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SCRIHS Protocol # **12-135**

TITLE **A Phase 1 and 2 Study of 5-Aminolevulinic Acid (5-ALA) to Enhance Visualization and Resection of Malignant Glial Tumors of the Brain***

* Nervous system neoplasms malignant: Anaplastic astrocytoma (10002224), Astrocytoma malignant NOS (10003572), Brain stem glioma (10006143), Ependymoma (10014967), Ependymoma malignant (10014968), Glioblastoma (10018336), Glioblastoma multiforme (10018337), Gliosarcoma (10018340), Anaplastic oligodendroglioma (10026659), Oligodendroglioma (10030286), Medulloblastoma (10027107), Mixed astrocytoma-ependymoma (10027743), Miscellaneous CNS primary tumor (10007959), Supratentorial primitive neuroectodermal tumor (10056672).

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IRB NUMBER: 12-135
IRB APPROVAL DATE: 01/22/2017

SCHEMA

There is a considerable body of literature that suggests that completeness of resection is a positive factor for longer term survival in individuals with malignant glioma. Unfortunately, it is often difficult to completely remove a malignant brain tumor because during surgery it is sometimes very difficult to distinguish tumor from normal brain. It would be very helpful if there would be some way to help the neurosurgeon make this distinction. Malignant glioma tumor cells (more so than normal cells) contain the biosynthetic pathways to produce protoporphorin from a naturally occurring amino acid, 5-aminolevulinic acid (5-ALA). Protoporphorin is the immediate precursor to hemoglobin and is fluorescent under deep blue light. When exogenous 5-ALA is provided at increased concentration, protoporphorin concentration in the malignant cell increases at a rate far greater than normal brain cells and renders the malignant cell fluorescent red under blue light. This feature distinguishes the tumor cells from normal cells intraoperatively and facilitates complete resection.

Recent studies in Germany have confirmed the utility of pre-operative oral 5-ALA and intraoperative brain tumor fluorescence in aiding the resection of malignant brain tumors. These studies have led to the approval of oral 5-ALA for this indication by the European Medicines Agency, but it has not been approved for this indication by the United States FDA. This proposal is a phase 1 and phase 2 trial that will hopefully lead to FDA approval of oral 5-ALA for intra-operative visualization of malignant brain tumors.

In the phase 1 part of this proposed study, a minimum of three to a maximum of **18 patients* will be administered oral 5-ALA two-three hours prior to surgery in cohorts of three at five escalating doses of 5-ALA (10, 20, 30, 40, or 50 mg/kg).

Dose-Escalation Schedule	
Dose Level	**Dose of 5-ALA**
Level 1	10 mg/kg
Level 2	20 mg/kg
Level 3	30 mg/kg
Level 4	40 mg/kg
Level 5	50 mg/kg

*(*Due to four patients receiving 40mg/kg rather than three, 19 total patients will be studied in phase 1. Protocol deviation approved by IRB on 17 Oct 2013.)*

The following data will be collected:

- Dose-limiting toxicity data.
- Tumor fluorescence assessed by neurosurgeon (0 to +++) in three distinct areas of fluorescence (Strong fluorescence, Weak fluorescence, No fluorescence).
- Tumor density from biopsies obtained by the neurosurgeon in the same three distinct areas of fluorescence and assessed by neuropathology (Solid tumor, Tumor mixed infiltrating normal brain, No tumor).
- Neurosurgeon's intra-operative estimate of residual tumor.
- Neuroradiologist's estimate of post-operative residual tumor on MRI.

This trial will evaluate:

- The toxicity of a single dose of oral 5-ALA given pre-operatively.

IRB NUMBER: 12-135
IRB APPROVAL DATE: 01/22/2017

- The sensitivity and specificity of 5-ALA - Protoporphyrin IX (Pp IX) as an intraoperative fluorescent detection agent and aid for resection of tumor tissue remaining in the walls of the resection cavity of primary and recurrent malignant brain tumors.
- The relationship of the neurosurgeon's estimate of the extent of malignant glioma resection (as guided by tumor fluorescence) to the actual extent of resection determined by post-operative imaging.

Following completion of the phase 1 portion of this trial, an additional 16 subjects will be entered at the recommended phase 2 dose level in order to further define the above parameters at the recommended phase 2 dose level.

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IRB NUMBER: 12-135
IRB APPROVAL DATE: 01/22/2017

TABLE OF CONTENTS

IRB NUMBER: 12-135
IRB APPROVAL DATE: 01/22/2017

IRB NUMBER: 12-135
IRB APPROVAL DATE: 01/22/2017

1. OBJECTIVES
   1.1 Primary Objectives:
      1.1.1 Establish a safe dose for oral 5-ALA administration.
      1.1.2 Determine the sensitivity and specificity of 5-ALA mediated fluorescence for malignant glioma tissue in the brain.
      1.1.3 Compare the neurosurgeon's intra-operative estimate of the extent of malignant glioma resection (as guided by tumor fluorescence) with the actual extent of resection determined by post-operative imaging.

2 BACKGROUND
   2.1 Aminolevulinic Acid (5-ALA)
   Aminolevulinic acid (also known as ALA, 5-ALA, 5-Aminolevulinic acid, D-Aminolevulinic acid, dALA or δALA) is a naturally occurring substance that is found in all organisms, including humans, and is necessary for part of the basic metabolic life processes (*Figure 1*). It is the first compound in the porphyrin synthesis pathway, the pathway that leads to hemoglobin in mammals and chlorophyll in plants. In non-photosynthetic eukaryotes such as animals, insects, fungi, and protozoa, as well as the α-proteobacteria group of bacteria, it is produced by the enzyme 5-ALA synthase, from glycine and succinyl CoA. This reaction is known as the Shemin pathway.[23]

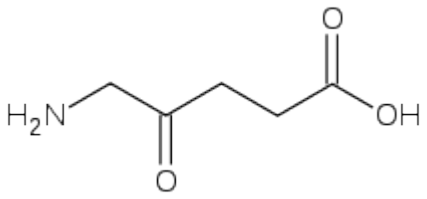

Figure 1. 5-Aminolevulinic acid (C5H9NO3, MW 167.6)

Approximately 350 mg of 5-ALA is synthesized in humans each day for endogenous heme production. Administration of exogenous 5-ALA results in the production of high intracellular (mitochondrial) concentrations of Protoporphyrin IX (Pp IX). 5-ALA (and other porphyrins) and Pp IX are excreted in the urine and the stool (via bile), respectively. PpIX has properties of strong fluorescence and can also act as a photosensitizing agent for photodynamic therapy - resulting in cell death.

   2.1.1 Pharmacology
   The metabolism of 5-ALA is the first step in the biochemical pathway resulting in heme synthesis. 5-ALA is not fluorescent or a photosensitizer, but rather a metabolic precursor of PpIX, which is fluorescent and a photosensitizer. The synthesis of 5-ALA is normally tightly controlled by feedback inhibition of the enzyme, 5-ALA synthetase, presumably by intracellular heme levels. 5-ALA, when provided to the cell, bypasses this control point and results in the accumulation of PpIX, which is converted into heme by ferrochelatase through the addition of iron to the PpIX nucleus (see Figure 2).

