Title: **Two-Part Study to Evaluate the Safety and Efficacy of Image Guided Surgery using Near-Infrared dyes for Intramolecular Imaging of Nervous System Tumors Compared to Standard of Care, (TumorGlow™)**

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List of Abbreviations

ACC – Abramson Cancer Center
AE – Adverse Event
APP – Advanced Practice Provider
BP – Blood Pressure
CRF – Case Report Form
CT – Computed Tomography
CTCAE – Common Terminology Criteria for Adverse Events
CTRC – Clinical Translational Research Center
CTSRMC - Clinical Trials Scientific Review and Monitoring
DOB – Date of Birth
DSMC – Data Safety Monitoring Committee
ER – Emergency Room
FDA – Food and Drug Administration
GCP – Good Clinical Practice
hCG – Human Chorionic Gonadotropin
H&E – Hematoxylin and Eosin
HIPAA – Health Insurance Portability and Accountability Act
HR – Heart Rate
HUP – Hospital of the University of Pennsylvania
HTN – Hypertension
ICH – International Conference on Harmonization
ID – Identification
IP – Investigational Product
IRB – Institutional Review Board
IV – Intravenous
LD_{50} – Median lethal dose
MRI – Magnetic Resonance Imaging
NIR – Near-Infrared
OR – Operating Room
PAH – Pennsylvania Hospital
PET – Positron Emission Tomography
PHI – Protected Health Information
PI – Principal Investigator
PPMC – Penn Presbyterian Medical Center
RR – Respiratory Rate
SAE – Serious Adverse Event
UADE – Unanticipated Adverse Device Effect
US – United States
USP – United States Pharmacopeia
VATS – Video Assisted Thoracoscopic Surgeries

Study Summary

Title	Two-Part Study to Evaluate the Safety and Efficacy of Image Guided Surgery using Near-Infrared Dyes for Intramolecular Imaging of Nervous System Tumors Compared to Standard of Care, (**TumorGlow™**)
Short Title	Indocyanine Green for All Nervous System Tumors
Protocol/IRB Number	822231/UPCC #09315
Phase	Phase I/II
Methodology	Open Label; Part 1 observational and Part 2 permitting deviation from surgical plan based on indocyanine green intraoperative imaging.
Study Duration	5 years
Clinical Sites	Penn Medicine: Hospital of the University of Pennsylvania Penn Medicine Pennsylvania Hospital Penn Medicine Presbyterian Hospital
Objectives	Part I: To determine safety/efficacy of high dose, delayed indocyanine green (second window ICG) during surgery of nervous system tumors. Part II: To determine diagnostic test characteristics (sensitivity/specificity) of delayed, high dose indocyanine green (second window ICG) as a diagnostic aid during surgery of nervous system tumors. Part IIb: To optimize timing and dose of second window IcG during surgery of nervous system tumors, based on sensitivity/specificity. Part IIc: Compare SOC dyes with indocyanine green to determine efficacy for tumor visualization, answering the question is one dye superior to another, more accurate and precise.
Number of Subjects	We intend on enrolling 500 subjects in this study.
Diagnosis and Main Inclusion Criteria	Patients presenting with suspected resectable nervous system tumors who are considered to be good surgical candidates and are at risk of local recurrence.
Investigational Product, Dose, Route, Regimen	The study drug is indocyanine green, single dose of up to 5 mg/kg administered up to 72 hours prior to resection or a single dose of 25 mg administered during induction, diluted into sterile water for intravenous (IV) injection prior to tumor resection. Dye and dose plan is determined by PI based on tumor type, surgical timing, and signal to background ratio.
Investigational Devices	• VS_3 Iridium – Visionsense Stereoscopic High Definition (3DHD) including IR Fluorescence Vision System • Stryker 1588 AIM (Advanced Imaging Modality) Video Camera with Infrared Compatibility with Stryker AIM HD Autoclavable Laparoscope • Zeiss INFRARED™ 800 for OPMI® PENTERO® 800 • Leica FL800 ICG Fluorescence Module with the Leica M530

	OH6 • Surgical Dual Imager
Statistical Methodology	Statistical methodology includes analyses of sensitivity, specificity, and predictive value as they relate to indocyanine green-mediated tumor identification as compared to histopathology.

1 Introduction

This study is conducted according to United States (US) and international standards of Good Clinical Practice (GCP) (Food and Drug Administration [FDA] Title 21 part 312 and International Conference on Harmonization [ICH] guidelines), applicable government regulations and institutional research policies and procedures.

1.1 Background

Primary malignant and non-malignant brain tumors account for an estimated 21.42 cases per 100,000 for a total count of 343,175 incident tumors based on worldwide population estimates [1]. These entities result in variable but disappointing rates of survival, particularly for primary brain tumors (5-year survival rates: anaplastic astrocytoma 27%; glioblastoma multiforme 5%) [2, 3]. Metastatic brain tumors outnumber primary brain tumors (estimates as high as 10:1) as they affect approximately 25% of patients diagnosed with cancer [4-6]. In terms of brain tumor surgery, the extent of surgical resection—a factor that is greatly impacted by a Neurosurgeon's ability to visualize these tumors—is directly associated with patient outcomes and survival [7-9]. Although spinal cord tumors are lower in terms of their incidence [10], data correlating extent-of-resection to outcomes and survival have been demonstrated in patients with intramedullary tumors [11].

Using systemically delivered compounds with a high sensitivity of detection by near-infrared (NIR) fluorescence, it would be possible for us to improve surgical resection thus minimizing chances of recurrence and improving survival. Simply, if the tumor cells will "glow" during surgery, the surgeons are more likely to identify tumor margins and residual disease, and are, therefore more likely to perform a superior cancer operation. By ensuring a negative margin through NIR imagery, it would make it possible to decrease the rates of recurrence and thus improve overall survival.

This concept of intraoperative molecular imaging requires two innovations:

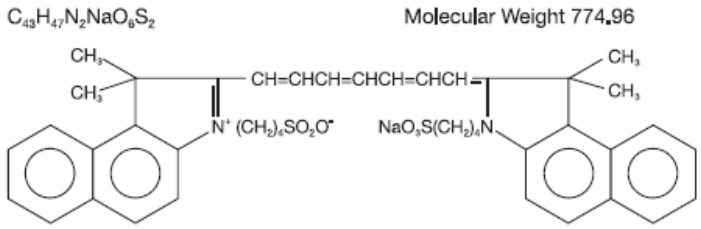
(i) a fluorescent contrast agent that can be injected systemically into the subject and that selectively accumulates in the tumor tissues, and
(ii) an imaging system that can detect and quantify the contrast agent in the tumor tissues.[12, 13]

Subjects undergo intraoperative imaging, receiving an injection of indocyanine green and then undergoing intraoperative imaging of the surgery site with a NIR imaging system. The imaging devices allow the operating field to be observed in real-time.

