOG/WHO performance status
  - Mini Mental Status Exam (MMSE)
- Blood tests: complete blood count with differential and platelet count, comprehensive metabolic profile
- 10 cc serum sample for possible later exploratory evaluations (e.g. identification of a biomarker), will be frozen and stored in MBTI biobank
- Urinalysis
- Pregnancy test (if applicable)

7.10.2. Surgical Period

Within 72 hours before surgery, the following procedures should be performed:

- Complete physical and neurological examination with vital signs, KPS
- Review of concomitant medications and toxicity assessment
- ECG
- Blood tests: complete blood count with differential and platelet count, comprehensive metabolic profile
- Blood sample for circulating tumor DNA

The procedure for tumor resection is standard of care and performed at the surgeons' discretion and according to institutional standards. During surgery, the following additional procedures will be performed:

- Temporary manual placement of sonication device and sonication
- Infusion of microbubbles
- Infusion of Abraxane® (ABX)
- Implant of SC9 sonication device

Part of surgical routine but requiring particular attention to detail and timing:

- Fluorescein injection (part of surgical routine, record exact timing)
- Tumor resection; tumor sample will be sent for routine histopathological sampling and identification of biomarkers.

Post-operative care: Patients will be hospitalized for about 3-5 days as standard of care for tumor resection. Patients will then be discharged to home or for rehabilitation as needed. Before hospital discharge, the following procedures shall be performed:

- Postoperative MRI (to be performed within < 48 hours of surgery as routine care to establish post-surgical tumor baseline for comparison
- Complete physical and neurological examination with vital signs
- Review of concomitant medications and toxicity assessment
- Blood tests: complete blood count with differential and platelet count, comprehensive metabolic profile

7.10.3. ABX/SC9 Treatment Period

On about Day 15 after surgery, the drug and ultrasound-based antitumor treatment period will begin. The first cycle will define the DLT-defining observation period.

Prior to each treatment cycle the following procedures and investigations will be performed (time window within 72 hours of administration):

- Complete physical and neurological examination including
    - Vital signs
    - Karnofsky performance status (KPS) and ECOG/WHO performance status
    - Mini Mental Status Exam (MMSE)
- Cycle 1 only: Gd-MRI (with and without contrast, for comparison to post-Gd-MRI for assessment of BBB opening). Occasionally, for technical or timing reasons, this imaging will be performed before and during cycle 2 or later.
- Symptom and toxicity assessment
- Review of concomitant medications
- Blood tests:
    - Complete blood count with differential and platelet count, comprehensive metabolic profile
    - Blood sample for circulating tumor DNA before

Treatment: Sonication and ABX administration

- Establish intravenous access (peripheral or via central venous access device (port-a-cath™)
- Microbubble administration and immediate sonication via the SC9 device
- ABX administration intravenously (over approximately 30 minutes)
- Cycle 1 only: Gd-MRI demonstrating the extent of BBB opening to follow immediately treatment administration. Optional Gd-MRIs to demonstrate the extent of BBB opening may be done in cycles 2-6 per investigator discretion and subject consent for the optional exam
- Blood sample for circulating tumor DNA after sonication
- Observation infusion suite/outpatient clinic for 6 hours post treatment administration in cycle 1
    - Subsequent cycles observation time may be shortened to 2-4 hours depending on prior tolerance, as clinically indicated
- Weekly complete blood count (CBC)

For Cycle 1, Days 8 and 15 (+/- 48 hours) only:

- Complete physical and neurological examination including:
- Vital signs

- Karnofsky performance status (KPS) and ECOG/WHO performance status
- Symptom and toxicity assessment
- Review of concomitant medications

Telehealth visits are allowed for Cycle 1 Days 8 and 15 at the investigator's discretion; if a telehealth visit is done for those days, then only symptom and toxicity assessment with review of concomitant medications will be done.

Imaging:

- Gd-MRI and evaluation of tumor response or progression after every 3 cycles, or more frequent when clinically indicated.

7.10.4. End of Study Treatment Period

Treatment will continue until unequivocal treatment progression, severe toxicity or unacceptable adverse event, intercurrent illness preventing further safe treatment administration, patient's decision to withdraw from treatment or physician's decision in the patient's best interest.

A total of 6 cycles of ABX/SC9 are planned. Prolonging therapy for up to another 6 cycles on a compassionate use basis may be considered if judged by the treating oncologist to be in the patient's best interest. In this case patients will continue careful observation and recording any potential related cumulative or long-term toxicity.

Device explantation: Once the treatment is completed, the implant may be removed upon patient's specific request or if medically indicated.

A formal end of study visit is to be planned 4 weeks (± 14 days) after the last treatment administration (or before starting cycle 7 for patients who remain on therapy, or before starting any subsequent line of antitumor therapy). The end of study visit includes:

- Complete physical and neurological examination including
    - Vital signs
    - Karnofsky performance status (KPS) and ECOG/WHO performance status
    - Mini Mental Status Exam (MMSE)
- Symptoms and toxicity assessment
- Review of concomitant medications
- Blood tests:
    - complete blood count with differential and platelet count, comprehensive metabolic profile
    - Chemistry/metabolic panel including liver and kidney function tests
- Gd-MRI (unless performed within 30 days)

Follow-up:

Patients will be followed as per local routine practice usually including monthly or bi-monthly visits, MR imaging is usually performed every 2-3 months. Special attention will be given to potential late treatment-induced toxicities, e.g. peripheral neuropathy, white matter changes on follow-up MRI. All patients will be followed for survival with regular follow-up phone calls, if not been actively followed in our institution. Additional information on subsequent treatments or late toxicities may be collected from other treating institutions, physicians or family members.

Patients removed from treatment for adverse events will be followed until resolution or stabilization of the adverse event. Survival information will also be collected.

The complete schedule of assessments is included in Appendix 1: Schedule of assessments.

8. ENDPOINTS

8.1. Safety and treatment emergent toxicity

Patients will be carefully followed and assessed throughout all interventions and treatments. Treatment-emergent symptoms and toxicity will be recorded and scored according to the Common Toxicity Criteria version 5. Relationship to the treatment will be attributed by the investigators as unlikely, possible, or probable.

8.2. Dose-limiting toxicity (DLT)

Treatment-emergent and possibly or probably or definitely related toxicity occurring during treatment cycle 1 (start of ABX treatment until the last day of cycle 1 (21 days = planned start of cycle 2) will be evaluated for potential DLT.

→ any surgery-related complications will be separately carefully recorded, however are not considered for DLT definition as surgery and device implantation has previously been demonstrated as safe and feasible; the focus of this protocol is the investigation of sonication in conjunction with ABX chemotherapy.

A DLT is defined as treatment-emergent adverse events that are considered possibly, probably or definitely related to the sonication procedure, ABX chemotherapy or the sonication procedure in conjunction with the ABX chemotherapy (see below).

Patients will be carefully followed and evaluated for toxicities throughout the study, and relevant adverse events will be recorded. Special emphasis will be directed to potential cumulative and late treatment-related toxicity.

8.2.1. DLT toxicity is defined as follows:

Treatment-emergent and possibly, probably or definitely related toxicity attributable to sonication or to the sonication plus Abraxane® procedure (excluding intraoperative procedure) occurring during the DLT period (defined as 21 days from the first SC9 sonication procedure associated with ABX treatment).

i) Any related toxicity ≥ grade 3 that does not respond to optimal medical management (including steroids) within 10 days, exceptions are enumerated here below.

ii) CNS toxicity of ≥ grade 2 that does not revert to grade ≤ 1 within 21 days, i.e. time for next treatment cycle

iii) Grade 4 CNS toxicity

iv) Any treatment-emergent and related toxicity (except hematotoxicity, nausea/vomiting, fatigue and hypersensitivity to Abraxane® or Definity® microbubble injections) > grade 2 that has not reverted to a grade ≤ 2 by day 22 of the first cycle.

→ Treatment-emergent toxicity/events that are unequivocally not related to the sonication or ABX (e.g. attributed to disease progression) will <u>not</u> be <u>considered</u> a <u>DLT</u>

Specific examples and definitions of toxicities included and excluded (→) in the DLT definition:

CNS toxicities:

- New or progressive neurological deficit starting within days after the procedure and persisting beyond day 15 (± 3 days)
    - associated with protocol therapy, ie. sonication and/or ABX therapy
    - symptoms do not respond to steroids, require prolonged hospitalization (>7 d),
- Any ≥ grade 3 clinically significant central nervous system (CNS) toxicity persisting for more than one week that is possibly or definitively related to ABX and/or the sonication procedure.
- Seizures ≥ grade 3 are considered DLT

    → Seizures that occur following sonication, that are self-limiting and do not require intervention other than appropriate antiepileptic therapy are not considered DLT
- Grade ≥ 3 CNS hemorrhage

    → Grade ≤ 2 (asymptomatic or minor symptoms) radiologic evidence of CNS hemorrhage (commonly seen after biopsy) is not a DLT.
- Assessment of CNS toxicity is complicated by the relative changes from the pre-treatment neurological status.

    → Worsening neurological symptoms that can be explained by the presence of the mass lesion and are compatible with the natural history of glioblastoma may require cessation of treatment in individual patients. Such occurrences would not be considered DLT unless they occur with a greater frequency than expected in this patient population.