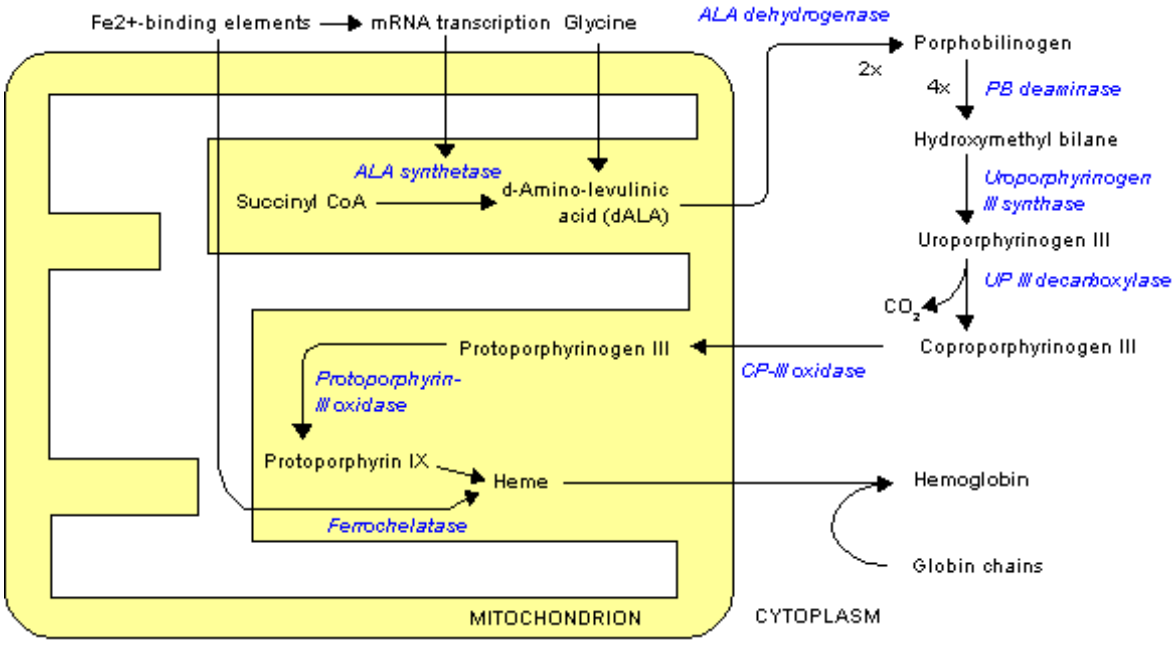

Figure 2. ALA synthase, the first enzyme of the heme biosynthetic pathway, catalyzes the condensation of glycine and succinyl coenzyme A to form ALA. This enzyme is localized in the inner membrane of mitochondria. Separate genes encode erythroid and nonerythroid ALA synthases. ALA dehydratase, a cytosolic enzyme, converts two molecules of ALA into a monopyrrole, porphobilinogen (PBG), with the removal of two molecules of water. PBG deaminase catalyzes the condensation of four molecules of PBG to yield a linear tetrapyrrole, hydroxymethylbilane (HMB). Uroporphyrinogen III cosynthase catalyzes the formation of uroporphyrinogen III from HMB. Uroporphyrinogen decarboxylase, a cytosolic enzyme, catalyzes four sequential decarboxylations of the carboxymethyl side chains in uroporphyrinogen III (an octacarboxyl porphyrin) to yield heptacarboxyl porphyrin, hexacarboxyl porphyrin, pentacarboxyl porphyrin, and, finally, coproporphyrinogen III (a tetracarboxyl porphyrin). This enzyme can also metabolize uroporphyrinogen I to coproporphyrinogen I. Coproporphyrinogen oxidase, a mitochondrial enzyme in mammalian cells, catalyzes the removal of the carboxyl group and two hydrogens from the proprionic groups of pyrrolerings A and B of coproporphyrinogen III to form vinyl groups at these positions, forming protoporphyrinogen. This enzyme is unable to metabolize coproporphyrinogen I. Protoporphyrinogen oxidase mediates the oxidation of protoporphyrinogen IX to protoporphyrin IX, catalyzing the removal of six hydrogen atoms from the porphyrinogen nucleus. Protoporphyrin, the final intermediate in the pathway, is the only intermediate that is an oxidized porphyrin. Porphyrins in the oxidized state are reddish and fluoresce when exposed to long-wave ultraviolet light. Porphyrinogens that leak into extracellular fluid undergo auto-oxidation and are excreted primarily as porphyrins.

2.1.2 Pharmacodynamics of 5-ALA

When exogenous 5-ALA is administered either orally, intravenously or topically, it is taken up by the cells and metabolized in the pathway described, resulting in a large temporary accumulation of PpIX in the mitochondria. Exogenous 5-ALA induces elevated PpIX levels in most (but not all) tissues that line body surfaces or body cavities. It can be shown that PpIX preferentially accumulates in the mucosa of the rat colon, but to a much lesser

IRB NUMBER: 12-135
IRB APPROVAL DATE: 01/22/2017

extent in the submucosa and muscle layers.[3] Mucosal selectivity occurs for other organs (stomach, bladder) in vivo when 5-ALA is administered either intravenously or orally.[17] Tissues that show no 5-ALA-induced PpIX include muscle (striated, smooth and cardiac), dermis, blood vessels, mature erythrocytes and most (but not all) other tissues of mesodermal origin.[19] However, since most of these mesodermal tissues have multiple components, a more detailed examination might reveal that a minority component in some tissues does in fact show a measurable degree of 5-ALA induced PpIX.

Malignant tissues usually accumulate more PpIX than do corresponding normal tissues from which they are derived. Increased PpIX accumulation can be found in many malignant cell lines, as compared to their non-malignant counterparts.[4] A number of studies have indicated that exogenous 5-ALA results in a selective and preferential accumulation of PpIX in diseased or neoplastic cells more than in normal cells. In terms of tumor-to-normal tissue (T/N) ratio of formed PpIX, 5-ALA-mediated accumulation of PpIX contrasts of 10:1 up to 90:1 have been reported depending on the pathologic condition, 5-ALA drug dose, and time post-administration.[1]

There are also certain non-malignant but altered tissues that show enhanced PpIX production in comparison to cells in their normal state. Examples of these tissues are psoriatic, actinic keratotic and pre-keratotic lesions. Thus, while an enhancement of 5-ALA induced PpIX accumulation is not specific for malignant tissues, in general it does indicate the presence of some abnormality.

It is unclear what the exact mechanism is for the selective accumulation of PpIX in neoplastic tissue over normal tissue when presented with exogenous 5-ALA. It is clear, however, that this selective accumulation is not due to differences in the cellular uptake of 5-ALA.[13] Whatever the mechanism, it is obvious from a kinetic point of view that the slowest step between the uptake of 5-ALA and formation of PpIX must be considerably faster than the transformation of PpIX into heme to attain a larger accumulation of PpIX and this occurs more robustly in neoplastic cells than in normal cells.[14]

PpIX is strongly fluorescent when activated by light at the far blue end of the visible spectrum in the Soret band (400nm). It emits a characteristic fluorescence spectrum with two prominent peaks in the red at 630nm and 700nm. This property has been used by various surgical specialties to help identify tumor for resection and to evaluate the extent of resection. Specifically for neurosurgery an oral form of 5-ALA (Gliolan, Medac GmbH, Wedel, Germany) has been approved by the European Medicines Agency in September 2007 for for visualisation of malignant tissue during surgery for malignant glioma (WHO grade III and IV) (http://www.emea.europa.eu/pdfs/human/press/pr/42748007en.pdf). The results of MC-ALS.8-I/GLI Phase I/II and subsequent Phase III trials in Europe have been summarized in a number of recent papers by Walter Stummer M.D. and his colleagues.[28,29,31,33] The European Medicines Agency comments can be found at: http://www.emea.europa.eu/humandocs/PDFs/EPAR/gliolan/H-744-en6.pdf (also see section 2.3) and the package insert for Gliolan can be found at http://www.emea.europa.eu/humandocs/PDFs/EPAR/gliolan/H-744-PI-en.pdf

The accumulation and fluorescence of PpIX in tumor cells is not the only biological property of this 5-ALA metabolite. Photodynamic therapy of tumors is also possible with the accumulation of PpIX in tumor cells. Photodynamic therapy involves the photoactivation of a photosensitizer which when activated will kill tumor cells. The fundamental building block of most red-absorbing, naturally occurring photosensitizers is 5-ALA. These sensitizers including PpIX are can cause cell death when exposed to light of certain frequencies. PpIX has the ability to absorb a photon of visible light and then transfer most of this absorbed energy to a molecule of oxygen. This causes a transient increase in the chemical reactivity of the oxygen molecule, and converts it into a relatively strong oxidizing agent, singlet oxygen. It is the singlet oxygen that kills cells by causing lethal oxidative damage to biologically important structures.