1.2 Name and Description of the Investigational Product

Indocyanine green is a water-soluble tricarbocyanine dye routinely used in clinical settings for measuring cardiac output, hepatic function, liver blood flow and ophthalmic angiography and has been in use for over 60 years [14]. This protocol utilizes Patheon's indocyanine green, NDC 17238-424. The chemical formula is

$C_{45}H_{47}N_2O_6S_2Na$ and the compound has a molecular weight of 774.96Da (CAS number 3599-32-4). It has a peak absorption in the NIR spectrum at 800nm and maximal emission at 835nm. Indocyanine green is rapidly and completely bound to plasma proteins (especially albumin) after intravenous (IV) injection in the blood [15].



Indocyanine Green for Injection USP is a sterile, lyophilized green powder containing 25 mg of indocyanine green with no more than 5% sodium iodide. It is packaged with Sterile Water for Injection, USP, which is used to dissolve the indocyanine green, and is to be administered intravenously.

1.3 Name and Description of the Investigational Devices

Imaging Systems Intended for Use in the Neurosurgical Operating Room (OR):

Investigational Device	**Manufacturer**	**FDA Product Classification / 510(k)**	**Use of Device (Visit 3: Operative Visit)**	**Hospital Used**
VS_3 Iridium – Visionsense Stereoscopic High Definition (3DHD) including IR Fluorescence Vision System	VisionSense (Medtronic)	Confocal Optical Imaging; Endoscope and accessories; Class II; VS3-IR 510(k): K152204	1) Dura View; 2) Cortex View; 3) Tumor View; 4) Margin View 5) Ex-vivo View	PAH; HUP
Stryker 1588 AIM (Advanced Imaging Modality) Video Camera with Infrared Compatibility with Stryker AIM HD Autoclavable Laparoscope	Stryker	Confocal Optical Imaging; Endoscope and accessories; Class II; VS3-IR 510(k): K173866	1) Dura View; 2) Cortex View; 3) Tumor View; 4) Margin View 5) Ex-vivo View	PPMC
INFRARED™ 800 for OPMI® PENTERO® 800	Carl Zeiss Surgical GMBH	System, X-Ray, Angiographic; Angiographic x-ray system; Class II; 510(k): K100468	Tumor View	PAH; HUP; PPMC
Leica FL800 ICG Fluorescence Module with the Leica M530 OH6	Leica Microsystems	System, X-Ray, Angiographic; Angiographic x-ray	Tumor View	PAH; HUP

Investigational Device	Manufacturer	FDA Product Classification / 510(k)	Use of Device (Visit 3: Operative Visit)	Hospital Used
		system; Class II; 510(k): K141136		
Surgical Dual Imager	Dr. Yodh Lab	Confocal Optical Imaging; Endoscope and accessories; Class II; 510(k): Not Yet Approved	1) Tumor View 2) Margin View 3) Ex-vivo View	PAH

HUP = Hospital of the University of Pennsylvania; NIR = near-infrared; PAH = Pennsylvania Hospital; PPMC = Penn Presbyterian Medical Center

1.4 Preclinical Data

The use of indocyanine green for imaging has been well-documented over the last 20 years. It is believed that indocyanine green, which is normally cleared quickly in vivo, gets "stuck" in tumors. The University of Pennsylvania laboratories have shown that indocyanine green can be used to safely and accurately image lung, breast, renal and ovarian cancers in mice and dogs. Work done at the Hospital of the University of Pennsylvania (HUP) in the lab of Dr. Sunil Singhal has shown 5 mg/kg (given to mice in his lab) is an optimal dose for tumor imaging [25, 28]. In addition, performing optical imaging within 72 hours following dosing of indocyanine green is optimal based on Penn's experience [25]. The LD_{50} after IV administration ranges between 60 and 80 mg/kg in mice, 50 and 70 mg/kg in rats and 50 and 80 mg/kg in rabbits [16, 22].

Studies in a rat glioma model demonstrated that indocyanine green remained within tumors with relatively few glioma cells seen beyond the indocyanine green-laden tumor margin. The doses utilized ranged from 60 to 120 mg/kg with imaging taking place at least 1 hour following the dose [22]. In Dr. Singhal's lab, indocyanine green-injection utilizing canine tumor models demonstrated reliable uptake into flank tumor xenografts with visualization of the tumor/normal tissue interface [23-25].

1.5 Clinical Data to Date

Indocyanine green has been used in the clinical settings since 1957. There is a wealth of data available attesting to the safety of this drug injected at its current clinically indicated dosing level. Indocyanine green has been shown to preferentially uptake in esophageal tumors as opposed to surrounding epithelial lumen after 1 minute of IV exposure [26]. Additionally, the same group reported being able to better characterize the vascularization of the tumor to further clarify the invasiveness of the cancer. Indocyanine green usage has been shown to be safe in a similar clinical setting by the Gotoh group, who used indocyanine green

to detect and characterize bullous and emphysematous lesion of the lung in video assisted thoracoscopic surgeries (VATS) with Infrared Thoracoscopy that were not previously detectable by white light [18]. Finally, indocyanine green has been utilized in applications relating to brain tumors. In a rat glioma model, areas imaged using indocyanine green correlated to the tumor margin within 1 mm [27]. Although dosing and the timing of administration of indocyanine green were variable, tumor demarcation was made possible with the use of intraoperative NIR imaging of indocyanine green [22]. Since then, Dr. Singhal's group has demonstrated uptake into human lung tumors [23]. In addition, Dr. Lee's group has demonstrated uptake in human gliomas, meningiomas and brain metastasis [20, 21, 25].

Previous studies conducted in full-term pregnant women demonstrated no maternal or fetal reactions to the dye. It appeared that indocyanine green clearance is a safe and sensitive test for evaluation of liver function during pregnancy [19].

Prior and ongoing studies with solid tumors at the University of Pennsylvania using indocyanine green and imaging devices have enrolled over 500 subjects to date (17, 20, 23, 25, 28-46). The data collected from these subjects have provided us with knowledge that this method of imaging can be helpful to surgeons in the operating room, including identifying inflammatory processes in lymph nodes and metastatic disease in the thoracic cavity that was not visible on CT or PET scans (17, 20, 23, 25, 28-46).