8.2.2. The following toxicities are not considered DLT:

Nervous system disorders

→ Steroid-resolving edema with recovery to baseline clinical status (with or without imaging)
→ Seizures that occur following sonication, that are self-limiting and/or do not require intervention other than appropriate anti-epileptic therapy
→ Hemorrhage or stroke seated outside the sonication zone (i.e. a 2-cm diameter cylindrical region with a length of 6 cm in front of each US emitter) and thus not related to sonication

Blood and lymphatic system disorders

→ Grade 3 thrombocytopenia without clinically significant bleeding
→ Grade 3 or 4 lymphopenia
→ Grade 3 neutropenia, without fever (< 101°F/38.3°C)

Other non-hematological toxicities

→ Nausea, vomiting or diarrhea/colitis responding to medical management
→ Injury, poisoning and complications from the tumor surgery
→ Deep Vein Thrombosis (DVT) or thromboembolic event
→ Metabolic and nutrition disorders

→ Electrolyte disturbances that are asymptomatic and that respond to replacement or intervention (e.g. IV fluids)
→ Steroid-induced hyperglycemia and other steroid-associated toxicities
→ Skin and subcutaneous tissue disorders
→ Acneiform or maculopapular rash

8.3. Survival Analyses

All patients will be followed for survival. Survival times are calculated from the day of surgery (tumor resection and SC9 implantation) until the date of progression or death or censored at last follow-up. Actuarial survival will be calculated according to the Kaplan-Meier method.

The primary endpoint for the Phase II part of the study will be overall survival (OS) at 1 year. Any patient who has had the SC9 device implanted and received at least one therapeutic dose of ABX will be evaluated for this endpoint.

8.4. Secondary Endpoints

The secondary endpoint for the Phase II study will be progression-free survival. Any patient who has had the SC9 device implanted and received at least one dose of ABX (beyond the test dose) will be evaluated for this endpoint.

8.5. Exploratory Endpoints

The exploratory endpoints will be the area covered by overlay the volume of significant ultrasound-induced signal enhancement (SUISE) map of US-based BBB disruption with maps of enhancing and non-enhancing Fluid-Attenuated Inversion Recovery (FLAIR)+ tumor from MRI. This is a semi-automatic method to map BBB disruption by US based on contrast-enhanced MRI performed 48 hours before and within approximately 30 minutes following sonication. This method derives from the principle that gadolinium contrast only permeates into brain parenchyma with defective BBB, and computes the field of BBB disruption as the volume of significant ultrasound-induced signal enhancement (SUISE), as shown in Figure 10.[11] We will use this approach and algorithm to image and create a 3D map of the field of US-based BBB disruption of the SC9 implantable US device, which is expected to be 9-fold larger than that for the first-generation US devices. To investigate whether residual tumor is exposed to BBB disruption and presumably elevated concentrations of PTX, we will overlay the SUISE volume map with maps of residual enhancing disease and non-enhancing tumor (visualized by FLAIR sequence) obtained on routine postoperative MRI.

Figure 10. Image processing algorithm for calculating volume of BBB disruption

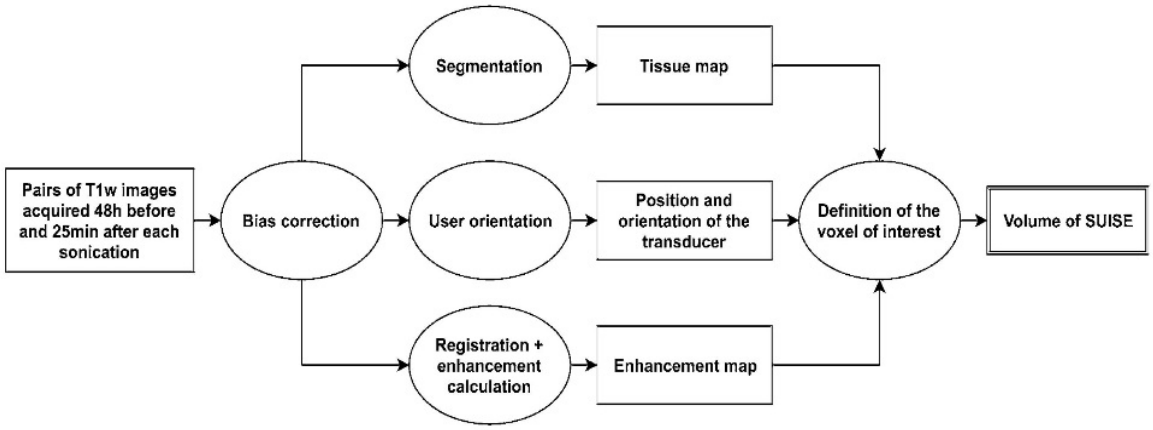


Image processing algorithm used to calculate the volume of SUISE (significant ultrasound-induced signal enhancement) and generate the 3D map of the field of US-based BBB disruption, as described in Asquier et al.[11]

8.5.1. Drug levels in tissue samples (tumor / peritumoral brain) and plasma

Statistical analysis and power considerations: Table 1 provides a summary of the number of patients, conditions and samples that will be collected and undergo quantification of fluorescein and PTX concentrations for these studies. We will use mixed-model regression methods to evaluate differences in drug concentrations for the comparisons described above: within-patient comparisons of fluorescent vs. non-fluorescent brain tissue, fluorescent (patients that underwent sonication) vs. non-fluorescent brain tissue (patients that underwent mock sonication), glioma tissue for patients that underwent sonication v. mock sonication. Fixed main effects will be specified for tissue type (fluorescent, non-fluorescent, gross tumor tissue) and treatment (sonication, mock sonication). Random effects for the model intercept will be specified according to subject to account for within-patient correlation of readouts from multiple tissue samples from the same patient. Main effects will be used to investigate whether a particular group of samples (e.g. fluorescent samples within sonicated peritumoral brain, or glioma tissue) has a higher drug concentration than a control group of samples (non-sonicated brain, or non-fluorescent brain in a patient that underwent intraoperative sonication). P-values <0.05 will be considered significant. Given the exploratory nature of these analyses, multiple comparisons adjustment will not be applied.

We expect to find that US-based BBB disruption in humans will lead to an increase in drug concentrations in peritumoral brain and glioma tissue. Given our pre-clinical work (Figure 8), we anticipate that the brain/plasma concentration ratios for these drugs will be similar, and approximately 0.3-0.5. We anticipate PTX concentrations to be 4 to 5-fold higher in fluorescent peritumoral brain and possibly higher in glioma tissue compared to non-sonicated brain, reaching concentrations in the therapeutic range in the context of in vitro studies for human cell lines (0.3-0.5 uM). Standard deviation estimates for PTX concentrations ranged 0.13-0.22 μM in preliminary data. For conservative power considerations assuming a standard deviation of 0.22 uM, if only one sample was available per patient, 8 patients in each treatment group (sonication vs. mock sonication) would provide 80% power at two-sided 5% Type I error to detect a difference in PTX concentration of 0.33 uM for between-patient comparisons and 0.26 uM for within-patient comparisons. Since we anticipate multiple measurements per patient, the precision of our drug

concentration estimate in each tissue type is likely to be more precise, hence smaller effect sizes will be detectable at similar power and Type I error.

Based on our experience with fluorescein mapping of BBB disruption and its effects on drug concentrations in mice, it is possible that BBB disruption might be partially present beyond areas of gross fluorescence. This potential pitfall will be addressed by analyzing the data comparing it to the group of patients that will undergo administration of PTX and fluorescein but will undergo mock sonication only. In addition to the effect of BBB disruption, drug concentration in the brain is influenced by other factors that might vary between patients, such as rate of metabolism and clearance of ABX, and exact time between infusion of chemotherapy and biopsy of tissue. To control for these confounding factors, in addition to performing a per-patient analysis, we will obtain plasma samples at the time of biopsy and normalize the drug levels in the brain by plasma levels for each patient. In our experience, these ratios provide reproducible results that show clear effects of sonication (Figure 8). Availability of fluorescent and adjacent non-fluorescent brain tissue within the same patient will be determined by the particular anatomy and surgical approach used for each patient, and thus, collecting all these samples might not always be possible. Given that our Phase I/II trial will enroll up to 41 patients, we will have an opportunity to perform this intraoperative study in sufficient patients as we only need n=16 including both sonication and mock sonication groups. Moreover, the analysis of pulled samples across patients including absolute drug concentrations and brain/plasma concentration ratios will overcome the limitation of having some samples missing for some patients.

8.5.2. Detection of circulating tumor DNA in peripheral blood

The collected patient blood samples will be analyzed for the following tumor derived nucleic acids; circulating tumor DNA and circulating tumor miRNA isolated from circulating tumor exosomes. This will be detected by quantitative and digital PCR analysis as well as with next generation sequencing techniques. The nucleic acids purified from patient plasma samples will be subjected to either digital droplet PCR assays, Whole-exome sequencing or RNA-seq.