A topical form of 5-ALA has been approved by the FDA for the photodynamic therapy (PDT) treatment of actinic keratoses 5-ALA (Levulan Kerastick Topical Solution, DUSA Pharmaceuticals New York, Inc. Valhalla NY 10595). According to the presumed mechanism of action, photosensitization following application of Levulan Topical Solution occurs through the metabolic conversion of 5-ALA to PpIX, which accumulates in the skin to which Levulan Topical Solution has been applied. When exposed to light of appropriate wavelength and energy, the accumulated PpIX produces a photodynamic reaction, a cytotoxic process dependent upon the simultaneous presence of light and oxygen. The absorption of light results in an excited state of the porphyrin molecule, and subsequent spin transfer from PpIX to molecular oxygen generating singlet oxygen, which can further react to form superoxide and hydroxyl radicals. Photosensitization of actinic (solar) keratosis lesions using the Levulan Kerastick, plus illumination with the BLU-U™ Blue Light Photodynamic Therapy Illuminator (BLU-U), is the basis for Levulan photodynamic therapy of skin lesions. It is presumed that the same mechanism could be utilized for photodynamic therapy of brain neoplasms using oral administration of 5-ALA, and this is the basis for future studies (but not this study).

It is the properties of the intracellular metabolism of 5-ALA, the subsequent intracellular accumulation of PpIX without extracellular spread, the selective and preferential accumulation of PpIX in neoplastic cells compared to normal cells and the fluorescent properties of the accumulated PpIX that form the basis for this proposal concerning the gross total resection of malignant brain tumors. The photodynamic therapy benefits of 5-ALA/PpIX in the treatment of malignant gliomas will likely be addressed in a subsequent proposal.

2.1.3 Pharmacokinetics of 5-ALA

The pharmacokinetics of 5-ALA have been well studied. 5-ALA is supplied as the HCl salt which yields an acidic solution. Intravenous administration of an unbuffered solution to animals can cause bradycardia and hypotension, whereas buffered 5-ALA does not. Unfortunately, aqueous solutions of 5-ALA are unstable when buffered to pH 7.4. This makes intravenous formulation difficult and sometimes inconsistent.[36] For this reason there have been a number of studies to determine the characteristics of intravenous vs. oral formulations. In dogs, the concentration of 5-ALA decreased rapidly after

IRB NUMBER: 12-135
IRB APPROVAL DATE: 01/22/2017

intravenous bolus (terminal half-life of 19.5 ± 2.5 min after dosing) and was not significantly different from drug delivered orally (terminal half-life of 40.7 ± 22.9 min after dosing).[5] In a human pharmacokinetic study (N= 4) using a 128 mg dose of sterile intravenous 5-ALA HCl and oral 5-ALA HCl (equivalent to 100 mg 5-ALA) in which plasma 5-ALA and PpIX were measured, the mean half-life of 5-ALA was 0.70 ± 0.18 h after the oral dose and 0.83 ± 0.05h after the intravenous dose.[36] The oral bioavailability of 5-ALA was 50-60% with a mean Cmax of 4.65 ± 0.94 µg/mL. PpIX concentrations were low and were detectable only in 42% of the plasma samples. PpIX concentrations in plasma were quite low relative to 5-ALA plasma concentrations, and were below the level of detection (10 ng/mL) after 10 to 12 hours. In another human study (N=6), the terminal half-life of an intravenous dose of 5-ALA was about 50 minutes for an intravenous dose and 45 minutes for an oral dose.[6] In this same study it was determined that peak plasma concentrations of 5-ALA were achieved at 0.83 ± 0.20 h after administration of the oral dose. And that about 60% of 5-ALA was absorbed after oral administration. The drug was either metabolized in the liver or other organs or rapidly excreted in the urine.[6]

5-ALA does not exhibit fluorescence, while PpIX has a high fluorescence yield. Time-dependent changes in surface fluorescence have been used to determine PpIX accumulation and clearance in actinic keratosis lesions and perilesional skin after application of Levulan Topical Solution in 12 patients. Peak fluorescence intensity was reached in 11 ± 1 h in actinic keratoses and 12 ± 1 h in perilesional skin. The mean clearance half-life of fluorescence for lesions was 30 ± 10 h and 28 ± 6 h for perilesional skin. The fluorescence in perilesional skin was similar to that in actinic keratoses. Therefore, the FDA has recommended that Levulan Topical Solution should only be applied to the affected skin. (Data from DUSA Pharmaceuticals New York, Inc., Valhalla, NY 10595.)

The normal brain synthesizes very little PpIX,[8,15,34] which provides good contrast to gliomas.[24,31] It is interesting that considerably more PpIX is found in the brain when the hexyl derivative of 5-ALA (hexaminolevulinate) is given. Clinical measurements of PpIX pharmacokinetics have been made in blood plasma and from some easily accessible sites by fluorescence spectroscopy after different routes of 5-ALA delivery.[6,9,20,21,35] One of the most important result is that PpIX has returned to baseline levels within 48 hours after dosage with 5-ALA, wherever measured and irrespective of the route of administration.[9,21,36]

2.1.4 Toxicity

In vivo pharmacological and toxicological effects that may be associated with elevated systemic levels of 5-ALA, Pp IX, and other intermediates of porphyrin biosynthesis in humans are well known, and characterize a group of metabolic diseases known as the porphyrias. An association between high systemic concentrations of 5-ALA and neurological abnormalities is observed in Acute Intermittent Porphyria (AIP). However, while there have been considerable interest in the association between elevated 5-ALA levels and the neurological symptoms of AIP, numerous attempts at clarifying the relationship have yielded inconsistent results and contradictory conclusions. Most studies have shown that 5-ALA and Porphobilinogen (PBG = condensation of 2 molecules

of 5-ALA) are not neurotoxic in vivo when administered in large amounts to animals and humans. Furthermore, the concentration of these precursors in the brain or cerebrospinal fluid of porphyric patients is substantially below that required for demonstrable toxicity in vitro. Litman and Correia hypothesize that depletion of hepatic heme, a hallmark of AIP, leads to elevated tryptophan content and 5-hydroxytryptamine turnover in the brain, and is responsible for the neurological symptoms of AIP.[16] It should be noted that on this basis, administration of exogenous 5-ALA would not lead to a depletion of hepatic heme and, therefore would not be expected to elicit the neurological symptoms of AIP. Photosensitivity, associated with high systemic levels of PpIX, is a typical finding in Erythropoietic Protoporphyria (EPP) which is associated with decreased activity of ferrochelatase, the enzyme that converts PpIX to heme. EPP is entirely cutaneous, with none of the hemolytic or neurological problems of the other porphorias.

Mild transient nausea and occasional vomiting have been observed following oral administration of high doses of 5-ALA.[10,21,37] In one study, the incidence of nausea following 30 mg/kg and 60 mg/kg (oral) was 7% and 19%, respectively.[37] No vomiting was observed with the lower dose, while 8% of the patients vomited following the higher dose. In all instances the nausea was mild and short lived (less than 15 min.). When vomiting occurred, it was mild and occurred only once in each patient. The nausea or vomiting occurred within *2.5* to 3 hours after receiving the drug.

Abnormalities in liver function have been observed in patients following ingestion of 5-ALA. Webber et al. observed that almost one-quarter of all patients who retained 5-ALA had at least one abnormality in liver function tests.[37] This was unrelated to 5-ALA dose in that more patients receiving low doses (< 30 mg/kg) developed abnormalities than those receiving high doses. Significant variations in the time of appearance of the abnormalities were also noted (12 to 120 hr. postoperatively). In all cases, liver function tests returned to normal 2 months after receiving 5-ALA.

The most common complaint, due to Pp IX photosensitization, is a very unpleasant pricking, itching or burning sensation under the skin after exposure to the sun. A systemic load above 10 mg/kg is required to develop skin photosensitization (DUSA Pharmaceuticals) which lasts from approximately 24 to 48 hours following 5-ALA administration. <u>It is for this reason that patients will be kept in subdued light conditions for 48 hours following surgery</u>.