The standard of care dye, Gleolan™, is the first *and only* FDA approved optical imaging agent for use in Fluorescence-Guided Surgery. Gleolan [aminolevulinic acid hydrochloride (ALA HCl)] is an optical imaging agent indicated in patients with glioma (suspected World Health Organization Grades III or IV on preoperative imaging) as an adjunct for the visualization of malignant tissue during surgery. It is orally administered 2-4 hours prior to anesthesia. A standard surgical operating microscope adapted with a blue light emitting light source and ancillary excitation and emission filters to visualize fluorescence excitation in the wavelength of 375 to 440 nm and for observation from 620 to 710 nm will be used to view Gleolan (50, 51, 52).

In this current study, we intend to compare SOC dyes with indocyanine green to find out which dye is more accurate and precise. This may lead to patients receiving more than one (1) drug prior to surgery and visualizing the tumor with different cameras during surgery.

There are no additional risks of administering both dyes. However, due to imaging with multiple cameras, the surgical procedure may be prolonged 15 minutes. In this study, there is no anticipated risk by extending the surgical procedure.

As of January 2019, this protocol completed Part 1, observational, and began Part 2, permitting deviation from the surgical plan based on indocyanine green intraoperative imaging, of the study. A total of 327 subjects consented with 296

subjects receiving infusion of indocyanine green and 26 non-evaluable subjects (4 subjects have been consented but not infused as of yet). The reasons for non-evaluable subjects are the following: iodine/shellfish allergy (4 subjects), surgical procedures cancelled (4 subjects), decline participation (9 subjects), scheduling conflicts (5 subjects), and clinical issues (4 subjects; i.e., pre-infusion hypertension [HTN]; no medical clearance for surgery).

Of the 296 subjects infused, 110 subjects were administered 2.5 mg/kg and 186 subjects were administered 5 mg/kg of indocyanine green. Of 296 infusions, there were 55 total adverse events (AEs) observed in 35 patients, which are presented in more detail in **Section 1.7** below.

1.6 Dose Rationale

In this study, the indocyanine green dose began at 5 mg/kg, aligned with previous nonclinical and clinical experience at the University of Pennsylvania. Given the incidence of HTN in our CNS cohort and seeking optimal signal to background ratio, we decreased to 2.5 mg/kg to assess efficacy and safety. Thus, we intend to evaluate dose ranges but will not exceed 5mg/kg. Alternately, based on neurosurgeon plan, patients may receive a 25 mg bolus of ICG at the time of induction administered intravenously by the anesthesiologist. This 25 mg dose of indocyanine green is similar to dosing for other intraoperative vascular visualization. This can cause less usage of the drug, lower risk of adverse event occurrences.

We make no changes to the route of administration.. The most important difference between what is labeled on the package and this protocol is the dose regimen (up to 5 mg/kg) and the amount of time elapsing before intraoperative imaging begins (up to 72 hours). This protocol began with 24-hour infusions the day before surgery based on previous nonclinical and clinical experience at the University of Pennsylvania. Interested in strength of signal to background ratio over extended or shortened timeframes, we allow for a range of up to 72 hours. The rationale for dosing timing prior to surgical intervention is based on the minimal risks with indocyanine exposure, to accommodate logistical scheduling of surgeries, and to collect data on optimal signal to background ratio in various tumor types.

Indocyanine green has been used in a dose-escalation trial up to a maximum dose of 10 mg/kg [22] and in other trials 3-5 mg/kg [13, 17-21]. Indocyanine green has a LD_{50} after IV administration of 50 to 75 mg/kg [22].

As Dr. Singhal and other researchers have used an IV injection of indocyanine green to obtain imaging sufficient to describe discrepancies in the structure of abnormal hyper-vascularized tissue [47], it therefore stands to reason that we would be able to accomplish our objective of imaging tumors with indocyanine green using a similar dose.

Interim data analysis (see **Section 7.2**), specifically the fluorescence signal to background ratio, will determine the optimal timing of indocyanine green infusion, which will guide future infusion in this protocol and future protocols.

1.7 Risk/Benefit Assessment

1.7.1 Known Potential Risks

Risks of Indocyanine Green:

The risks of using indocyanine green are well described and exceedingly rare [15, 28]. Indocyanine green is known to have a rare but serious side effect known as anaphylaxis and because of this, patients with a history of iodide allergies will not be considered candidates in this study. There is no connection with any other particular contrast agent or drug or food allergy. Anaphylaxis is an allergic reaction to a substance that can cause swelling of the skin, shortness of breath, dizziness, increased heart rate (HR), rash, and/or death. The indocyanine green dye will be administered in a procedure room with trained nurses who will closely monitor the subject's vital signs (HR, blood pressure [BP], oxygen level [pulse oximetry], and skin color) to check for any signs of anaphylaxis. In the event of an anaphylactic reaction, the indocyanine green injection will be stopped and medication will be used to control the reaction. One study placed the rate of adverse reactions for indocyanine green injections at fewer than 4 in 240,000 [29].

Adverse Events (as of January 2019):

The AEs table below outlines the number of events that occurred by Common Terminology Criteria for Adverse Events (CTCAE) v4.0 event category in this protocol as of January 2019. Subjects could have had more than one AE. Of 296 infusions, there were 55 total AEs observed in 35 patients: 36 events in the 5 mg/kg dose cohort and 19 events in the 2.5 mg/kg dose cohort. Out of the 55 AEs, one was a serious adverse event (SAE) (HTN). One patient experienced two unique AEs during infusion (HTN-General Disorder).

Overall, in 296 patients, HTN accounted for 5.7% of AEs; mild arm pain/discomfort accounted for 7.4% of AEs, general disorders accounted for 2.7%, administration site conditions accounted for 2% of AEs and 0.6% patients reported a gastrointestinal issue.

Of the patients in the **5 mg/kg dose cohort**, 5.4% reported vascular disorders, 9.1% reported mild arm discomfort or pain, 3.2% had general disorders and 1.6% had administration site conditions.

Of the patients in the **2.5 mg/kg dose cohort**, 6.4% reported vascular AEs, 4.6% of them had mild arm discomfort or pain, 1.8% had general disorders, 2.7% had administration site conditions and 1.8% had gastrointestinal AEs.

Table 1 Adverse Events (Protocol #822231, as of February 14, 2019)

	Total	Total %	Total	%	Total	Total %
Dose (mg/kg)			**5**		**2.5**	
# of infusion	**296**		**186**		**110**	
Vascular disorder: HTN	17	5.7	10	5.38	7	6.36
Mild arm discomfort/pain	22	7.4	17	9.14	5	4.55
General disorders	8	2.7	6	3.23	2	1.82
Admin site conditions	6	2	3	1.61	3	2.73
GI	2	0.6	0	0.00	2	1.82

Out of the 55 AEs, one subject in the 5.0 mg/kg dosage cohort experienced a serious adverse event (SAE). This patient, with a past medical history of HTN, began the infusion with high BP. At the direction of the Principal Investigator (PI), infusion was started and directed discontinuation if the patient's systolic BP exceeded 200. The patient's baseline pre-infusion BP was 177/89. The patient received 5.0 mg/kg for a total dose of 276 mg out of the prescribed 533 mg dose ordered (mg/kg). Mid-infusion, the patient's systolic BP was 220 and the infusion was discontinued. During post-infusion observation, BP remained high without return to baseline of 177/89, and although the patient was asymptomatic, was evaluated and treated in the ER then discharged home. Patient arrived for planned craniotomy procedure following day as scheduled. This event was Grade 3.