We will obtain plasma samples (10 ml) during surgery, before and after sonication (a total of 6 samples), so that we can determine what is the optimal time to detect circulating tumor DNA. At subsequent treatment cycles we will also collect a blood sample before and within 2 hours after each sonication procedure (10 ml per sample). Whenever possible, the additional blood will be drawn at time points of other scheduled routine blood analyses. Depending on the results of the first analyses we may refine the time points for collection of the post-sonication samples. This will allow us to determine whether the circulating tumor DNA can predict response to therapy and recurrence. Each measure of tumor-derived nucleic acids will be correlated with radiographic imaging, time to tumor progression and overall survival to determine if they are predictive of response to US-mediated ABX therapy.

9. ADVERSE EVENTS

This study will be conducted in compliance with the study-specific standard operating procedure (SOP) and per the DSMC guidelines of the Robert H. Lurie Comprehensive Cancer Center of Northwestern University (https://www.cancer.northwestern.edu/docs/research/data-safety-monitoring-plan.pdf)

The level of risk attributed to this study requires High Risk Monitoring as outlined in the SOP. In addition, the study will abide by all safety reporting regulations, as set forth in the Code of Federal Regulations.

9.1. Adverse Event Monitoring

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of subjects enrolled in the studies as well as those who will enroll in future studies using similar agents. Adverse events are reported in a routine manner at scheduled times during a trial (see Table 9 for time points). In addition, certain adverse events must be reported in an expedited manner to allow for optimal monitoring and patient safety and care.

All patients experiencing an adverse event, regardless of its relationship to study drug, will be followed until:

- the adverse event resolves or the symptoms or signs that constitute the adverse event return to baseline;
- any abnormal laboratory values have returned to baseline;
- there is a satisfactory explanation other than the study drug for the changes observed; or
- death.

9.2. Definitions & Descriptions

9.2.1. Device Deficiency

A device deficiency is defined as an inadequacy of an investigational medical device related to its identity, quality, durability, reliability, safety, or performance. This may include malfunctions, use error, omission of an act that results in a different medical device response than intended by the manufacturer or expected by the user, or inadequacy in the information supplied by the Sponsor. A use error includes slips, lapses, and mistakes. Any Device deficiency must be immediately reported to CarThera.

9.2.2. Adverse Event

An adverse event (AE) is any untoward medical occurrence in a patient receiving study treatment and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an experimental intervention, whether or not related to the intervention.

Recording of AEs should be done in a concise manner using standard, acceptable medical terms. In general, AEs are not procedures or measurements, but should reflect the reason for the procedure or the diagnosis based on the abnormal measurement. Pre-existing conditions that worsen in severity or frequency during the study should also be recorded (a pre-existing condition that does not worsen is not an AE). Further, a procedure or surgery is not an AE; rather, the event leading to the procedure or surgery is considered an AE.

All AEs will be collected from the signing of the ICF until the end-of-study visit at the time points specified in Table 9.

If a specific medical diagnosis has been made, that diagnosis or syndrome should be recorded as the AE whenever possible. However, a complete description of the signs, symptoms and investigations which led to the diagnosis should be provided. For example, if clinically significant elevations of liver function tests are known to be secondary to hepatitis, "hepatitis" and not "elevated liver function tests" should be recorded. If the cause is not known, the abnormal test or finding should be recorded as an AE, using appropriate medical terminology (e/g/ thrombocytopenia, peripheral edema, QT prolongation). In addition, appropriate attribution of the AE to either the surgical procedure, sonication, or drug delivery should be made as shown in Table 4.

For all AEs/ADEs and serious adverse events (SAEs)/SADEs relatedness evaluation, the investigator should also assess attribution of the three study procedures interventions: 1- surgical procedure, 2- the sonication procedure (Ultrasound emission and BBB opening) and 3- Abraxane delivery. In his/her assessment, the investigator should consider timing of event as illustrated in Table 4.

Table 4. Attribution of Adverse Events

Attribution	Expected Timing	Examples
Surgical Procedure	From time to surgery to first visit of treatment (cycle 1)	Pain/Discomfort at Scar Subdural Hematoma Hemorrhage
Sonication	Within 2 days of sonication	Pain at Connection or Sonication Transient Facial Palsy Peritumoral Edema
Delivery of Abraxane	Within 3 weeks of Abraxane delivery	Neutropenia Diarrhea
Study procedures	Any event occurring with delayed onset in regards to surgery, sonication or Abraxane® infusion, and may be attributed to different categories, should be attributed to "*Study Procedures*"	*e.g.* delayed infection, focal neurological deficit, brain edema occurring at day 15 post sonication, etc.

9.2.2.1. Severity of AEs

Adverse events will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5, available at: https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf

If no CTCAE grading is applicable, the severity of an AE is graded as follows:

- Mild (grade 1): the event causes discomfort without disruption of normal daily activities.
- Moderate (grade 2): the event causes discomfort that affects normal daily activities.
- Severe (grade 3): the event makes the patient unable to perform normal daily activities or significantly affects his/her clinical status.
- Life-threatening (grade 4): the patient was at risk of death at the time of the event.
- Fatal (grade 5): the event caused death.

9.2.2.2. Serious Adverse Events (SAEs)

All SAEs, regardless of attribution, occurring from time of signed informed consent, through 30 days after the last administration of study drug, must be reported upon discovery or occurrence. An SAE is defined in regulatory terminology as any untoward medical occurrence that:

- Results in *death*.

  → However: If death is due to disease progression and not due to treatment emergent toxicity, this is not considered an SAE.

- Is *life-threatening*. The patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.

- Requires *in-patient hospitalization* or *prolongation of existing hospitalization* for ≥ 24 hours.

- Results in *persistent or significant disability or incapacity*.

- Is a *congenital anomaly/birth defect.*

- Is an important medical event. Any event that does not meet the above criteria, but that in the judgment of the investigator jeopardizes the patient, may be considered for reporting as a serious adverse event. The event may require medical or surgical intervention to prevent one of the outcomes listed in the definition of "Serious Adverse Event."

  For example: allergic bronchospasm requiring intensive treatment in an emergency room or at home; convulsions that may not result in hospitalization; development of drug abuse or drug dependency.

- The following events <u>are not considered SAE</u>, unless in direct causality with the device or treatment:
  - Symptoms and decline due to disease progression
  - Death due to disease progression
  - Surgery for disease progression and removal of the device
  - Elective removal of the device at the end of study or on the patient's request

9.2.2.3. Pregnancies

Pregnancies and suspected pregnancies (including a positive pregnancy test regardless of age or disease state) of a female subject occurring while the subject is receiving Abraxane®, or within 60 days from last IP administration are considered immediately reportable events. IP is to be discontinued immediately. The pregnancy, suspected pregnancy, or positive pregnancy test must be reported to Celgene Drug Safety immediately by facsimile, or other appropriate method, using the Pregnancy Initial Report Form, or approved equivalent form. The female subject may be referred to an obstetrician-gynecologist (not necessarily one with reproductive toxicity experience) or another appropriate healthcare professional for further evaluation.

The Investigator will follow the female subject until completion of the pregnancy and must notify Celgene Drug Safety immediately about the outcome of the pregnancy (either normal or abnormal outcome) using the Pregnancy Follow-up Report Form, or approved equivalent form.

If the outcome of the pregnancy was abnormal (e.g., spontaneous or therapeutic abortion), the Investigator should report the abnormal outcome as an AE. If the abnormal outcome meets any of the serious criteria, it must be reported as an SAE to Celgene Drug Safety immediately by facsimile, or other appropriate method, within 24 hours of the Investigator's knowledge of the event using the SAE Report Form, or approved equivalent form

All neonatal deaths that occur within 28 days of birth should be reported, without regard to causality, as SAEs. In addition, any infant death after 28 days that the Investigator suspects is related to the in utero exposure to the IP should also be reported to Celgene Drug Safety immediately by facsimile, or other appropriate method, within 24 hours of the Investigator's knowledge of the event using the SAE Report Form, or approved equivalent form.

Male Subjects

If a female partner of a male subject taking investigational product becomes pregnant, the male subject taking IP should notify the Investigator, and the pregnant female partner should be advised to call their healthcare provider immediately. Male patients treated with nab-paclitaxel are advised not to father a child during and up to 6 months after treatment.

9.2.2.4. Overdose

The risk of ultrasound overdose is not applicable. For Definity® microbubbles overdose, please refer to corresponding label. For Abraxane®, an overdose is defined as a dose ≥ 10% over the protocol-specified dose of Abraxane® assigned to a given patient, regardless of any associated adverse events or sequelae.

On a schedule or frequency basis, an overdose is defined as anything more frequent than the protocol required schedule or frequency.

On an infusion rate basis, an overdose is defined as any rate faster than the protocol-specified rate. For nab-paclitaxel, an infusion completed in less than 25 minutes may increase Cmax by approximately 20%, therefore a nab-paclitaxel infusion completed in less than 25 minutes will meet the infusion rate criterion for an overdose.

Complete data about drug administration, including any overdose, regardless of whether the overdose was accidental or intentional, should be reported in the case report form.

9.2.2.5. Unanticipated Problems Involving Risks to Subject or Others[5]

A UPIRSO is a type of SAE that includes events that meet ALL of the following criteria:

- Is *unanticipated* in terms of nature, severity, or frequency

[5] Anticipated toxicity please refer to the most recent version of the Sonocloud-9 Investigators' Brochure (Risk Assessment section), and the respective package inserts/SMPC of Abraxane®, Definity® and Fluoroscite®.