2.2 Brain Tumors

2.2.1 Background

Malignant gliomas are brain neoplasms that originate from the parenchymal elements of the brain. Malignant gliomas account for approximately 70% of the 22,500 new cases of malignant primary brain tumors that are discovered in United States adults each year.[38] Although these tumors are relatively uncommon, the prognosis is generally very poor with a median survival of 12-15 months for individuals diagnosed with glioblastoma multiforme (GBM) and 2 to 5 years for those with anaplastic astrocytoma (AA). The median survival of a patient undergoing biopsy of GBM without surgical resection or further treatment is about 8 weeks. For those patients who undergo surgery alone, the median

survival increases to 17 weeks. The addition of radiation therapy post-surgical resection raises median survival to approximately 7 to 12 months; chemotherapy adds an additional few weeks to this median survival. Overall, the 5-year relative survival rate for a primary malignant glioma is age dependent. Survival rates are 63.1% for age 0-19, 50.4% for age 20-44, 14.2% for age 45-64, and 4.9% for age >65. Recurrence of the tumor at the site of surgical resection is the rule. The recurrence is usually within 2 cm of the margin of the resection cavity in 80% of cases. For patients with recurrent tumors undergoing repeat surgical resection, the re-operation may add as much as 14 to 30 weeks of additional survival. Nevertheless, this response is met with fatal growth in virtually all patients. The 5-year survival rate is less than 5%.[2,22,38]

2.2.2 Current Treatment of Malignant Brain Tumors

The current treatment of patients with malignant brain gliomas is traditionally divided into surgical, radiotherapeutic, chemotherapeutic, and experimental treatments.[38] Surgery is usually performed to either fully resect or debulk the contrast-enhancing portion of the tumor. Although surgery alone rarely cures a malignant brain tumor, reduced tumor burden allows the body's own immune system or adjuvant therapies a greater chance of success. Biopsies are usually reserved for tumors in areas of the brain that are considered eloquent (harboring critical cerebral functions; i.e. language, motor, vision, functions, etc.) or when the patient's medical condition does not predict a safe surgical outcome. Radiotherapy plays a central role in the management of brain tumors in individuals older than 4 years of age. Radiotherapy is generally regional, with a boost to the tumor bed, for a total of 6,000 cGy over 30 treatments in 6 weeks. Chemotherapy continues to be offered to brain tumor patients as adjuvant treatment. It is usually delivered intravenously but oral agents such as Temodar have recently shown some efficacy and are now the standard of care. Chemotherapy impregnated wafers (BCNU) are sometimes implanted at the time of surgery. Experimental protocols, including immunotherapy and gene therapy, round out the efforts attempting to better treat these deadly neoplasms.

There is general agreement amongst neurosurgeons and neuro-oncologists that the extent of resection is a major factor in the prognosis of individuals with malignant gliomas.[2,22,30,38] Usually the extent of resection refers to resection of the contrast-enhancing portion of the tumor. The ultimate goal in surgery is to achieve a "gross total" resection of the tumor which is defined as complete absence of the contrast-enhancing portion of the tumor on the immediate post-operative scan.

There are a number of methods that the neurosurgeon uses to achieve the goal of gross total resection of a malignant glioma. The first method is to dissect around the tumor and to develop the plane between the obvious tumor tissue and the surrounding normal brain. In many instances this can be done – usually with the help of the operating microscope. At times, however this plane can be very difficult to establish – especially when the dissection is close to the intracerebral ventricle where tumor is often difficult to distinguish from the normal surrounding brain. It is very important in these instances to use other methods to help to identify residual tumor. Some neurosurgeons have used intra-operative ultrasound with limited success. Other neurosurgeons have employed the use of an intra-operative MRI scanner which is very expensive and cumbersome.

IRB NUMBER: 12-135
IRB APPROVAL DATE: 01/22/2017

In the past decade a group of neurosurgeons in Europe led by Walter Stummer, M.D. have explored the use of intra-operative tumor fluorescence from 5-ALA-PpIX to identify residual malignant glioma.[24-33] These studies have established that this technique can be very successful in helping to achieve gross-total resection in malignant gliomas with great safety and effectiveness. These studies have generated interest in this technique amongst neurosurgeons in the United States. Independently, Dr. John Ruge from the Lutheran General Hospital in Park Ridge, Illinois and also the Principal Investigator of this proposal, Dr. Jeffrey Cozzens has traveled to Dusseldorf, Germany to observe brain tumor surgery with 5-ALA-PpIX fluorescence by Dr. Stummer. Dr. Ruge has, in conjuction with DUSA Pharmaceuticals, initiated a Phase I/II study with the FDA for studying the use of 5-ALA in brain tumor surgery (see http://www.clinicaltrials.gov/ct2/show/NCT00671710?term=5-ALA&rank=12). (ClinicalTrails.gov identifier NCT00671710). There is also a similar trial underway in Cleveland, Ohio under the direction of the National Cancer Institute and Dr. Robert Maciunas (see http://www.clinicaltrials.gov/ct2/show/NCT00752323?term=5-ALA&rank=8) (ClinicalTrials.gov identifier NCT00752323)

2.3 Rationale for the Use of 5-ALA in the Surgery of Brain Tumors

Aminolevulinic acid (5-ALA) is a naturally occurring amino acid and a precursor in heme biosynthesis. 5-ALA is produced at the cytosolic surface of the mitochondrial membrane and then transported to the cellular cytosol for heme biosynthesis. The availability of 5-ALA in the cell cytoplasm is the rate limiting factor in heme biosynthesis in all but erythropoetic cells. The heme biosynthetic enzymes are more active in glial tumors of the brain than in normal brain tissue. As a result, addition of 5-ALA (the rate-limiting precursor) to malignant glial cells leads to an increase in the intracellular accumulation of protoporphorin IX (PpIX), a fluorescent intermediary in heme biosynthesis. As a result, malignant glial cells show red fluorescence relative to dark normal brain tissue when viewed under deep blue light (440 nm). This effect allows the neurosurgeon to distinguish tumor from normal brain and therefore enables more complete surgical removal of malignant glial tumor from within normal brain tissue.

In a study in Europe for approval of 5-ALA by the European Medicines Agency, a similar study to this proposed study was performed (also see http://www.emea.europa.eu/humandocs/PDFs/EPAR/gliolan/H-744-en6.pdf

Study MC-ALS.28/GLI
The aim of this prospective, single-arm, uncontrolled multicentre (n = 4) phase II study was to determine the positive predictive value of tissue fluorescence, defined as the percentage of patients showing positive tumour cell identification in all biopsies taken from areas of weak and strong fluorescence.

The primary efficacy parameter was the positive predictive value of tissue fluorescence, defined as the percentage of patients with positive tumour cell identification in all biopsies taken from areas with weak and strong fluorescence. A biopsy was termed "positive tumour cell identification" if the reference neuropathological institute observed a tumour cell content greater than 0%.

The mean tumour cellularity per patient (i.e. mean area on a section occupied by tumour cells) in strongly fluorescing biopsies was 79.1% ± 20.1%, whereas mean tumour cellularity in weakly fluorescing biopsies was 30.78% ± 27.88% per patient. A minimum

tumour cellularity of 4.5% was detectable by virtue of its (weak) fluorescence by the neurosurgeon. Taking all biopsies together, mean tumour cellularity with strong or weak fluorescence was 79.1% ± 19.8%.

The number of truly-positive specimens (biopsies) was higher if the quality of fluorescence was strong (n=32) rather than weak (n=25). Consequently, the positive predictive value of strong fluorescence was higher (100.0%; 90% CI: 91.1% - 100.0%) than that of weak fluorescence (83.3%; 90% CI: 68.1% - 93.2%). In total, there were 28 patients where all fluorescing biopsies showed tumor in all biopsies taken from areas of any fluorescence (strong or weak fluorescence) resulting in a positive predictive value of 84.8% (90% CI: 70.7% - 93.8%).

Among 185 evaluated biopsies, seven sections did not reveal any tumour cells (false-positive specimens). In all false-positive specimens, the quality of fluorescence in was weak. Strongly fluorescing false-positive biopsies did not occur in this study.

The positive predictive value of tissue fluorescence at the biopsy level, defined as the number of tumour positive biopsies among all biopsies taken from areas of any fluorescence (weak and strong fluorescence), was 96.2% (90% CI: 93.0% - 98.2%). The positive predictive value was higher, if only strong fluorescent biopsies were taken into account (100.0%; 90% CI: 96.9% - 100.0%) compared to weak fluorescent biopsies only (92.2%; 90% CI: 85.9% - 96.3%).