Of the 296 patients who received indocyanine green under this protocol, we observed an average of 4% decrease in pulse oximetry from baseline compared to end of infusion. At discharge, this group demonstrated an average of 0.20% decrease from pre-infusion in pulse oximetry. Between the two dose cohorts, we observed that the 2.5 mg/kg cohort has less deceleration in pulse oximetry than the 5 mg/kg cohort. To date, 186 patients were infused with 5 mg/kg indocyanine green. These patients experienced a 5% deceleration from pre-infusion to during infusion. From pre-infusion to discharge, we observed an average of 0.38% decrease in pulse oximetry. To date, 110 patients were infused with 2.5 mg/kg indocyanine green. Of those, a 2.4% deceleration from pre-infusion to during infusion was observed. These patients experienced an average of 0.08% increase in pulse oximetry from pre-infusion to discharge.

Risks of Gleolan (aminolevulinic acid hydrochloride (ALA HCl) (5-ALA) [53]:

- Adverse reactions occurring in >1% of patients in the week following surgery were pyrexia, hypotension, nausea, and vomiting.
- Adverse reactions occurring in < 1% of patients in the first 6 weeks after surgery were: chills, photosensitivity reaction, solar dermatitis, hypotension, abnormal liver function test, and diarrhea.

- Neurologic events related to the surgical procedure occurred in 29% of patients and included: aphasia, hemiparesis, hemianopia, headache, seizure, hemiplegia, monoparesis, hypoesthesia, and brain edema.
- Elevated liver enzymes occurred in clinical studies.

There are no anticipated risks of dosing of simultaneous dosing of Indocyanine green and 5-ALA.

Risks of the Use of NIR Imaging Systems:

The video images that we will take intraoperatively may result in the surgeon changing the operation. He/she will not change the scale of the operation to do anything that was not described to the subject during the surgical consent process beforehand. The possible risks associated with taking these images are as follows:

- There is a chance that an area without any cancer in it will glow in the video images. If that happens, the surgeon may remove healthy tissue that did not need to be removed and that would not have been removed in a standard surgery.
- There is a chance that while removing additional tissue that glows in the video images, the subject could be put at an increased risk of having a surgical complication. A surgical complication includes, but is not limited to bleeding, infection, pain, injury to a major organ, nerve damage, blood clot in the arms/legs/other region of the body, and/or death.
- There is a chance that being under anesthesia for the extra 15-20 minutes that it takes to obtain the images for this study could put the subject at an increased risk of having of a common side effect associated with anesthesia. These common side effects include, but are not limited to nausea and vomiting after surgery, sore throat and hoarseness, shivering/chills, confusion, increased BP, and/or muscle aches.
- Finally, there is a chance that the imaging technique could falsely reassure the surgeon that all of the cancer has been taken out, so not as much tissue will be removed. In other words, not all of the cancer could be taken out.

Oncologic surgery in the brain is always balanced by the potential neurologic/functional risks of additional resection, and for this reason neurosurgeons are always weighing the risks and benefits of additional tumor resection. The addition of the fluorescent dye does not change the calculus for the treating physician/neurosurgeon. The surgeon will continue to weigh the risks and minimize these risks. If he/she thinks that removing the extra tissue is too risky, they will not remove the extra tissue. If removal of additional tissue has minimal risk, the surgeon may choose to remove the additional tissue for diagnostic test considerations.

1.7.2 Known Potential Benefits

The purpose of this study is not to investigate a possible benefit for each subject. We do not know whether these video images truly identify cancerous tissue, so we cannot guarantee any benefits from being part of this study. However, there is a chance that because of the video images, the surgeon will remove additional cancerous tissue that they would not have removed during a standard operation.

1.7.3 Assessment of Potential Risks and Benefits

The PI weighs the risks of participating in the study with the potential benefits of participating in the study for each subject enrolled. We feel that the very low risk of adverse reactions expected from the study compared to the practical potential benefit of improving nervous system tumor resection and related outcomes using a non-toxic imaging agent are acceptable.

2 Study Objectives

The primary study objective is to determine safety/efficacy of high dose, delayed indocyanine green (second window ICG) during surgery of nervous system tumors.

The first objective is to determine diagnostic test characteristics (sensitivity/specificity) of delayed, high dose indocyanine green (second window ICG) as a diagnostic aid during surgery of nervous system tumors. Additionally, to optimize timing and dose of second window IcG during surgery of nervous system tumors, based on sensitivity/specificity. Lastly, to compare SOC dyes with indocyanine green to determine efficacy for tumor visualization, answering the question is one dye superior to another, more accurate and precise.

3 Study Design

3.1 General Design

This study is a single center, open-label, two-part study to assess image guided surgery of intramolecular imaging in nervous system tumors. Subjects with a diagnosis of a resectable nervous system tumor who are at risk of recurrence are included. The primary goal is to observe what tissues fluoresce in the OR, and then to identify if that tissue is cancerous/tumor or normal when the histopathology is performed.

This study is split into two parts. The purpose of Part 1 is to confirm dose, assess timing of the operation and assess the feasibility of using indocyanine green as a fluorescent for neurosurgical tumor resection without extending surgery beyond what is seen on the standard of care MRI. The purpose of Part 2 is expansion of the margin view phase of the operation, where residual fluorescence can be resected at the discretion of the surgeon; in addition, further evaluation of optimal dosing and timing.

Candidates are those with a presumed tumor in the nervous system that requires surgery. There is no randomization or control group.

Subjects undergo indocyanine green injection within 72 hours prior to surgery. Permitting infusion time within 72 hours of operation is believed to be adequate for tissue glow/surgical visualization. Alternately, patients may receive 25 mg of indocyanine green during induction. Dose and time of administration will be determined by the neurosurgeon during surgical planning. This 25 mg dose of indocyanine green is similar to dosing for other intraoperative vascular visualization.

3.2 Primary Study Endpoints

The primary end-point of the study is to determine the test characteristics (i.e., sensitivity, specificity, positive predictive value, negative predictive value) of second window indocyanine green in detection of tumors using intraoperative NIR imaging as compared to the standard of care histopathology report.