- Places the research subject or others at a different or *greater risk of harm*
- Is deemed to be *at least possibly related* to participation in the study.

9.3. Adverse Event Reporting

9.3.1. Routine Reporting

All routine adverse events, such as those that are expected, or are unlikely or definitely not related to study participation, are to be reported on the appropriate eCRF. Routine AEs will be reviewed by the Data Monitoring Committee (DMC) according to the study's phase and risk level, as outlined in the Data Safety Monitoring Plan (DSMP).

9.3.2. Determining if Expedited Reporting is Required

This includes all events that occur within 30 days of the last dose of protocol treatment. Any event that occurs more than 30 days after the last dose of treatment and is attributed (possibly, probably, or definitely) to the agent(s) must also be reported accordingly.

1) Identify the type of adverse event using the NCI CTCAE v5.

2) Grade the adverse event using the NCI CTCAE v5.

3) Determine whether the adverse event is related to the protocol therapy.

   Attribution categories are as follows:

   - Definite: AE is clearly related to the study treatment.
   - Probable: AE is likely related to the study treatment.
   - Possible: AE may be related to the study treatment.
   - Unlikely: AE not likely to be related to the study treatment.
   - Unrelated: AE is clearly NOT related to the study treatment.

4) Determine the prior experience of the adverse event.

   Expected events are those that have been previously identified as resulting from administration of the agent. An adverse event is considered unexpected, for expedited reporting purposes only, when either the type of event or the severity of the event is not listed in:

   - the current protocol
   - the drug package insert
   - the current Investigator's Brochure

9.3.3. Expedited Reporting of SAEs/Other Events

9.3.3.1. Reporting to the Northwestern University QAM/DMC

All SAEs must be reported to the assigned Quality Assurance Manager (QAM) within 24 hours of becoming aware of the event. Completion of the NU CRO SAE Form, provided as a separate document, is required.

The completed form should assess whether or not the event qualifies as a UPIRSO. The report should also include:

- Protocol description and number(s)

- The patient's identification number (provided at registration)
- A description of the event, severity, treatment, and outcome (if known)
- Supportive laboratory results and diagnostics
- The hospital discharge summary (if available/applicable)

All SAEs will be reported to, and reviewed by, the DMC at their next meeting.

9.3.3.2. Reporting to the Northwestern University Institutional Review Board

The following situations require reporting to the Northwestern University Institutional Review Board (IRB):

- Any death of a NU subject that is unanticipated in nature and at least possibly related to study participation will be promptly reported to the NU IRB within 24 hours of notification.
- Any death of a NU subject that is actively on study treatment (regardless of whether or not the event is possibly related to study treatment)
- Any death of a non-NU subject that is unanticipated and at least possibly related and any other UPIRSOs will be reported to the NU IRB within 5 working days of notification.
- All other deaths of NU subjects not previously reported, other non-NU subject deaths that were unanticipated and unrelated, and any other SAEs that were not previously reported as UPIRSOs will be reported to the NU IRB at the time of annual continuing review.

9.3.3.3. Reporting to the FDA

The FDA will be notified within 7 calendar days of any SAE that is associated with study treatment, is unexpected, and is fatal or life-threatening.

The FDA will be notified within 15 calendar days of any SAE that is associated with the study treatment, unexpected, and serious but *not fatal or life-threatening*. This includes any previous SAEs that were not initially deemed reportable, but are later determined to meet the criteria for reporting (i.e. by the DMC).

All other SAEs will be reported on an annual basis as part of the annual FDA report.

9.3.3.4. Reporting to the drug manufacturer

Celgene Corporation
Global Drug Safety and Risk Management
86 Morris Avenue
Summit, New Jersey 07901
Fax: (908) 673-9115
E-mail: drugsafety@celgene.com
Telephone: 1-908-673-9667
Toll Free: 1-800-640-7854

9.3.3.5. Reporting to the device manufacturer

CarThera
Hôpital Pitié-Salpêtrière
Institut du Cerveau et de la Moelle épinière - ICM iPEPS

47-83 bd de l'Hôpital - CS21414
75646 PARIS Cedex – France
E-mail: safety@carthera.eu
Telephone (US): +1 (262) 212 9105
Telephone (France): +33 (4) 72 62 62 68

9.3.3.6. Expedited Reporting by Investigator to Celgene

For the purpose of regulatory reporting, Celgene Drug Safety will determine the expectedness of events of being related to ABRAXANE® based on the Investigator Brochure. In the United States, all suspected unexpected serious adverse reactions (SUSARs) will be reported in an expedited manner in accordance with 21 CFR 312.32.

Serious adverse events (SAE) are defined above. The investigator must inform Celgene in writing using a Celgene SAE form or MEDWATCH 3500A form of any SAE within 24 hours of being aware of the event. The written report must be completed and supplied to Celgene by facsimile within 24 hours. The initial report must be as complete as possible, including an assessment of the causal relationship between the event and the investigational product(s). Information not available at the time of the initial report (e.g., an end date for the adverse event or laboratory values received after the report) must be documented on a follow-up report. A final report to document resolution of the SAE is required. The Celgene tracking number (AX-XX-XX- PI-#####) and the institutional protocol number should be included on SAE reports (or on the fax cover letter) sent to Celgene. A copy of the fax transmission confirmation of the SAE report to Celgene should be attached to the SAE and retained with the patient records.

9.3.3.7. Expedited Reporting by Investigator to CarThera

Serious adverse events (SAE) are defined above. The investigator must inform CarThera in writing of any SAE within 24 hours of being aware of the event. Information not available at the time of the initial report (e.g., an end date for the adverse event or laboratory values received after the report) must be documented on a follow-up report.

10. ABRAXANE DRUG INFORMATION

(for further details please refer to the separate Investigators Brochure)

Albumin bound Paclitaxel

Other names

Abraxane® for Injectable Suspension (paclitaxel protein-bound particles for injectable suspension)

Classification

Paclitaxel is classified as a plant alkaloid, a taxane and an antimicrotubule agent.

10.1. Summary Prescribing Information for Abraxane®

nab-Paclitaxel (ABRAXANE® for Injectable Suspension [Abraxis BioScience, LLC, a wholly owned subsidiary of Celgene Corporation, Summit, New Jersey, United States; hereafter referred to as "Celgene"], ABI-007) is a proprietary solvent-free, protein-stabilized formulation of paclitaxel comprised of paclitaxel in a non-crystalline amorphous state and human albumin with a mean particle size of approximately 130 nanometers. *nab*-Paclitaxel has been developed to improve the therapeutic index of paclitaxel, also reducing the toxicities associated with Taxol and the CrEL and ethanol vehicle. This may be achieved in part by taking advantage of endogenous transport pathways to deliver higher doses of paclitaxel to the tumor. Because *nab*-paclitaxel does not contain a solvent vehicle, micellar entrapment observed with Taxol does not occur (Ibrahim, 2002; Sparreboom, 1999; ten Tije, 2003).[36,43,44] *nab*-Paclitaxel displays linear pharmacokinetic (PK) characteristics. The novel albumin-bound particle formulation of paclitaxel in *nab*-paclitaxel conferred the ability to achieve a higher MTD based on every 3-weeks dosing: 300 mg/m^2 for *nab*-paclitaxel (Study DM97-123) versus 175 mg/m^2 for Taxol (Nyman, 2004).[45] The use of albumin-bound paclitaxel also enables *nab*-paclitaxel to be given in a shorter, more convenient infusion time of 30-40 minutes compared with 3 hours to 24 hours with Taxol. Due to its distinct pharmacological and PK properties and therapeutic index, *nab*-paclitaxel has been approved by regulatory authorities worldwide in over 40 countries/regions as a new product, rather than as a generic formulation of Taxol. *nab*-Paclitaxel may be given without steroid and anti-histamine premedication, which is required for Taxol to prevent solvent-related HSRs (Taxol US prescribing information). Cremophor EL has been shown to leach plasticizers, specifically di(2-ethylhexyl) phthalate (DEHP), from polyvinyl chloride (PVC) bags and polyethylene-lined tubing (Gelderblom, 2001; Venkataramanan, 1986; Pfeifer, 1993; Allwood, 1996; Song, 1996; Xu, 1998). Although no controlled epidemiologic toxicity studies have been conducted in humans exposed to DEHP, severe effects (e.g., carcinogenicity, cardiopulmonary toxicity, hepatotoxicity, and nephrotoxicity) have been observed in experimental models. The Taxol prescribing information instructs users to prepare, store, and administer solutions in glass, polypropylene, or polyolefin containers; non-PVC-containing infusion sets (e.g., those with polyethylene lining) should be used (Taxol US prescribing information). By comparison, standard tubing and intravenous (IV) bags may be used for the IV administration of *nab*-paclitaxel (Ibrahim, 2002; Nyman, 2004).[36,45]

As of October 2014, *nab*-paclitaxel is approved under the trade name of ABRAXANE® in 51 countries worldwide for the treatment of patients with metastatic breast cancer. ABRAXANE® is also approved in 8 countries worldwide for the first-line treatment of locally advanced or metastatic non-small cell lung cancer (NSCLC), and in 40 countries for the first-line treatment of metastatic adenocarcinoma of the pancreas, and it is approved in Japan for treatment of advanced gastric cancer.