This study only provides data for the positive predictive value. Nothing was obtained for the negative predictive value. The results showed that the strong fluorescent area has a much higher cellularity than the comparators areas and this makes the positive predictive value of strong fluorescence 100%. It satisfactorily shows that the fluorescence is highly specific for tumour. Since the major problem is to remove healthy tissue taken as tumour (false positives) the high specificity is reassuring. The issue of real sensitivity remains but it less critical for the clinical use.

2.4 Correlative Studies Background

This study will run in conjunction with another study (currently being developed) that will look at the feasibility and effectiveness of digital subtraction magnetic resonance imaging (dsMRI) of the brain. Currently the radiological assessment of the extent of brain tumor resection is performed utilizing the neuroradiologist's subjective interpretation of the differences between a post-operative gadolinium enhanced image and a post-operative non-enhanced image. dsMRI has the potential to digitally and objectively define and indentify the differences between these two images. It also has the potential to measure the volume of any residual tumor.

IRB NUMBER: 12-135
IRB APPROVAL DATE: 01/22/2017

3 PATIENT SELECTION

3.1 Eligibility Criteria

Patients must have clinically documented primary brain tumor for which resection is clinically indicated. The anticipated histology at resection should include: Anaplastic astrocytoma (10002224), Astrocytoma malignant NOS (10003572), Brain stem glioma (10006143), Ependymoma (10014967), Ependymoma malignant (10014968), Glioblastoma (10018336), Glioblastoma multiforme (10018337), Gliosarcoma (10018340), Anaplastic oligodendroglioma (10026659), Oligodendroglioma (10030286), Medulloblastoma (10027107), Mixed astrocytoma-ependymoma (10027743), Miscellaneous CNS primary tumor (10007959), Supratentorial primitive neuroectodermal tumor (10056672)

3.1.1 Prior therapy is not a consideration in protocol entry.

3.1.2 Age ≥ 18 years. Because no dosing or adverse event data are currently available on the use of 5-ALA in patients <18 years of age, children are excluded from this study but will be eligible for future pediatric phase 1 single-agent trials.

3.1.3 ECOG performance status ≤2 (Karnofsky ≥60%, see Appendix A).

3.1.4 Life expectancy is not a consideration for protocol entry.

3.1.5 Patients must have normal organ and marrow function as defined below:

Leukocytes	≥3,000/mcL
Absolute neutrophil count	≥1,500/mcL
Platelets	≥100,000/mcL
Total bilirubin	within normal institutional limits
AST (SGOT)/ALT (SGPT)	≤2.5 X institutional upper limit of normal
Creatinine	within normal institutional limits OR
Creatinine clearance	≥60 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal.

3.1.6 The effects of 5-ALA on the developing human fetus are unknown. 5-ALA has unknown teratogenic or abortifacient effects. For this reason, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.

3.1.7 Ability to understand and the willingness to sign a written informed consent document.

3.2 Exclusion Criteria

3.2.1 Prior therapy is not an exclusion criterion.

3.2.2 Patients may not be receiving any other investigational agents at the time of entry into the study.

3.2.3 History of allergic reactions attributed to compounds of similar chemical or biologic composition to 5-ALA.

3.2.4 Personal or family history of porphyrias.

3.2.5 Uncontrolled intercurrent illness including, but not limited to ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.6 Pregnant women are excluded from this study because 5-ALA is of unknown teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother

with 5-ALA, breastfeeding should be discontinued if the mother is treated with 5-ALA.

3.2.7 Patients who are cancer survivors or those who are HIV positive are not excluded from this study.

3.2.8 Patients with significant cognitive impairment (which in the judgement of the principal investigator leaves them incapable of giving informed consent) are excluded from this study.

3.3 Inclusion of Women and Minorities

Both men and women and members of all ethnic groups are eligible for this trial. The proposed study population is illustrated in the table below.

Race/Ethnicity (%)

Gender	White, not of Hispanic Origin	Black, not of Hispanic Origin	Hispanic	Asian or Pacific Islander	Unknown	Total
Male	**25**	**5**	**5**	**5**	**10**	**50**
Female	**25**	**10**	**5**	**5**	**5**	**50**
Total	**50**	**15**	**10**	**10**	**15**	**100**

4 TREATMENT PLAN

4.1 Aminolevulinic Acid (5-ALA) Administration

Treatment will be administered on an inpatient basis. Reported adverse events and potential risks are described in Section 6. One-time, single-dose administration of 5-ALA is planned, which precludes the need for dose modification. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy in the peri-operative period.

4.1.1 Treatment Protocol

A maximum of thirty-three subjects with malignant brain tumors will participate in this trial. Each patient will have been evaluated and found to have a malignant brain tumor by history and recent imaging studies (MRI) and deemed a surgical candidate based on current neurosurgical standards of care. Consent will be obtained by the principal investigator or co-investigator. Three subjects will be enrolled in each of the five phase 1 dose levels seen below according to the rules seen in the table in section 4.2.1. After establishment of the safest effective dose, an additional 16 subjects will be enrolled at that dose level for the phase 2 study. The protocol is divided into two parts; the surgical procedure part and the post operative treatment part.

Dose-Escalation Schedule	
Dose Level	**Dose of 5-ALA**
Level 1	10 mg/kg
Level 2	20 mg/kg
Level 3	30 mg/kg
Level 4	40 mg/kg
Level 5	50 mg/kg

IRB NUMBER: 12-135
IRB APPROVAL DATE: 01/22/2017

4.1.2 Surgical Phase

5-ALA will be mixed in the minimum volume of sterile water or juice immediately before use and given as a single bolus orally approximately three hours prior to induction of anesthesia for surgical resection of a malignant brain tumor. Image-guided microsurgical resection of the tumor will be undertaken. The intensity of the white and blue light from the microscope will be measured at the shortest focal length of the microscope with a digital light meter before resection of the tumor is performed. During tumor resection under the surgical microscope, the tumor bed will be illuminated with deep blue light via a high pass optical filter in the microscopes light chain. The neurosurgeon will examine the tumor bed with a barrier filter in the optical chain of the surgical microscope (see Appendix B).

Under white light the neurosurgeon will take small biopsies from areas of obvious tumor and areas in the wall of the resection cavity that are judged to be normal (but possibly edematous), non-eloquent brain.

Under blue light, small biopsies will be taken from areas of strong fluorescence, weak fluorescence and non-fluorescing regions in the tumor resection cavity.

At all times, the neurosurgeon will use their best judgment to remove or biopsy only that tissue that would normally be removed or biopsied as part of any brain tumor procedure that was not involved or part of an investigational study.

Specimens will be collected regardless of fluorescence. All biopsy specimens, including those taken from the solid tumor, will be reviewed by a neuro-pathologist. Residual fluorescent tissue in the tumor cavity will be surgically removed only if in the judgment of the neurosurgeon these areas would have been resected using white light alone and that this would lead to a more complete resection of tumor. Pathologic confirmation of tumor type will be made by neuropathology.

After surgical resection of the tumor, the neurosurgeon will judge the extent of resection by estimating the volume of remaining obvious tumor seen under white light or fluorescent tissue seen under blue light.

Data Collection in Operating Room

Under White Light	Obvious Tumor	Obvious Normal Brain (possibly edematous)	
Biopsy for Histology	A	B	
Tumor Content of Sample	X	X	
Specimen Labeled	X	X	
Under Blue Light	Strongly Fluorescent Tissue	Weakly Fluorescent Tissue	Non-Fluorescent Tissue
Biopsy for Histology	A (This can be the same biopsy as A under white light or it can be a new biopsy D)	C	B (This can be the same biopsy as B under white light or it can be a new biopsy E)
Fluorescence Intensity by Neurosurgeon	X	X	X
Tumor Content of Sample	X	X	X
Specimen Labeled	X	X	X
Estimate made of extent	X	X	X

of Fluorescent Tissue/Tumor Resected			

Specimens will be collected regardless of fluorescence.