3.3 Secondary Study Endpoints

The secondary end-point of the study is to identify based on signal to background noise the optimal timing for surgery following infusion of indocyanine green and dose for optimal safety without toxicity for visualization of indocyanine green during intraoperative imaging in nervous system tumors.

3.4 Additional Study Endpoints

Additional study end-points is to compare standard of care dyes with indocyanine green to determine which dye is more accurate and precise.

4 Study Population and Duration of Participation

4.1 Inclusion Criteria

1. Adult patients 18 years of age and older.
2. Patients presenting with a nervous system tumor presumed to be resectable and at risk for local recurrence on pre-operative assessment.
3. Good operative candidate as determined by the treating physician and multidisciplinary team.
4. Subject capable of giving informed consent.

4.2 Exclusion Criteria

1. Pregnant women as determined by urinary or serum beta hCG within 48 hours of surgery.
2. Subjects with a history of iodide allergies.

3. Vulnerable patient populations.
    a. Patients unable to participate in the consent process.
4. History of uncontrolled hypertension (requiring ER admission or ≥ 3 blood pressure medications)

4.3 Subject Recruitment and Screening

Subject recruitment is performed by the patient's treating physician in clinical practice during consultation at a Penn Medicine location. All eligible candidates are invited to participate in the research in the following manner:

1. During the patient's initial surgical consultation, the treating physician or a member of the study team describes the study and its risks and benefits.
2. The potential subject is given a copy of the consent form to review and have all questions answered by the PI or Sub-I. If the patient decides to participate, the consent form is signed.
3. The subject is screened for history of iodine/shellfish allergy by clinical staff.
4. The subject is scheduled for injection at a Penn Medicine location and advised to call with any questions.

4.4 Duration of Study Participation

Each subject participates in the treatment phase of the trial for approximately 2-4 weeks until surgical resection then is followed for 5 years for survival and recurrence.

4.5 Total Number of Subjects and Sites

It is expected that up to 500 subjects will be enrolled in this study, see **Section 7.3** for the sample size determination.

4.6 Vulnerable Populations

Pregnant women, prisoners, children, or other vulnerable populations will not be included. Although not directly targeted, mentally disabled persons, economically or educationally disadvantaged persons, and/or employees or students of the University of Pennsylvania will not be denied enrollment and any special protections and/or additional safeguards will be undertaken in order to protect the rights and welfare of these subjects from coercion or undue influence as appropriate. Students or employees of Penn are informed that their decision to participate does not affect their standing with the University in any way.

4.7 Evaluable Subjects

4.7.1 Non-Evaluable: When and How to Withdraw Subjects

Subjects will be not evaluable if study visits 2 and 3 are not complete. Reasons may include:

1. The subject decides that he/she does not wish to have their surgery.

2. The subject experiences a SAE or an AE during the injection, which would require an intervention of steroids or epinephrine.
3. The subject fails to meet eligibility criteria.
4. The PI or sub-I determine it is in the subject's best interest to not continue in the study.
5. Other reasons such as logistical or scheduling conflicts.

4.7.2 Data Collection

Subject study data is entered into Penn CTMS and a RedCap database as part of the Center of Precision Surgery in order to capture demographic and study data as well as surgeon impression from surgery, which are both encrypted and password protected databases maintained on secure servers.

Data collection and access to medical records will continue for all evaluable subjects on survival and recurrence data for up to five years after their surgery.

5 Study Intervention

5.1 Investigational Product

5.1.1 Description

Indocyanine green is a NIR contrast agent. It is a water-soluble tricarbocyanine dye supplied as a lyophilized powder in 25 mg vials. The powder will be reconstituted in sterile diluent.

5.1.2 Treatment Regimen

The dose is a single infusion of up to 5 mg/kg reconstituted indocyanine green injected intravenously in the dark, within 72 hours of the operation. Alternately, subjects may receive a 25 mg dose of indocyanine green administered by the anesthesiology team during induction. If subjects experience any AEs, please reference **Section 8.2** for protocol intervention. There is no second dose.

5.2 Approved Product

5.2.1 Description

Gleolan (aminolevulinic acid hydrochloride) is an optical imaging agent for oral solution. The 50-mL, clear vial contains 1,500 mg of lyophilized ALA HCl powder (equivalent to 1,170 mg ALA). After reconstitution, the product has a concentration of 30 mg ALA HCl per mL (equivalent to 23.4 mg ALA per mL).

5.2.2 Treatment Regimen

The recommended reconstituted oral dose of Gleolan is 20 mg/k administered 3 hours (range 2 to 4 hours) before anesthesia. If subjects experience any AEs, please reference **Section 8.2** for protocol intervention. There is no second dose.

5.3 Method for Assigning Subjects to Treatment Groups

As this is an open-label study, there will be no randomization of treatment.

5.4 Preparation and Administration of Study Drug

Penn Investigational Drug Service (HUP and Penn Presbyterian Medical Center [PPMC]) or Pennsylvania Hospital (PAH) Pharmacy prepares the study intervention upon notification. The study intervention is administered after preparation once a member of the study team picks up the drug(s) and delivers to the HUP CTRC or the in-house INCU RN, Farm Journal Building at PAH or the in-house ICU RN, or the Abramson Cancer Center (ACC) at PPMC in a light sensitive bag(s), where the staff nurse administers the drug.

5.5 Prior and Concomitant Therapy

Any therapies that are normally not exclusionary criteria for surgical resection are permitted in this study.

5.6 Packaging

Indocyanine green is supplied in a kit (NDC 17238-424) containing six 25mg indocyanine green vials and six 10mL sterile water plastic vials. The label is provided in **Section 15**.

Gleolan is supplied as 1,500 mg of lyophilized ALA HCl powder (equivalent to 1,170 mg ALA), for oral solution in a 50-mL clear, colorless, glass vial with a rubber stopper and an aluminum crimp seal.

5.7 Blinding of Study Drug

As this is strictly an open-label imaging study, no blinding of the drug is necessary.

5.8 Receiving, Storage, Dispensing and Return

Penn Investigational Drug Service (HUP and PPMC) or PAH Pharmacy is responsible for all aspects of receiving, storage, dispensing and return of the study intervention.

6 Study Procedures

When the infusion is done pre-operatively (Table 2a), study participation consists of 1) a screening visit, followed by 2) an infusion visit 72 hours prior to surgery, and 3) the operative visit.