10.2. Mode of action

ABRAXANE is a microtubule inhibitor that promotes the assembly of microtubules from tubulin dimers and stabilizes microtubules by preventing depolymerization. This stability results in the inhibition of the normal dynamic reorganization of the microtubule network that is essential for vital interphase and mitotic cellular functions. Paclitaxel induces abnormal arrays or "bundles" of microtubules throughout the cell cycle and multiple asters of microtubules during mitosis.

10.3. Storage and stability

Store the vials in original cartons at 20ºC to 25ºC (68ºF to 77ºF). Retain in the original package to protect from bright light.

Unopened vials of ABRAXANE® are stable until the date indicated on the package when stored between 20ºC to 25ºC (68ºF to 77ºF) in the original package. Neither freezing nor refrigeration adversely affects the stability of the product.

Stability of Reconstituted Suspension in the Vial

Reconstituted ABRAXANE® in the vial should be used immediately but may be refrigerated at 2ºC to 8ºC (36ºF to 46ºF) for a maximum of 24 hours if necessary. If not used immediately, each vial of reconstituted suspension should be replaced in the original carton to protect it from bright light. Discard any unused portion.

Stability of Reconstituted Suspension in the Infusion Bag

The suspension for infusion when prepared as recommended in an infusion bag should be used immediately but may be refrigerated at 2°C to 8°C (36°F to 46°F) and protected from bright light for a maximum of 24 hours. The total combined refrigerated storage time of reconstituted ABRAXANE® in the vial and in the infusion bag is 24 hours. This may be followed by storage in the infusion bag at ambient temperature (approximately 25°C) and lighting conditions for a maximum of 4 hours. Discard any unused portion.

10.4. Protocol – specific dosing

Abraxane® provided for free by the manufacturer will be infused intravenously over approximately 30 minutes at:

- 40-80 mg/m^2 for the intraoperative test dose
- At escalating dose levels every 3 weeks, no earlier than 2 weeks (± 3 days) postoperatively at 40 mg/m^2, 80 mg/m^2, 135 mg/m^2, 175 mg/m^2, 215 mg/m^2 and 260 mg/m^2.

10.5. Preparation

ABRAXANE® is a cytotoxic drug and, as with other potentially toxic paclitaxel compounds, caution should be exercised in handling ABRAXANE®. The use of gloves is recommended. If ABRAXANE® (lyophilized cake or reconstituted suspension) contacts the skin, wash the skin immediately and thoroughly with soap and water. Following topical exposure to paclitaxel, events may include tingling, burning and redness. If ABRAXANE® contacts mucous membranes, the membranes should be flushed thoroughly with water.

ABRAXANE is supplied as a sterile lyophilized powder for reconstitution before use. Steps to prepare are:

1. Aseptically, reconstitute each vial by injecting 20 mL of 0.9% Sodium Chloride Injection, USP.
2. Slowly inject the 20 mL of 0.9% Sodium Chloride Injection, USP, over a minimum of 1 minute, using the sterile syringe to direct the solution flow onto the inside wall of the vial.
3. DO NOT INJECT the 0.9% Sodium Chloride Injection, USP, directly onto the lyophilized cake as this will result in foaming.

4. Once the injection is complete, allow the vial to sit for a minimum of 5 minutes to ensure proper wetting of the lyophilized cake/powder.

5. Gently swirl and/or invert the vial slowly for at least 2 minutes until complete dissolution of any cake/powder occurs. Avoid generation of foam.

6. If foaming or clumping occurs, stand solution for at least 15 minutes until foam subsides.

Each mL of the reconstituted formulation will contain 5 mg/mL paclitaxel.

The reconstituted suspension should be milky and homogenous without visible particulates. If particulates or settling are visible, the vial should be gently inverted again to ensure complete resuspension prior to use. Discard the reconstituted suspension if precipitates are observed. Discard any unused portion. Calculate the exact total dosing volume of 5 mg/mL suspension required for the patient and slowly withdraw the dosing volume of the reconstituted suspension from the vial(s) into a syringe: Dosing volume (mL)=Total dose (mg)/5 (mg/mL). Inject the appropriate amount of reconstituted ABRAXANE into an empty, sterile intravenous bag [plasticized polyvinyl chloride (PVC) containers, PVC or non-PVC type intravenous bag]. The use of specialized DEHP-free solution containers or administration sets is not necessary to prepare or administer ABRAXANE infusions. The use of medical devices containing silicone oil as a lubricant (i.e., syringes and intravenous bags) to reconstitute and administer ABRAXANE may result in the formation of proteinaceous strands.

Visually inspect the reconstituted ABRAXANE suspension in the intravenous bag prior to administration. Discard the reconstituted suspension if proteinaceous strands, particulate matter or discoloration are observed.

10.6. Abraxane® Administration

ABRAXANE® is injected into a vein [intravenous (I.V.) infusion] over approximately 30 minutes. The use of an in-line filter is not recommended. Following administration, the intravenous line should be flushed with sodium chloride 9 mg/ml (0.9%) solution for injection to ensure complete administration of the complete dose, according to local practice.

Nursing implications

- Given the possibility of extravasation, it is advisable to closely monitor the infusion site for possible infiltration during drug administration. Limiting the infusion of ABRAXANE to approximately 30 minutes, as directed, reduces the likelihood of infusion-related reactions.
- Premedication to prevent hypersensitivity reactions is generally not needed prior to the administration of ABRAXANE. Premedication may be needed in patients who have had prior hypersensitivity reactions to ABRAXANE. Patients who experience a severe hypersensitivity reaction to ABRAXANE should not be re-challenged with this drug.
- ABRAXANE causes myelosuppression. Monitor CBC and withhold and/or reduce the dose as needed.
- Sensory neuropathy occurs frequently and may require dose reduction or treatment interruption.
- Sepsis occurred in patients with or without neutropenia who received ABRAXANE in combination with gemcitabine; interrupt ABRAXANE and gemcitabine until sepsis

resolves, and if neutropenia, until neutrophils are at least 1500 cells/mm3, then resume treatment at reduced dose levels.

- Pneumonitis occurred with the use of ABRAXANE in combination with gemcitabine; permanently discontinue treatment with ABRAXANE and gemcitabine.
- Severe hypersensitivity reactions with fatal outcome have been reported. Do not re-challenge with this drug.
- Exposure and toxicity of paclitaxel can be increased in patients with hepatic impairment; therefore administer with caution.
- ABRAXANE contains albumin derived from human blood, which has a theoretical risk of viral transmission.
- ABRAXANE can cause fetal harm. Advise patients of potential risk to a fetus and to use effective contraception.

10.7. Availability & Supply

ABRAXANE is commercially available and will be supplied by the hospital pharmacy. Contact information and instructions for ordering drug will be supplied in a separate pharmacy manual.

10.8. Side effects

The most common adverse reactions (≥ 20%) in metastatic breast cancer were reported to be alopecia, neutropenia, sensory neuropathy, abnormal ECG, fatigue/asthenia, myalgia/arthralgia, AST elevation, alkaline phosphatase elevation, anemia, nausea, infections, and diarrhea.

The most common adverse reactions (≥ 20%) in NSCLC were reported to be anemia, neutropenia, thrombocytopenia, alopecia, peripheral neuropathy, nausea, and fatigue.

The most common (≥ 20%) adverse reactions of ABRAXANE in adenocarcinoma of the pancreas were reported to be neutropenia, fatigue, peripheral neuropathy, nausea, alopecia, peripheral edema, diarrhea, pyrexia, vomiting, decreased appetite, rash, and dehydration.

10.9. Abraxane®-related references

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Ibrahim NK, Desai N, Legha S, et al. Phase I and pharmacokinetic study of ABI 007, a Cremophor-free, protein-stabilized, nanoparticle formulation of paclitaxel. Clin Cancer Res.2002;8:1038−1044.

Nyman DW, Campbell KJ, Hersh E, et al. A Phase I trial of ABI-007, nanoparticle paclitaxel, administered to patients with advanced non-hematologic malignancies. J Clin Oncol. 2004;22(14S):2027.

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Xu QA, Trissel LA, Zhang Y. Paclitaxel Compatibility with IV Express Filter Unit. Int J Pharm Compounding. 1998;2:243-245.

11. CORRELATIVES / SPECIAL STUDIES

Samples of brain tumor and peritumoral tissue will be collected at the time of surgery, processed and analyzed for paclitaxel concentration. Plasma samples obtained at the time of surgical resection will be obtained, processed and analyzed for paclitaxel concentration, and compared to tissue concentrations. A separate lab manual will be developed.