Areas where biopsies have been made of fluorescent and non-fluorescent tissue within the tumor cavity will be graded by the neurosurgeon at the time of biopsy as 0 to +++ (3+) fluorescence. This will be recorded in the operating room each specimen identified with a code. The pathologist who reviews each specimen will be blinded as to the extent of fluorescence in the area where the biopsy was obtained.

Grade	Definition	
0	No fluorescence. No difference between any of the tissues visualized.	No fluorescence
+	Low fluorescence. Red glow is barely distigushed from surrounding non-fluorescent tissue.	Weak fluorescence
++	Moderate fluorescence. Dull red glow of fluorescent tissue.	
+++	High fluorescence. Bright red glow of fluorescent tissue	Strong fluorescence

The neurosurgeon is responsible for:

- An overall assessment of the detection of tumor within the cavity provided by the fluorescence imaging.
- Judging the extent of resection of the tumor or fluorescent tissue.
- Under white light:
  - Identifying at least one area of obvious tumor and biopsying that site. Then switch to blue light and grading the fluorescence at the site.
  - Identifying at least one area of obviously normal (but possibly edematous) brain in the wall of the tumor resection cavity in an area of non-eloquent brain and biopsying that site. Then switch to blue light and grading the fluorescence at the site.
- Under blue light:
  - Identifying at least one fluorescent area within the tumor cavity in an area of non-eloquent brain and biopsying and grading that site's fluorescence. It may not be necessary to biopsy this area if this same area has already been biopsied under white light.
  - Identifying at least one non-fluorescent area within the tumor cavity in an area of non-eloquent brain and biopsying and grading that site's fluorescence. It may not be necessary to biopsy this area if this same area has already been biopsied under white light.
  - Identifying one area of weak fluorescence in an area of non-eloquent brain and biopsying and biopsying and grading that site's fluorescence. It may not be necessary to biopsy this area if this same area has already been biopsied under white light.
  - Intra-operative photographs of tumor fluorescence will be made during each surgery to help compare the degree of fluorescence from one patient to the other.

The neuro-pathologist is responsible for:

- Determining the extent of tumor cellularity for each specimen in categories of
  - Solid tumor
  - Infiltrating tumor/normal brain
  - No tumor (edematous peritumoral normal brain)

4.1.3 Post-Operative Follow-up

Patients will be assessed for completeness of tumor resection within 24 hours following surgery using MRI both with and without gadolinium enhancement. A 24 post-surgical MRI within 24 hours of surgery is the standard of care for anyone undergoing surgical resection of a brain tumor. Subsequently, patients will be re-imaged periodically to assess tumor progression according to the specific standard of care tumor treatment protocol determined by the Neuro-Oncologist. At these same intervals, when appropriate to the normal standard of care, routine liver function tests and physical examination will be performed.

4.1.4 Survival

Survival will be determined over two years from entry into the study by either periodic medical record review for patients seen in the SIU Neuro-Oncology Clinic, or by search of the Social Security Death Index (SSDI).
Volumetric analysis will be performed with Tera-recon software on gadolinium contrast-enhanced T1W MRI to assess extent of resection **(**EOR) and residual tumor volume (RTV). These findings will then be correlated with progression free survival (PFS) and overall-survival (OS).

4.2 Definition of Dose-Limiting Toxicity

Dose-Limiting Toxicity (DLT) is determined by Adverse Events which are defined and graded following the CTEP, NCI Guidelines: Adverse Event Reporting Requirements of January 1, 2005 (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/newadverse_2006.pdf) and the CTC/CTCAE Criteria. Expected Adverse Events (AE) have not been identified for 5-ALA by the NCI Comprehensive Adverse Event and Potential Risk list (CAEPR) but are identified in section 11.1.2 and 11.1.3 of this protocol. Those AEs which are determined to be Grade 2 or higher and directly attributed to 5-ALA or its metabolites will be considered to be dose-limiting for the purposes of determining dose escalation. At present, CTEP does not have a CAEPR or ASAEL for this agent (see Appendix D).

Since this study involves the one-time dose of the study drug, further dose modifications associated with the above adverse events will not be required.

4.2.1 Dose-Escalation Scheme

Dose escalation will proceed within each cohort according to the following scheme. Three subjects will be entered sequentially at each of the five dose levels (10, 20, 30, 40, and 50 mg/kg). This dose escalation scheme is more aggressive than standard accelerated dose escalation schemas published because we are already aware of safety data from Europe demonstrating no toxicity with oral doses of 50 mg/kg. An additional three subjects will be added to the recommended phase 2 dose level according to the following scheme. Three subjects are planned at each dose level in order to ensure that sufficient samples are available to determine fluorescent intensity differences between normal and malignant tissue at each dose level.

IRB NUMBER: 12-135
IRB APPROVAL DATE: 01/22/2017

Each dose escalation will be approved by a Data Safety Monitoring Board determined by SCRIHS (see Section 11.5).

Dose Escalation and Modification Rule Table

# of Patients with DLT at a Given Dose Level	Escalation Decision Rule
0 out of 3	Enter 3 patients at the next dose level.
≥ 2 out of 3	Dose escalation will be stopped. This dose level will be declared the maximally administered dose (highest dose administered). Three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
1 out of 3	Enter at least 3 more patients at this dose level. - If 0 of these 3 patients experience DLT, proceed to the next dose level. - If 1 or more of this group suffer DLT, then dose escalation is stopped, and this dose is declared the maximally administered dose. Three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
≤1 out of 6 at highest dose level below the maximally administered dose	This is generally the recommended phase 2 dose. At least 6 patients must be entered at the recommended phase 2 dose.

4.2.2 Phase 2 accrual of 16 additional subjects at the recommended phase 2 dose will commence once the phase 1 portion of this trial is completed. All correlative studies will be continued in the phase 2 portion of the study in order to define the differential fluorescence between normal and malignant brain tissue, enhancement of tumor resection, and survival impact of preoperative 5-ALA.

4.3 Supportive Care Guidelines

Patients will be managed by standard of care procedures for post-operative brain resection patients. In addition, patients will be sequestered in “low light” environment for 48 hours following the administration of 5-ALA.

Low light exposure conditions will start in the holding area immediately after ingestion of the study drug and will continue in all patient environments for at least 48 hours. These conditions include the following:

- Covering as much of the patient’s skin, mucous membranes and eyes as possible except for those areas that need to be exposed for clinical care and evaluation (ie. placement of intravenous access, preparation for general anesthesia, preparation for surgery, observation during and after surgery, etc.). This will include the patient wearing dark glasses when awake.
- Windows in patient care areas will be covered and doors closed.
- Overhead room lights will be turned off in patient care areas. Lights that are necessary for patient care may be used at the minimal intensity required.
- Patient care areas do not need to be completely dark. Minimal light for patient, caregiver and visitor safety is allowed. Televisions, monitors and medical equipment may be used as necessary.

IRB NUMBER: 12-135
IRB APPROVAL DATE: 01/22/2017

4.4 Duration of Therapy

A one-time, single-dose administration of oral 5-ALA 3 hours prior to induction of anesthesia for surgical resection of a malignant glioma is planned.

4.5 Duration of Follow-up

Patients will be followed for 2 years after surgery or until death, whichever comes first.

4.6 Criteria for Removal from Study

Because this study involves the one-time dose of the study drug followed by surgery, the only criteria for removal from the study would be if the patient either could not ingest the drug or could not undergo surgery.

5 DOSING DELAYS/DOSE MODIFICATIONS

One-time, single-dose administration of 5-ALA is planned, which precludes the need for intra-patient dose modification or delay.

6. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

The following list of AEs (Section 6.1) and the characteristics of an observed AE (Section 6.2*)* will determine whether the event requires expedited (via Medwatch) reporting in addition to routine reporting.

6.1 Comprehensive Adverse Events and Potential Risks List (CAEPR)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single, complete list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system.

We have contacted CTEP and we have been informed that CTEP does not have a CAEPR list for 5-ALA (see Appendix D).

6.2 Adverse Event Characteristics

CTCAE term (AE description) and Grade

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 will be utilized for AE reporting. All appropriate treatment areas will have access to a copy of the CTCAE version 3.0. A copy of the CTCAE version 3.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

Expectedness - AEs can be ‘Unexpected’ or ‘Expected’ for expedited reporting purposes only. ‘Expected’ AEs are listed in sections 11.1.2 and 11.1.3 of this protocol.