Table 2a Pre-Op Infusion Schedule of Evaluations

Study Procedure	Visit 1: Screening	Visit 2: Infusion Visit	Visit 3 Operative Visit	Annual Assessment (Until Year 5)
Informed Consent	X			
Review Inclusion/Exclusion Criteria	X			

Study Procedure	Visit 1: Screening	Visit 2: Infusion Visit	Visit 3 Operative Visit	Annual Assessment (Until Year 5)
Demographics/Medical History	X			
Physical Examination	X			
Vital Signs: BP, HR, RR	X	X	X	
Height and Weight	X			
Pregnancy Test	X			
Prior/Concomitant Medications	X			
Iodine/Shellfish Allergy Test	X			
Dispense IP		X		
AE/Unanticipated Problems		X	X	
Annual Medical Record Review: Survival, Recurrence				X

AE = adverse event; BP = blood pressure; HR = heart rate; IP = investigational product; RR = respiratory rate

When the infusion is done intraoperatively (Table 2b), study participation consists of 1) a screening visit, followed by 2) the operative visit in which the anesthesiology team may infuse the NIR drug intra-operatively. This will be a 25mg infusion.

Table 2b Intra-op Infusion Schedule of Evaluations

Study Procedure	Visit 1: Screening	Visit 2: Operative Visit	Annual Assessment (Until Year 5)
Informed Consent	X		
Review Inclusion/Exclusion Criteria	X		
Demographics/Medical History	X		
Physical Examination	X		
Vital Signs: BP, HR, RR	X	X	
Height and Weight	X		
Pregnancy Test	X		
Prior/Concomitant Medications	X		
Iodine/Shellfish Allergy Test	X		
Dispense IP to Anesthesia for injection intra-op		X	
AE/Unanticipated Problems		X	
Annual Medical Record Review: Survival, Recurrence			X

AE = adverse event; BP = blood pressure; HR = heart rate; IP = investigational product; RR = respiratory rate

6.1 Visit 1: Initial Consult (approximately 2 weeks prior to operation)

Patients are given the consent form to examine at their leisure and are encouraged to ask questions and have time to have all questions answered. The PI or member of the study team obtains consent from the patient. He/she will be screened for history of iodine/shellfish allergy by clinical staff.

6.2 Visit 2 (CTRC, Infusion visit within 72 hours before operation)

For subjects receiving ICG with 72 hours of surgery, they will arrive at the Perelman Center for Advanced Medicine Clinical & Translational Research Center, 3400 Civic Center Blvd., Philadelphia, PA 19104 or the ACC, Farm Journal Building at PAH, 230 W. Washington Square, Philadelphia PA, 19106, or the ACC at PPMC, 1st floor CUPP Building, 51 N 39th St, Philadelphia, PA 19104 for their infusions at their respective infusion locations within 72 hours prior to the scheduled surgery to receive their injection of indocyanine green by trained nursing staff. The nursing staff places a peripheral IV and calls the pharmacy for the scheduled infusion of up to 5 mg/kg of indocyanine green diluted in sterile water administered intravenously in the dark, over 40 minutes. The nurse monitors subjects pre infusion, during the 40-minute infusion and for 30 minutes post infusion for any signs of anaphylaxis. For all AEs and prescribed treatment, please see **Section 6.5** and **8.2** Expected Adverse Events and Treatment).

During the study visit, subjects' vital signs are monitored as follows: pre infusion; then at 5 minutes after infusion starts, then at every 15 minutes over the 40-60 minute infusion time frame. During the post infusion 30 minute observation period, vital signs are completed at 10-minute intervals and the subject is monitored for any signs of anaphylaxis. The following vital signs are monitored and recorded during each time point: HR, BP, skin color, and oxygen levels (pulse oximetry) and the subject is observed for any adverse sign or symptoms that could include but is not limited to HTN, mild pain or discomfort at infusion site and signs of anaphylaxis. Symptoms are treated according to prescribed interventions outlined in the ICG Treatment Plan.

The Neurosurgeon may want to compare both indocyanine green with other Standard of Care NIR dyes (ex: 5-ALA) intra-operatively to see which dye localizes more accurately and precisely at the region of interest. This may result in using more than one dye for your surgery.

6.3 Visit 3: Operative Visit

Patients will come to one of the University of Pennsylvania Heath System hospitals to undergo removal of their tumor as clinically indicated. Surgical approach and exposure are performed according to standard of care. The entire procedure is photo-documented and recorded as per standard of care. There are five phases of NIR imaging; however, one or more of the phases may be omitted. Patients receiving intra-op ICG by anesthesia will report day of surgery and not have an infusion suite visit.

Dura View: upon opening the skull at the level of the dura but prior to opening the dura, a brief view of the dura is obtained with the NIR imaging system – Dura View.

Cortex View: upon opening the dura but prior to violating the cortex of the brain, another brief view of the cortex is with the NIR imaging system – Cortex View.

Tumor View: Prior to initial resection but upon identification of the tumor, the NIR imaging system (i.e. VS_3 Iridium, Stryker 1588 AIM) is used to visualize the tumor in its native and untouched form. The lights in the OR are turned off and the infrared imaging system is turned on. The video screen displays the images of the tumor area, and the surgeon measures the spectral reading from the tumor. The imaging system captures the infrared signal that is emitted from the indocyanine green that has been taken up by the tumors. Initial tumor fluorescence will be recorded and visualized. After initial visualization, surgical resection will proceed as per standard of care. A NIR microscope (i.e. Leica FL800, Zeiss INFRARED™ 800) will be used to assist with the surgical resection.

Margin View: After completion of the surgery based on standard techniques, the NIR imaging system (i.e. VS_3 Iridium, Stryker 1588 AIM) will be used to determine if there is any residual fluorescence in the resection bed. In Part 1 of this study, the surgeon will proceed to biopsy suspected areas of residual or abnormal tissue or abnormal fluorescence without extending surgery beyond what is seen on the standard of care MRI. In Part 2 of this study, the surgeon will proceed to resect suspected areas of residual or abnormal tissue or abnormal fluorescence. These biopsy and/or resected sites will be correlated with the final pathology, which will be considered the gold standard. Fluorescence of the biopsy and/or resected specimen with the NIR imaging system will be studied both in vivo and ex vivo. In addition, surgeon's judgment as to whether the biopsy and/or additional resection specimen represents tumor will be recorded in a binary outcome questionnaire (yes/no).

Final View: Last, prior to closing a final view of the cavity will be obtained with the NIR imaging system – Final View.

Ex Vivo View: Specimens will be placed in a specimen cup. These specimens will be imaged on a back table with the NIR imaging system.

Once the tumor is resected, a small section of the path specimen will be divided and appropriately labelled: 1) histopathology, 2) research lab specimen. Each tissue sample will be placed in its own unique specimen cup. The tissue will be sent to pathology for standard processing, following OSHA and Institutional policy and procedures for tissue handling and transport.