Figure 11. Correlative Samples - Details for Lab Manual

Correlative Samples - Details for Lab Manual			
Correlative study (sample type)	Tumor and peritumoral tissue	Plasma	Plasma
Mandatory or Optional	Mandatory	Mandatory	Mandatory
Timing (+/- windows)	Surgical visit	Surgical visit	Before/ after each sonication
Volume Needed (blood only)	NA	4 ml	20 ml
Tube type needed (blood only)	NA	Lavender or green tube (or any tube with anti-coagulant)	
Tissue thickness and/or # slides (tissue only)	Block as available	NA	NA
Processing center (e.g. PCF-CTU)	Sonabend lab	Sonabend lab	Sonabend lab
Sample handling/processing instructions	To be determined	To be determined	To be determined
Shipping/delivery info	NA	NA	NA
Storage needs	-80^{o} freezer	-80^{o} freezer	-80^{o} freezer

12. STATISTICAL CONSIDERATIONS

12.1. Statistical design and power

This document describes statistical design and power considerations for the proposed investigation of albumin-bound paclitaxel (ABX) toxicity after sonication with the ultrasound (US) device SC9 for opening the blood-brain barrier in glioblastoma patients. The proposed clinical trial framework utilizes a cohesive Bayesian paradigm to identify the MTD for ABX (Phase I) and then evaluate preliminary efficacy of the treatment approach (Phase II). The proposed methodologies balance statistical innovation and sophistication with practicality of clinical trial implementation. Since these Bayesian study designs rely on statistical summaries throughout trial conduct, statistical estimation methods are embedded in the text below along with the design and the explanation of implementation. What follows includes delineation of primary endpoints, Phase I and II trial designs and analysis strategies, and a summary of overall sample size considerations.

12.2. Primary endpoints

The primary endpoint of the Phase I study is clinically significant central nervous system (CNS) toxicity of ABX after sonication with the US device SC9.

DLT period

The DLT period spans from day 1 of the first therapeutic sonication and ABX infusion until end of cycle 1 (day 22).

DLT definition (see section 8.2)

The primary endpoint for the Phase II study will be OS at 1 year. This survival rate will be compared to a historical benchmark of a 45% 1-year survival rate.[46–50]

12.3. Phase I Trial Design

ABX dose levels to be considered include: 40 mg/m^2, 80 mg/m^2, 135 mg/m^2, 175 mg/m^2, 215 mg/m^2 and 260 mg/m^2.

The proposed Phase I trial uses a Bayesian Optimal Interval (BOIN) design. This novel Bayesian dose-finding approach has comparable performance to the well-known model-based continual reassessment method design in terms of accurately identifying the MTD, but with the added benefit of having a lower risk of assigning patients to sub-therapeutic or overly toxic doses. Since the BOIN design is algorithm-based in terms of identifying dose escalation/de-escalation rules, like a traditional 3+3 Phase I design, it is straightforward to implement. In a recent review of Phase I trial designs, the BOIN design was shown to be simple to implement while maintaining superior operating characteristics for identifying MTD.[51–53]

Our proposed BOIN Phase I design uses a target DLT rate for the MTD of $\leq$20%. Decisions for dose escalation, de-escalation, or continuation at the same dose level (DL) will be made after DLT evaluation for each individual patient. Up to 17 patients will be included in the Phase I component of the trial. This portion of the study will be stopped early and the MTD determined if 12 patients are treated at a given dose. The patient in the first cohort will be treated at the lowest ABX dose level (DL) of 40 mg/m^2 (DL 1); additional dose levels of 80 mg/m^2 (DL 2), 135 mg/m^2 (DL 3). Doses of 175 mg/m^2 (DL 4), 215 mg/m^2 (DL 5) and 260 mg/m^2 (DL 6) will also be evaluated

as tolerated. To assign a dose to the next patient, we will decide on escalation/de-escalation according to the rules displayed in Table 5. Table 5 indicates decisions to be made within the current dose level. If the current dose is the highest DL 6 and the algorithm indicates escalation, DL 6 will be maintained. Parallel inclusion during this expansion phase is allowed as long no dose limiting toxicity has been observed in more than 2 patients. No more than 2 patients shall be receiving postsurgical chemotherapy cycle 1 at a given time, while more than 2 patients may have been registered for the trial and have undergone surgery and device implantation. Once a total of 12 patients have been safely treated at a given dose level, this will conclude the phase 1 part of the trial. The phase 2 part will be initiated after formal DMC review. Figure 12 describes the conduct of the trial. Specific numeric parameters guiding the decisions to escalate, retain dose level or de-escalate, i.e. a DLT rate between (0.157,0.238), were calculated using the online R shiny app software (http://trialdesign.org/one-page-shell.html#BOIN) and are based on Bayesian regression methods described.[52] The proposed BOIN design for our setting applies an accelerated titration feature in which dosing is re-evaluated after each individual patient. This is appropriate in our setting due to the brief DLT period (21 days), and because preliminary data support the safety of the highest dose level 260 mg/m^2.

Table 5. Dose escalation rules for BOIN Phase I Trial

No (#) of pts treated at current dose	1	2	3	4	5	6	7	8	9	10	11	12
Escalate if # of DLT <=	0	0	0	0	0	0	1	1	1	1	1	1
Deescalate if # of DLT >=	1	1	1	1	2	2	2	2	3	3	3	3
Eliminate if # of DLT >=	NA	NA	2	3	3	3	4	4	4	5	5	5
*These escalation rules apply within the current dose. When none of the actions (i.e., escalate, de-escalate or eliminate) is triggered, stay at the current dose for treating the next patient. "NA" means that a dose cannot be eliminated before treating 3 evaluable patients.												

Operating Characteristics for the Phase I Design

Table 6 shows the operating characteristics of the proposed design for the Phase I BOIN trial under five scenarios for assumed true DLT rates for 6 dose levels. These operating characteristics are based on 1000 simulations of the trial using parameters described above. The operating characteristics show that, under the range of assumed true DLT probabilities at each dose level, the BOIN design selects the true MTD with highest probability, allocates more patients to the dose levels with the DLT rate closest to the target of 0.2 and maintains an acceptable number of DLTs given the target. We expect that the most realistic scenario will be similar to the gray-shaded rows in Table 6 with assumed true DLT rates of 0.02, 0.04, 0.06, 0.08, 0.10 and 0.20 for the six dose levels, respectively.

Table 6. Operating characteristics of the BOIN Phase I Design

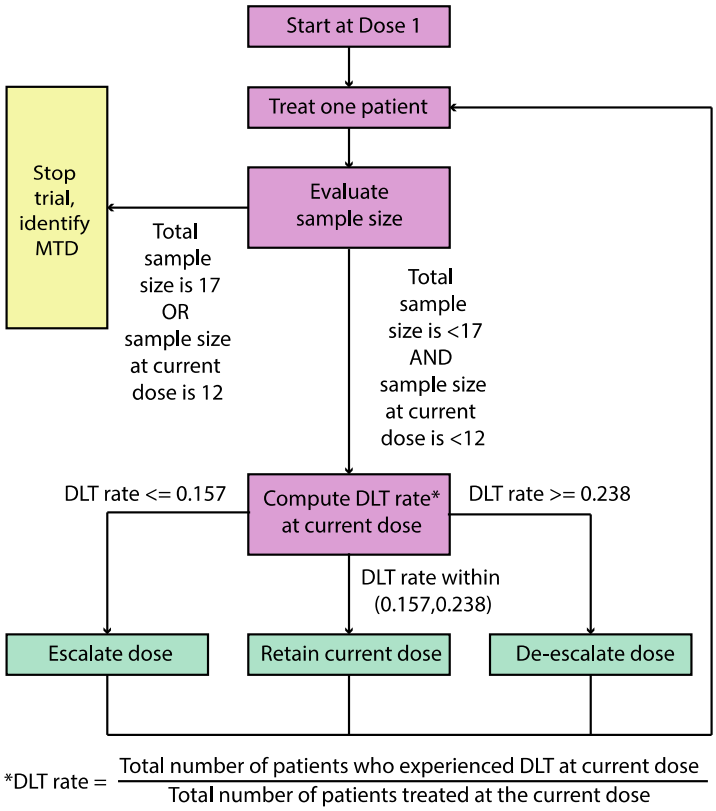
	Dose level						Total # of patients	% Early stopping
	1	2	3	4	5	6		
Assumed true DLT rate	**0.2**	0.34	0.4	0.45	0.5	0.55		
MTD selection	**47.8**	22.3	9.3	4	1.7	0.1		

probability								
% patients treated at dose	45.7	26.5	14.5	7.6	3.8	1.8	14.6	14.8
Assumed true DLT rate	0.09	**0.2**	0.35	0.41	0.48	0.54		
MTD selection probability	21.3	**44.7**	21.2	7.9	3.2	0.4		
% patients treated at dose	25.7	33.6	21.6	10.7	5.7	2.8	16.5	1.3
Assumed true DLT rate	0.02	0.08	**0.2**	0.37	0.43	0.49		
MTD selection probability	2	22.2	**45.9**	19.2	8.3	2.4		
% patients treated at dose	10.9	23.2	30.8	19.2	10.2	5.7	16.9	0
Assumed true DLT rate	0.01	0.05	0.09	**0.2**	0.38	0.51		
MTD selection probability	0.4	4.6	23.2	**45.9**	22.5	3.3		
% patients treated at dose	8.1	12.1	21.9	28.5	20.3	9	17	0.1
Assumed true DLT rate	0.01	0.04	0.07	0.1	**0.2**	0.37		
MTD selection probability	0.3	1.8	8.1	22.9	**45.9**	20.9		
% patients treated at dose	7.4	9.8	13.1	20	28.2	21.5	17	0.1
Assumed true DLT rate	0.02	0.04	0.06	0.08	0.1	**0.2**		
MTD selection probability	0.5	1.5	4.2	8.4	25.3	**59.9**		
% patients treated at dose	7.7	9.3	10.9	13.21.38.1	3.2	5.9	17	0.2

Phase I Trial Statistical Analyses

After the Phase I trial is completed, we will select the MTD based on isotonic regression as previously specified.[52] Specifically, we will select as the MTD the dose for which the isotonic estimate of the toxicity rate is closest to the target toxicity rate. If there are ties, we select the higher dose level when the isotonic estimate is lower than the target toxicity rate; and we will select the lower dose level when the isotonic estimate is greater than the target toxicity rate. Clinical and demographic data will be summarized for all Phase I participants using tables of frequencies and counts for categorical data and means and standard deviations or medians and ranges for continuous variables. Adverse events will be monitored throughout the conduct of the trial and will be reported on a timely manner, and subject to DSMC oversight. These will be tabulated for final review upon completion of the trial.