Attribution of the AE
Definite – The AE is clearly related to the study treatment.
Probable – The AE is likely related to the study treatment.
Possible – The AE may be related to the study treatment.
Unlikely – The AE is doubtfully related to the study treatment.
Unrelated – The AE is clearly NOT related to the study treatment.

6.3 Expedited Adverse Event Reporting

6.3.1 Phase 1 and phase 2 studies with investigational agents (Including hospitalization defined below):
- For hospitalization only – Any medical event equivalent to CTC grade 3, 4, or 5 which precipitated hospitalization (or prolongation of existing hospitalization) must be reported regardless of designation as expected or unexpected and attribution.
- The FDA safety information and adverse event reporting system, Medwatch, FDA form 3500A will be used for reporting within 24 hours.

6.3.2 Expedited Reporting Guidelines – Medwatch Reporting Requirements for Adverse Events That Occur Within 30 Days[1] of the Last Dose of the Investigational Agent on Phase 1 Trials

Phase 1 Trials								
	Grade 1	**Grade 2**	**Grade 2**	**Grade 3**		**Grade 3**		**Grades 4 & 5[2]**
				Unexpected		**Expected**		
	Unexpected and Expected	**Unexpected**	**Expected**	**with Hospitalization**	**without Hospitalization**	**with Hospitalization**	**without Hospitalization**	**Unexpected and Expected**
Unrelated Unlikely	Not Required	Not Required	Not Required	10 Calendar Days	Not Required	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days
Possible Probable Definite	Not Required	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days	24-Hour; 5 Calendar Days	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days

[1] **Adverse events with attribution of possible, probable, or definite that occur <u>greater</u> than 30 days after the last dose of treatment with an agent under a CTEP IND require reporting as follows:**
AdEERS 24-hour notification followed by complete report within 5 calendar days for:
- Grade 3 unexpected events with hospitalization or prolongation of hospitalization
- Grade 4 unexpected events
- Grade 5 expected events and unexpected events

[2] Although an AdEERS 24-hour notification is not required for death clearly related to progressive disease, a full report is required as outlined in the table. December 15, 2004

Note: All deaths on study require both routine and expedited reporting regardless of causality. Attribution to treatment or other cause must be provided.

Expedited AE reporting timelines defined:
"24 hours; 5 calendar days" – The investigator must initially report the AE via Medwatch within <u>24 hours</u> of learning of the event followed by a complete Medwatch report within <u>5 calendar days</u> of the initial 24-hour report.
"10 calendar days" - A complete Medwatch report on the AE must be submitted within 10 calendar days of the investigator learning of the event.

Any medical event equivalent to CTCAE grade 3, 4, or 5 that precipitates hospitalization (or prolongation of existing hospitalization) must be reported regardless of attribution and designation as expected or unexpected with the exception of any events identified as protocol-specific expedited adverse event reporting exclusions.

6.4 Routine Adverse Event Reporting
All Adverse Events must be reported in routine study data submissions. AEs reported

through Medwatch must also be reported in routine study data submissions.

7. PHARMACEUTICAL INFORMATION
   7.1 Drug (5-ALA) - 5-ALA for oral administration will be provided by DUSA Pharmaceuticals New York, Inc., Valhalla, NY 10595.

   7.2 Drug Availability - The study drug 5-aminolevulinic acid will be provided by DUSA Pharmaceuticals New York, Inc., Valhalla, NY 10595.

   7.3 Drug Ordering - The study drug 5-aminolevulinic acid will be provided by DUSA Phamaceuticals, Inc. to the Memorial Medical Center or St. John's Hospital Pharmacy.

   7.4 Drug Accountability - The Investigator, or a responsible party designated by the Investigator, in cooperation with the Memorial Medical Center or St. John's Hospital Pharmacy will maintain a careful record of the inventory and disposition of all agents received from DUSA Pharmaceuticals, Inc.

8. CORRELATIVE/SPECIAL STUDIES
   8.1 Assessment of Visual Tumor Discrimination
      8.1.1 Overall assessment of Tumor Discrimination - With the tumor and tumor cavity exposed, the neurosurgeon will visualize the tumor and tumor cavity under white light and again under deep blue light illumination and assess the ability of fluorescent illumination to distinguish tumor from normal brain tissue. This subjective assessment will be graded by the neurosurgeon at the time of visualization from 0 to +++ (3+).

Grade	Definition	
0	No fluorescence. No difference between any of the tissues visualized.	No fluorescence
+	Low fluorescence. Red glow is barely distigushed from surrounding non-fluorescent tissue.	Weak fluorescence
++	Moderate fluorescence. Dull red glow of fluorescent tissue.	
+++	High fluorescence. Bright red glow of fluorescent tissue	Strong fluorescence

      8.1.2 Surgical Assessment of Fluorescence in Biopsy Specimens:
      Each biopsy specimen taken for this trial under blue light will be graded for fluorescence intensity at the time of biopsy by the neurosurgeon on a scale of 0 to +++ (3+).
      8.1.3 Pathologic Assessment of Biopsy Specimens:
      All biopsy specimens, including those taken from the bulk tumor, will be reviewed by a neuropathologist.

9. STUDY CALENDAR
   Baseline evaluations are to be conducted within two weeks prior to start of protocol therapy. Scans and x-rays must be done within one week prior to the start of therapy. Adverse events will be recorded up to 30 days following surgery.

	Pre-	Day 1	Day 2	Week 5

IRB NUMBER: 12-135
IRB APPROVAL DATE: 01/22/2017

	Study	(Surgery)		
5-ALA [a]		X		
Informed consent	X			
Demographics	X			
Medical history	X			
Physical exam	X	X		X
Vital signs	X	X	X	X
Concurrent Meds	X	X	X	
Height	X			
Weight	X	X		X
Performance Status	X	X		X
Adverse Event Evaluation	X	X	X	X
CBC w/diff, platelets	X	X	X	X
Serum chemistry	X	X	X	X
EKG (as indicated)	X			
MRI Brain gadolinium	X		X	
B-HCG	X			
Other correlative studies		X		

10. MEASUREMENT OF EFFECT

In this study, the study drug 5-ALA is not expected to have any direct effect on tumor cells. Future studies may be designed to investigate the potential of 5-ALA and its photosensitizing metabolite PpIX in a study of photodynamic therapy, but in this study no attempt will be made to photo-activate the tumor cells.

The study will look at the specificity and sensitivity of 5-ALA/PpIX for malignant glioma cells.

10.1 Definitions

Response and progression will be primarily evaluated in this study using MRI volumetric techniques which measure the volume of residual enhancing tumor.

10.1.1 Extent of Resection

Volumetric MRI techniques measure difference in the areas of bright MRI signal when a post-operative T1 non-enhanced image is compared to a post-operative T1 gadolinium enhanced image. These differences may be measured by post-processing digital subtraction techniques (dsMRI – see section 2.4) or by outlining areas of new bright signal as judged by the neuroradiologist. Extent of resection is divided into three categories which are defined as follows:

- Gross-total tumor resection (GTR) of tumor is defined as no measureable residual contrast enhancement in the immediate post-operative MRI.

IRB NUMBER: 12-135
IRB APPROVAL DATE: 01/22/2017

- Near-total resection (NTR) is defined as measureable post-operative residual contrast enhancement greater than or equal to the volume of one MRI voxel (0.175 cm^3) and less than or equal to 2.5 cm^3.
- Incomplete resection (IR) is defined as measureable post-operative residual contrast enhancement greater than 2.5 cm^3.

10.2 Guidelines for Evaluation of Measurable Disease

All neuroradiological measurements should be taken and recorded in metric notation using standardized volumetric techniques. These techniques have been discussed previously.[7,11,12]

10.3 Confirmatory Measurement/Duration of Response

10.3.1 Confirmation - N/A

10.3.2 Duration of Overall Response - N/A

10.3.3 Duration of Stable Disease - N/A

11. REGULATORY AND REPORTING REQUIREMENTS

Adverse event (AE) reporting for this study is via the FDA safety information and adverse event reporting system, Medwatch, FDA form 3500A. The descriptions and grading scales found in the revised NCI Common Toxicity Criteria (CTC) version 3.0 will be utilized for adverse event reporting and are available in the Clinical Research Department and available on line at http://ctep.cancer.gov/reporting/ctc.html. Expected adverse events specific for 5-ALA are found below (Section 11.1.2).