Of note, it is not always possible to be sure of diagnosis prior to surgical sampling and pathologic analysis. Nevertheless, all subjects and specimens will contribute to the final analysis. For example, if the subject is thought to harbor a malignant

glioma, but at the time of surgery, an intraoperative diagnosis of lymphoma is made, this subject's pathology and indocyanine green fluorescence status will still contribute to the final analysis plan.

6.4 Annual Assessments and Subject Follow-Up

For a period of up to 5 years after enrollment, the PI and research team will review the subjects' medical records annually to obtain information about their medical condition. Re-occurrence will be tracked with the subject's oncologist. We utilize the standard protocol used by the oncologist for tracking re-occurrence, which includes, but is not limited to MRI, CT, or PET scans.

6.5 Rescue Therapy

There is a possibility of anaphylaxis in patients with iodide allergies and these patients are not considered candidates in this study. Anaphylaxis is a reversible condition when treated quickly, and our subject population is monitored at the time of injection (see **Section 8.2**).

7 Statistical Plan

7.1 Primary Endpoint

The primary endpoint is the sensitivity, specificity, positive predictive value, negative predictive value of indocyanine green fluorescence as compared to the gold standard – H&E histopathology.

We compute estimates of the sensitivity (fraction of path-positive margins that are called correctly) and specificity (fraction of path-negative margins that are called correctly), and calculate Bayesian 90% probability intervals around them. We estimate the sensitivity of our method by evaluating the fraction of marginal biopsy and/or resected specimens that we call correctly, and similarly estimate the specificity by the fraction of path-negative biopsy and/or resected specimens that we call correctly.

7.2 Secondary Endpoints

For the secondary endpoint, we will use descriptive data on signal to background noise and safety data by tumor type to identify optimal dosing and timing of indocyanine green for intraoperative imaging in nervous system tumors. We will conduct an interim analysis after approximately 185 subjects are treated at the 2.5mg dose and 185 at the 5mg/kg dose.

Other important endpoints include safety and toxicity that includes review of vital signs and any reported or observed AE.

7.3 Sample Size Determination

There is no sample size calculation for this study as the purpose here is to assess safety and feasibility of using indocyanine green as an imaging agent for viewing

with NIR imaging systems during nervous system tumor surgery. To accomplish this goal, we propose a 500 patient non-randomized prospective design with annual follow-up for 5 years. This is intended to inform the design and planning of a future randomized study.

7.4 Statistical Methods

All data are summarized, with means and standard deviations for continuous variables and proportions for categorical variables. Graphical techniques will be used for data exploration. Analyses will be primarily descriptive. The remainder of the statistical approaches is described above.

8 Safety and Adverse Events

8.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others
Any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the Institutional Review Board (IRB)-approved protocol or consent form, the investigators brochure, etc.)
- Related or possibly related to participation in the research (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research)
- Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm).

Adverse Event
An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as AEs. Abnormal results of diagnostic procedures are considered to be AEs if the abnormality:

- results in study withdrawal
- is associated with an SAE
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

A pre-existing condition should be recorded as an AE if the frequency, intensity or the character of the condition changes.

Serious Adverse Event (SAE)
Adverse events (AEs) are classified as serious or non-serious. An ***SAE*** is any AE that, in the view of either the investigator or the sponsor, is:

- fatal
- life-threatening
- requires or prolongs hospital stay

- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All AEs that do not meet any of the criteria for serious should be regarded as *non-serious adverse events*.

Unanticipated Adverse Device Effect (UADE)
A UADE is any serious adverse effect on health or safety, or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application, or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

Preexisting Condition
A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an AE if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings
At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an AE must also be recorded and documented as an AE.

Post-study Adverse Event
All unresolved AEs are followed by the investigator until the events are resolved, the subject is lost to follow-up, or the AE is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

Hospitalization, Prolonged Hospitalization or Surgery
Any AE that results in hospitalization or prolonged hospitalization is documented and reported as an SAE unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an AE if the condition meets the criteria for an AE.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an AE in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an AE if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

8.2 Expected Adverse Events and Treatment

For all expected AEs, the treatment decisions would be to watchful wait, slow the rate of infusion or discontinue infusion, as appropriate.

Anaphylactic or Urticarial Reactions
Anaphylactic or urticarial reactions have been reported in patients with or without history of allergy to iodides. If such reactions occur, treatment with the appropriate agents (e.g., epinephrine, antihistamines, and corticosteroids) should be administered.

Hypertension
Infusion of indocyanine green may need to be stopped if patient becomes symptomatic at any level or, in the asymptomatic elevation of diastolic BP to ≥100 or systolic BP ≥200. The PI/APP are notified when the BP elevations are reaching levels of concern.

Mild Arm Discomfort/Pain
If patients experience any arm/shoulder pain or discomfort at the IV site, the nursing staff are instructed to apply a hot pack or compress at the area of pain. Infusion of indocyanine green may need to be stopped if patient cannot handle pain or pain has not subsided.

General Disorders
If subjects become symptomatic (fatigue, headache, fever-like symptoms, itching, etc), the PI/APP would be consulted for treatment and infusion decisions.

Administration Site Conditions
If subjects experience tenderness or muscle spasms during the infusion, the nursing staff evaluates as necessary and provides hot pack/compress to the IV site. If subjects experience extravasation of the drug, the infusion may be changed to the other arm or discontinued.

Gastrointestinal Disorders
If subjects become nauseous during the infusion, the infusion is discontinued.

Impact of Indocyanine Green on Pulse Oximetry Readings
A slight decrease of pulse oximetry readings is expected. To date, no treatment has been necessary, as pulse oximetry readings return to normal following completion of infusion.

8.3 Recording of Adverse Events

Safety is assessed by monitoring and recording potential adverse effects using the CTCAE Version 4.0 at each study visit. Participants are monitored by medical histories, physical examinations, and other studies. If CTCAE grading does not exist for an AE, the severity of mild, moderate, severe, life-threatening, and death, corresponding to Grades 1-5, is used whenever possible.

At each contact with the subject, the investigator must seek information on AEs by non-directive questioning and, as appropriate, by examination. Adverse events may also be detected when they are volunteered by the subject during the screening process or between visits, or through physical examination, laboratory test, or other assessments. Information on all AEs should be recorded immediately in the source document, and also in the appropriate AE module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results are recorded in the source document, grouped under one diagnosis.