Figure 12. Flowchart for implementing BOIN Phase I Design



12.4. Phase II Trial Design

The Phase II component of the trial will build on the Bayesian analysis paradigm established for the Phase I trial, and apply a 'BOP2' Bayesian optimal design that will jointly monitor both efficacy and toxicity.[54] This novel Bayesian design accommodates an arbitrary number of interim analyses. In addition, BOP2 controls Type I error and maximizes power given a fixed sample size, features that are missing in other Bayesian Phase II trial designs. The Phase II portion of the trial will use OS as a primary efficacy endpoint, assuming US-based ABX delivery at the MTD determined by the Phase I study. Using historical data, we estimate that 1-year OS is 45% in this recurrent GBM population.[46–50,55]

Table 7. Optimized stopping boundaries for BOP2 design

# patients treated	Stop if # responses <=	OR # toxicity >=
12	4	5
18	8	6
24	12	7

The Phase II trial will evaluate preliminary indication of 65% OS at 1-year as a definition of promising efficacy with a 20% toxicity probability. We will plan to perform interim analyses after enrollment of 12, 18 and 24 patients. Under these parameters, at 10% Type I error with a 30% toxicity probability under the null hypothesis considered too high, these parameters yield approximately 67% statistical power and support the stopping boundaries listed in Table 7. Based on this table, we will perform interim analyses when the number of subjects that reaches 12 and

18 patients with evaluable 1-year survival outcomes, and stop the Phase II trial if <=4 or <=8 patients survive less than 1 year respectively, or if >= 5 or >=6 patients experience toxicity as defined for the Phase II trial, respectively.

When and if the total number of patients reaches the maximum sample size of 24, we will reject the null hypothesis and conclude that the treatment is promising if the number of subjects with OS greater than 1 year exceeds 12 and if the number of patients experiencing toxicity is less than 7; otherwise we will conclude that the treatment is not promising, either due to efficacy or safety. Table 8 reports the operating characteristics of the design based on 10000 simulations using the BOP2 web application, which is available at http://www.trialdesign.org. In Table 8, joint probabilities of efficacy and toxicity are slightly higher than the product of their individual values to reflect a positive correlation in the two events.

Table 8. BOP2 Phase II Trial Operating Characteristics

Probability of efficacy	Probability of toxicity	Probability of efficacy & toxicity	Early stopping (%)	Claim promising (%)	Sample size
0.45	0.3	0.13	78.8	8.0	16.3
0.65	0.2	0.18	21.0	67.6	22.2

Phase II Trial Statistical Analyses

The Phase II trial will be conducted as described above. We will use descriptive approaches for clinical and demographic data as well as adverse events as described for the Phase I trial. For evaluation of the primary OS endpoint for the Phase II trial, Kaplan-Meier methods will be used to estimate the OS distribution and median OS for the Phase II trial participants. All patients treated at the MTD or recommended phase 2 dose (RP2D) will be included in the phase 2 efficacy analyses.

Overall sample size

In the Phase I trial, we intend to enroll a total sample size of approximtely 17. Simulations suggest that 6 of these will be treated at the MTD. We will use data from individuals at the MTD/recommended dose from the Phase I part for analyses in the Phase II setting; this suggests we will need to enroll 18 more patients in the Phase II trial to meet a total sample size of 24 for the Phase II analyses. Thus, we anticipate that our total sample size will be 17+18=35 patients. Anticipating that 15% of enrolled patients may be not evaluable for various reasons, we will plan to enroll a total sample size of 41 patients.

13. STUDY MANAGEMENT

13.1. Protocol Steering Committee

The oversight of the protocol will be assured by the protocol *Steering Committee* that is composed of the two principal investigators and the head of clinical research operations in the department of neurosurgery. The Steering Committee meets weekly as part of the oncological protocol review that includes other neuro-oncologist and neurosurgical experts, as well as the appropriate trial coordinators of the Lurie Cancer Center and the Department of Neurological Surgery.

13.2. Institutional Review Board (IRB) Approval and Consent

It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations. The IRB should approve the consent form and protocol.

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s) and should adhere to Good Clinical Practice (GCP) and to ethical principles that have their origin in the Declaration of Helsinki.

Before recruitment and enrollment onto this study, the patient will be given a full explanation of the study and will be given the opportunity to review the consent form. Each consent form must include all the relevant elements currently required by the FDA Regulations and local or state regulations. Once this essential information has been provided to the patient and the investigator is assured that the patient understands the implications of participating in the study, the patient will be asked to give consent to participate in the study by signing an IRB approved consent form.

Prior to a patient's participation in the trial, the written informed consent form should be signed and personally dated by the patient and by the person who conducted the informed consent discussion.

13.3. Amendments

The Principal Investigator will formally initiate all amendments to the protocol and/or informed consent. All amendments will be subject to the review and approval of the appropriate local, institutional, and governmental regulatory bodies, as well as by CarThera. Amendments will be distributed by the lead institution (Northwestern) to all affiliate sites upon approval by the Northwestern University IRB.

13.4. Registration, Data Collection and Monitoring Procedures

Registering a Patient for the Phase I Portion of the Study

For potential patients for the Phase I portion of this study, study teams are asked to inform the QAM of the date and time that the patient will need to be registered (croqualityassurance@northwestern.edu).

BEFORE a patient can be treated on study, please complete and submit the following items to confirm eligibility and receive an identification number:

- Patient's signed and dated informed consent form (upload to NOTIS and keep original hard copy in a secure location/study chart)
- Eligibility checklist (signed and dated by the treating physician uploaded to NOTIS)
- Eligibility eCRF (complete in NOTIS)
- Copy of the pathology report (upload to NOTIS)

The QAM will review all source documentation required to confirm eligibility that is readily available in the patient's electronic medical record (EMR). Any information that is not available in the EMR must be de-identified and emailed to the QAM. Once the QAM confirms the patient is eligible, he or she will register the patient, assign a subject identification number, and send a confirmation of registration to involved personnel.

Registration will then be complete and the patient may begin study treatment. Cohort assignment (dose level) will be assigned prior to start of cycle 1.

Registering a Patient to the Phase II Portion of the Study

For potential patients for the Phase II portion of this study, study teams are asked to inform the QAM of the date and time that the patient will need to be registered (croqualityassurance@northwestern.edu).

BEFORE a patient can be treated on study, please complete and submit the following items to confirm eligibility and receive a subject identification number:

- Eligibility eCRF (complete in NOTIS)
- Eligibility checklist (signed and dated by the treating physician and uploaded in NOTIS
- Signed and dated informed consent document (upload in NOTIS)
- Pathology Report (upload in NOTIS)

The QAM will review the registration, register the patient, assign an identification number, and send a confirmation of registration to involved personnel. Registration will then be complete and the patient may begin study treatment.

Data Submission

Once a subject is confirmed and registered to the study, eCRFs should be submitted according to the detailed data submission guidelines (provided in a separate document). Generally, all data for Phase I patients during the time period patients are evaluated for Dose Limiting Toxicities (DLTs) must be submitted on a weekly basis. Generally, for all Phase II patients, data are due at the end of every cycle.

Data Management and Monitoring/Auditing

This study will be conducted in compliance with the DSMP of the Robert H. Lurie Comprehensive Cancer Center of Northwestern University (please refer to NOTIS for additional information). The level of risk attributed to this study requires High-Risk Monitoring as outlined in the DSMP. The assigned QAM, with oversight from the Data Monitoring Committee, will monitor this study in accordance with the study phase and risk level. Please refer to the NOTIS for additional data submission instructions.

Adherence to the Protocol

Except for an emergency situation in which proper care for the protection, safety, and well-being of the study patient requires alternative treatment, the study shall be conducted exactly as described in the approved protocol.

Emergency Modifications

Investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior IRB approval.

For any such emergency modification implemented, an IRB modification form must be completed within 5 business days of making the change, and the QAM must be notified within 24 hours of such change.

Other Protocol Deviations

All other deviations from the protocol must be reported to the assigned QAM using the appropriate form.

A protocol deviation is any unplanned variance from an IRB approved protocol that:

- Is generally noted or recognized after it occurs.
- Has no substantive effect on the risks to research participants.
- Has no substantive effect on the scientific integrity of the research plan or the value of the data collected
- Did not result from willful or knowing misconduct on the part of the investigator(s)

A protocol deviation may be considered an instance of Promptly Reportable Non-Compliance if it:

- Has harmed or increased the risk of harm to one or more research participants.
- Has damaged the scientific integrity of the data collected for the study.
- Results from willful or knowing misconduct on the part of the investigator(s).
- Demonstrates serious or continuing noncompliance with federal regulations, State laws, or University policies.