As much as possible, each AE or follow-up information will be evaluated to determine:

1. Severity grade (CTCAE Grade 1-5)
2. Duration (start and end dates)
3. Relationship to the study treatment or process – [Reasonable possibility that AE is related: No (unrelated/ not suspected) or Yes (a suspected adverse reaction)]. If yes (suspected) - is the event possibly, probably or definitely related to the investigational treatment?
4. Expectedness to study treatment or process – [Unexpected – if the event severity and/or frequency is not described in the investigator brochure (if applicable) or protocol].
5. Action taken with respect to study or investigational treatment or process (none, dose adjusted, temporarily interrupted, permanently discontinued, unknown, not applicable)
6. Whether medication or therapy taken (no concomitant medication/non-drug therapy, concomitant medication/non-drug therapy)
7. Whether the event is serious

All AEs occurring during the study period are recorded. The clinical course of each event is followed until resolution, stabilization, or until the cause is determined. Serious adverse events (SAEs) ongoing at the end of the study period are followed up to determine the final outcome.

8.4 Reporting of Serious Adverse Events (SAEs) and Unanticipated Problems

Reporting period

Adverse events are reported from the time of informed consent until study completion. For this study, study completion is defined as the conclusion of surgery.

Investigator reporting:

Every SAE, regardless of suspected causality (e.g., relationship to study drug(s) or study procedure or disease progression) is reported to the Pharmacovigilance Officer within **24 hours** of learning of its occurrence.

Recurrent episodes, complications, or progression of the initial SAE is reported as the follow-up to the original episode within 24 hours of the investigator receiving the follow-up information. An SAE considered completely unrelated to a previously reported one is reported separately as a new event.

Send the notification (MedWatch or CIOMS form) to the Pharmacovigilance Officer.

New information regarding the SAE is reported as it becomes available and in the same manner that the initial SAE (i.e. MedWatch or CIOMS). The investigator follows the event to resolution or until the event is deemed and documented irreversible, whichever is longer.

Investigator Reporting: Local Reporting Requirements

The investigator reports AEs and SAEs to his/her IRB of record and other local regulatory groups per the local requirements.

8.5 Reporting Serious Adverse Events (SAEs) to the Data Safety Monitoring Committee (DSMC) at the Local PI site

The following are reported to the DSMC:

- All **SAEs** defined as reportable in the protocol.
- All AEs grade 3 or higher within 10 days of knowledge.
- All unexpected deaths within 24 hours of knowledge.
- All other deaths within 30 days of knowledge.

8.6 Pregnancy

Pregnancy, in and of itself, is not regarded as an AE unless there is suspicion that study drug or device or process may have interfered with the effectiveness of a contraceptive medication or method. When a pregnancy is confirmed in a subject during maternal or paternal exposure to study drug and/or process, data on fetal

outcome should be collected for regulatory reporting and drug safety evaluation. Follow-up should be conducted for each pregnancy to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications.

8.7 Unblinding Procedures

As this is an open-label study, there is no need to specify unblinding procedures.

8.8 Medical Monitoring

It is the responsibility of the PI to oversee the safety of the study at his site. This includes careful assessment and appropriate reporting of AEs.

9 Data Handling, and Record Keeping

9.1 Confidentiality

Information about study subjects is kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

The potential subjects are reassured that their personal information is protected by de-identifying study documents by replacing the subject's identifiable information with a unique subject ID number. In an effort to protect each subject's privacy, the study coordinator maintains an enrollment log on an encrypted hard-drive that links the subject ID number with the subject's name, date of birth (DOB), and gender.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

9.2 Data Collection and Management

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these

original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

The study CRF in RedCap and Penn CTMS, for all safety information, is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

Subject study data is entered into an excel database as well as a RedCap database. Data will be stored on-site for 5 years after the last follow-up visit. After 5 years, it may be archived. Data will be de-identified and each subject will be given a subject identification (ID) number immediately after consent is signed and they are enrolled in the study. Only the study staff will have access to the identifying information that would link the subject to their unique study ID number; this information will be kept in a secure location.

9.3 Records Retention

It is the investigator's responsibility to retain study essential documents per Penn policy.

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

This study is monitored according to the monitoring plan in **Section 15**. The investigator allocates adequate time for such monitoring activities. This study will be audited by the Department of Compliance and Monitoring on behalf of the ACC Data and Safety Monitoring Committee every 6 months from the first subject enrolled and every 6 months thereafter for the life of the study. The Investigator ensures monitors or other compliance or quality assurance reviewers are given access to all noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

10.2 Auditing and Inspecting

The investigator permits monitoring, audits, and inspections by the IRB, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator ensures the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

10.3 Reporting of Exceptions and Deviations

Exception

A one time, **intentional** action or process that departs from the IRB and Clinical Trials Scientific Review and Monitoring (CTSRMC) approved study protocol, intended for **one** occurrence. If the action disrupts the study progress, such that the study design or outcome may be compromised, or the action compromises the safety and/or welfare of study subjects, advance documented approval per institutional guidelines, is required.

Deviation

A one time, **unintentional** action or process that departs from the IRB and CTSRMC approved study protocol, involving one incident and **identified retrospectively**. If the deviation disrupts the study progress, such that the study design or outcomes may be compromised, or the deviation compromises the safety and/or welfare of study subjects, the deviation must be reported to the CTSRMC within 5 business days of PI knowledge and to local regulatory review committees per institutional guidelines.

Report the following information on the exception/deviation form:

- Protocol number
- Subject number
- Description of the exception or deviation
- Impact on subject safety
- Impact on data integrity

Deviations that are assessed by the PI to not disrupt the study progress, such as not affecting the study design or outcome, or compromising the safety and/or welfare of study subjects, should be documented in site records and contain documentation of the PI's assessment.

11 Ethical Considerations

This study is to be conducted according to US and international standards of GCP (FDA Title 21 part 312 and ICH guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent IRB, in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the investigator before commencement of this study.

11.1 Informed Consent Process / HIPAA Authorization

After a patient is determined to be a good candidate for the study, the informed consent / HIPAA authorization process is conducted by the PI or treating physician in their clinic. The potential subject is given the consent form to go over in detail during the initial consultation visit. He/she is encouraged to ask questions throughout the consent process and is given adequate time afterwards to ask any additional questions about the study. If the patient agrees to participate, the PI or treating physician obtains consent from patient and prints, signs, and dates the top line of the informed consent form. The consent form is given to the patient to print, sign, and date on the second line of the consent form. Documentation of this process occurs in each subject's electronic medical record in the office note of the day that consent was obtained.

12 Study Finances

12.1 Funding Sources

This clinical trial is being funded through a Penn Medicine Neuroscience Center Grant.

12.2 Conflict of Interest

All University of Pennsylvania Investigators follow the University of Pennsylvania Policy on Conflicts of Interest Related to Research. The Investigator states conflict, if any.

13 Publication Plan

No part of this study will be published without conformation with the Department of Neurosurgery of the University of Pennsylvania standards for publishing.

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15 Attachments

- Study Flowchart
- CRFs
- DSMP
- Informed Consent Form (ICF)
- Equipment