13.5. Investigator Obligations

The Principal Investigator is responsible for the conduct of the clinical trial at the site in accordance with Title 21 of the Code of Federal Regulations and/or the Declaration of Helsinki. The PI is responsible for personally overseeing the treatment of all study patients. The PI must assure that all study site personnel, including sub-investigators and other study staff members, adhere to the study protocol and all FDA/GCP/NCI regulations and guidelines regarding clinical trials both during and after study completion.

The Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected, entered onto the appropriate eCRFs, and submitted within the study-specific timeframes. Periodically, monitoring visits may be conducted and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. The study may also be subject to routine audits by the Audit Committee, as outlined in the DSMP.

13.6. Publication Policy

All potential publications and/or data for potential publications (e.g. manuscripts, abstracts, posters, clinicaltrials.gov releases) must be approved in accordance with the policies and processes set forth in the Lurie Cancer Center DSMP. For trials that require high intensity monitoring, the assigned QAM will prepare a preliminary data summary (to be approved by the DMC) no later than 3 months after the study reaches its primary completion date (the date that the final subject is examined or receives an intervention for the purposes of final data collection for the primary endpoint). If the investigator's wish to obtain DMC-approved data prior to this point (or prior to the point dictated by study design), the PI must send a written request for data to the QAM which includes justification. If the request is approved, data will be provided no later than 4 weeks after this request approval. The data will be presented to the DMC at their next available meeting, and a final, DMC-approved dataset will be released along with any DMC decisions regarding publication. The investigators are expected to use only DMC-approved data in future

publications. The investigators should submit a copy of the manuscript to the biostatistician to confirm that the DMC-approved data are used appropriately. Once the biostatistician gives final approval, the manuscript may be submitted to external publishers.

13.7. Criteria for Stopping the Trial

If any of the following scenarios occur with a reasonable possibility of a causal relationship with the study treatment, the trial will be stopped:

- If 1 or more patients experience a device-related death or life-threatening conditions, or if 2 or more patients experience any unexpected device-related severe AEs.
- If 1 or more patients undergo medical or surgical intervention necessary to preclude permanent impairment or damage suspected to be due to the use of a medical device.

13.8. Data Safety Monitoring Board

The study conduct will be overseen by an independent standing Data Safety Monitoring Committee of the Robert H. Lurie Comprehensive Cancer Center. Safety data will be submitted for review at regular intervals and before each dose escalation to the DSMC for review and approval. The data will also be reviewed by the Study (PIs) and Steering Committee, who may comment on the findings and interpretation.

Details and functioning of the DSMC are outlined in Appendix 5: Data Safety Monitoring Plan.

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APPENDICES

Appendix 1: Schedule of assessments

Table 9. Schedule of assessments

	Baseline	**Surgical Period**			**ABX Treatment Period**				**Off Treatment**	
	Screening	Prior	During	Before discharge	Cycle 1 d1¶	Cycle 1 d8¶	Cycle 1 d15¶	Cycles 2-6¶	End of Treatment	Follow-up
Informed Consent	X									
Demographics / Medical History	X									
Physical and Neuro Exam[1]	X	X		X	X	X	X	X	X	
Mini Mental Status Exam (MMSE)	X				X			X	X	
WHO + Karnofsky Performance Status	X	X			X	X	X	X	X	
Symptom & Toxicity assessment[2]	X	X		X	X	X	X	X	X	
Concomitant medications	X	X		X	X	X	X	X	X	
CBC incl. differential and platelets	X	(X†)		X	X	X	X	X	X	
Chemistry panel	X	(X†)		X	X			X	X	
Urinalysis	X									
Pregnancy test[3]	X	(X†)			X			X	X	
ECG	X	(X†)								
Gd-MRI (with/without contrast)*	X	(X†)		X	X			(X)[5, 9]	X	X
Post-sonication MRI[5]					X			X		
ABX/Sonication			X[4]		X			X		
Tumor sample			X[4]							
Circulating tumor DNA plasma sample		X[7]	X[7]		X[8]			X[8]	(X)	(X)
Survival status										X[6]

[1] Includes vital signs (BP, pulse, resp., temp), height / weight, physical and neurological exam; waived if visit is done via telehealth.
[2] nomenclature and grading according to NCI Common Toxicity Criteria, version 5
[3] Serum or urine test for females of child-bearing potential.
[4] may not be feasible in every patient
[5] Gd-MRI (limited sequences) to demonstrate BBB opening, 1st cycle mandatory, subsequent cycles optional
[6] Patients will be followed (routine clinic visit or phone call) every 3 months to document survival
[7] A total of 6 samples (10 ml each) to be collected perioperatively
[8] Before sonication and within max 2 hours after each sonication
[9] Disease assessment per RANO criteria done after Cycle 3 and Cycle 6 only
† to be repeated only if > 21 days since screening exams
¶ ± 48 hours
(X) optional
*Cycle 1 Pre-sonication MRI to be done 24-48 hours prior to post-sonication MRI

Appendix 2: Performance Status Scales and Definitions

Table 10. Performance Status Scales and Definitions

WHO (ECOG) Criteria		Karnofsky Performance Status	
		%	
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	**100**	Normal no complaints; no evidence of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	**90**	Able to carry on normal activity; minor signs or symptoms of disease.
		80	Normal activity with effort; some signs or symptoms of disease.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	**70**	Cares for self; unable to carry on normal activity or to do active work.
		60	Requires occasional assistance, but is able to care for most of his personal needs.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	**50**	Requires considerable assistance and frequent medical care.
		40	Disabled; requires special care and assistance.
4	100% bedridden. Completely disabled. Cannot carry on any self -care.	**30**	Severely disabled; hospital admission is indicated although death not imminent.
		20	Very sick; hospital admission necessary; active supportive treatment necessary.
5	Dead	**10**	Moribund; fatal processes progressing rapidly.
		0	Dead

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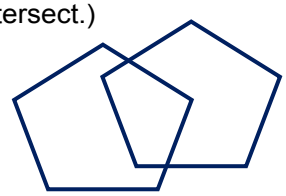
Appendix 3: Mini-Mental State Examination (MMSE)

Mini-Mental State Examination (MMSE)

Patient's Name:_____ Date: _____

Instructions: Score one point for each correct response within each question or activity.

Maximum Score	Patient's Score	Questions
5		"What is the year? Season? Date? Day? Month?"
5		"Where are we now? State? County? Town/city? Hospital? Floor?"
3		The examiner names three unrelated objects clearly and slowly, then the instructor asks the patient to name all three of them. The patient's response is used for scoring. The examiner repeats them until patient learns all of them, if possible.
5		"I would like you to count backward from 100 by sevens." (93, 86, 79, 72, 65, …) Alternative: "Spell WORLD backwards." (D-L-R-O-W)
3		"Earlier I told you the names of three things. Can you tell me what those were?"
2		Show the patient two simple objects, such as a wristwatch and a pencil, and ask the patient to name them.
1		"Repeat the phrase: 'No ifs, ands, or buts.'"
3		"Take the paper in your right hand, fold it in half, and put it on the floor." (The examiner gives the patient a piece of blank paper.)
1		"Please read this and do what it says." (Written instruction is "Close your eyes.")
1		"Make up and write a sentence about anything." (This sentence must contain a noun and a verb.)
1		"Please copy this picture." (The examiner gives the patient a blank piece of paper and asks him/her to draw the symbol below. All 10 angles must be present and two must intersect.) 
30		TOTAL

Appendix 4: Common Toxicity Criteria Adverse Events

The CTCAE version 5.0 can be downloaded with the following link:

https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf

Appendix 5: Data Safety Monitoring Plan

This study will be conducted in compliance with the Data Safety Monitoring Plan (DSMP) of the Robert H. Lurie Comprehensive Cancer Center of Northwestern University:
(https://www.cancer.northwestern.edu/docs/research/data-safety-monitoring-plan.pdf).

The assigned quality assurance manager (QAM), with oversight from the Data Safety Monitoring Committee (DMSC), will monitor this study in accordance with the Phase I/II guidelines for high risk monitoring. Aspects of the guidelines are as follows:

- All routine adverse events, such as those that are expected, or are unlikely or definitely not related to study participation, are to be reported on the appropriate electronic case report form (eCRF).
- Routine AEs will be reviewed by the Data Safety Monitoring Board (DSMC).
- All SAEs must be reported to the assigned QAM within 24 hours of becoming aware of the event. Reporting is done by email. The report will indicate whether or not the event qualifies as unanticipated problem involving risks to subjects or others (UPIRSO). The report will also include: 1) A description of the event, severity, treatment, and outcome (if known); 2) Supportive laboratory results and diagnostics; and 3) The hospital discharge summary (if available/applicable). All SAEs will be reported to, and reviewed by, the DSMC at their next meeting.
- The DSMC will review the data on a cohort basis or after no more than 6 subjects. The standing DSMC meets twice a month to allow for continuous and efficient review. The DSMC has the authority to suspend or close the trial if serious safety concerns are identified.
- Decision to move to the next cohort can be made only by the DSMC upon review of data.