11.1 Expedited Adverse Event Reporting (AE; formerly known as Adverse Drug Reaction)

11.1.1 Expedited Reporting Guidelines – Phase 1 studies with investigational agents (Including hospitalization defined in bullet 1 below)

- For Hospitalization only – Any medical event equivalent to CTC grade 3, 4, or 5 which precipitated hospitalization (or prolongation of existing hospitalization) must be reported regardless of designation as expected or unexpected and attribution.
- The FDA safety information and adverse event reporting system, Medwatch, FDA form 3500A will be used for reporting within 24 hours.

11.1.2 Agent-Specific Expected Adverse Events List

The list below guides the investigator in determining which AEs require expedited reporting. Those AEs that do not require expedited reporting are reported in routine study data submissions.

- Skin reaction - Grades 1 and 2.
- AST/ALT elevation - Grades 1, 2, or 3.
- Nausea - Grades 1 and 2.

11.1.3 Other Expected Adverse Events

For this protocol, the following more serious expected adverse events are risks associated with surgical removal of a malignant brain tumor and do not require expedited reporting:

- Adverse reaction to anesthesia - Both local and general anesthesia involve risk. There is a possibility of complication or injury from all forms of anesthesia and sedation.
- Balance problems - Difficulty with balance or vertigo may occur as a result of the tumor itself or from the surgery to correct it. Nausea and vomiting may also occur after surgery.
- Bleeding - It is possible, though unusual, to experience an episode of bleeding, which may be extensive during or after surgery. Bleeding may

require additional treatment or transfusion. Certain medications, such as anti-inflammatory drugs, may increase the risk of bleeding.

- Blood clot development - Blood clots may occur with any type of surgery. Blood clots at the site of the surgery can block blood flow and cause complications, including pain, swelling, inflammation and tissue damage (stroke).
- Brain injury - There is a risk that the procedure will cause injury of the surrounding brain. The symptoms that result from the injury depend on the location of the tumor.
- Cardiac complications - There is a chance that having the procedure could cause an irregular heartbeat or a heart attack.
- Functional loss - It is possible to experience problems such as difficulty opening the mouth or chewing after surgery. Also, speech, language and memory difficulties may occur after surgery.
- Hydrocephalus - It is-possible that the normal flow of spinal fluid around the brain may be altered. Additional treatment, including surgery, may be necessary to correct this.
- Infection - Infection may occur not only at the incision site, but also within the bone flap. Infection-related risks also include the development of meningitis (an infection which causes inflammation of the membranes covering the brain and spinal cord) or a brain abscess (localized collection of pus).
- Loss of nerve function - Some loss of function may occur in the facial nerves as a result of the procedure.
- Paralysis - it is possible that some paralysis or numbness may occur as a result of the surgery.
- Post-operative neurologic decline - There is a small risk that neurologic function will decline following surgery and may produce such symptoms as personality changes or loss of cognitive function. These problems are sometimes caused by post-operative hemorrhage (bleeding into or on the surface of the brain) or cerebral edema (accumulation of fluid that results in swelling and pressure on the brain).
- Post-operative pain - It is possible, though unlikely, that pain or other symptoms will increase following the procedure. A headache may linger anywhere from one week to one month following craniotomy and occasionally for a longer period of time.
- Recurrence - There is a chance that the tumor may return to the same site. However, the risk of recurrence depends on the type of tumor and how much of it can be removed in the initial operation.
- Respiratory difficulties - Breathing difficulties, which are usually temporary or post-operative pneumonia may occur as a result of surgery. Pulmonary embolus could occur from blood clotting in the veins.
- Seizure activity - Abnormal electrical activity in the brain, which can trigger seizures, may develop in response to the tumor itself or its removal.
- Visual disturbances - There may be some changes in visual function caused by the surgery or by the tumor itself.

IRB NUMBER: 12-135
IRB APPROVAL DATE: 01/22/2017

UNEXPECTED EVENT		EXPECTED EVENT	
GRADES 2 – 3 **Attribution of Possible, Probable or Definite**	**GRADES 4 and 5** **Regardless of Attribution**	**GRADES 1 - 3**	**GRADES 4 and 5** **Regardless of Attribution**
Grade 2 - Expedited report within 10 working days. **Grade 3** - Report by phone to IDB within 24 hrs. Expedited report to follow within 10 working days. (**Grade 1** – Adverse Event Expedited Reporting NOT required.)	Report by phone to IDB within 24 hrs. Expedited report to follow within 5 calendar days. This includes all deaths within 30 days of the last dose of treatment with an investigational agent regardless of attribution. Any late death attributed to the agent (possible, probable, or definite) should be reported within 5 calendar days .	Adverse Event Expedited Reporting NOT required.	Report by phone to IDB within 24 hrs. Expedited report to follow within 5 calendar days. This includes all deaths within 30 days of the last dose of treatment with an investigational agent regardless of attribution. Any late death attributed to the agent (possible, probable, or definite) should be reported within 5 calendar days.

11.1.4 Forms - None additional
11.1.5 Secondary AML/MDS – N/A

11.2 Data Reporting - In addition to adverse event reporting, all reports will be provided to the FDA as required by the Code of Federal Regulations.

11.3 CTEP Multicenter Guidelines - N/A

11.4 Cooperative Research and Development Agreement (CRADA)/Clinical Trials Agreement (CTA) N/A The agent, aminolevulinic acid (5-ALA), used in this protocol is provided by Collaborator, DUSA Pharmaceuticals New York, Inc., Valhalla, NY 10595.

11.4.1 Aminolevulinic acid (5-ALA) may not be used for any purpose outside the scope of this protocol, nor can aminolevulinic acid (5-ALA) be transferred or licensed to any party not participating in the clinical study. Collaborator data for aminolevulinic acid (5-ALA) are confidential and proprietary to Collaborator and shall be maintained as such by the investigators.
11.4.2 Any data provided to Collaborator(s) for Phase 3 studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.

11.5 Data Safety Monitoring Plan
A minimum of 3 to a maximum of **18 subjects* will be enrolled in this study. Oral 5-ALA will be administered 2-3 hours prior to surgery in cohorts of three at five escalating doses (10, 20, 30, 40, or 50 mg/kg). *(*Due to four patients receiving 40mg/kg rather than three, 19 total patients will be studied in phase 1. Protocol deviation approved by IRB on 17 Oct 2013.)*

Study investigators and study personnel will carefully screen each patient for side effects and adverse events. Expected adverse events specific for 5-ALA are described in the Protocol (Section 11.1.2) and other expected adverse events are

listed in Section 11.1.3. Subjects will be assessed for any unexpected serious adverse events such as significant changes in vital signs, nausea, vomiting, rashes, skin eruptions, and any long term changes in liver function. Unauthorized disclosure of protected health information is also a risk of this study.

It is the responsibility of the Principal Investigator (PI), Dr. Jeffrey Cozzens to oversee the safety of this Phase I study. However, since Dr. Cozzens holds the IND for the investigational drug, an independent data safety monitoring committee (DSMC) has been assigned to this study. The DSMC will consist of Dr. Krishna Rao (hematology/oncology physician), Dr. Kathy Robinson (oncology research study specialist), and Dr. Chad Noggle (neuropsychologist).

The DSMC will meet after each cohort (every third subject) has received the study drug to assess safety data including side effects and fluorescence ratings. The DSMC will discuss each adverse event and determine causality in consultation with the Principal Investigator. In addition, the DSMC will review study progress (including data quality and timeliness, recruitment, accrual and retention); study procedures (including subject privacy and data confidentiality protection); outcomes of adverse events and any new literature to assess change to the risk/benefit ratio and to determine if modifications need to be made or the study terminated. If any serious adverse events or unanticipated problems are identified and determined to be associated with the study protocol, the study will be stopped and immediately reported to SCRIHS and the FDA. The DSMC has the authority to stop the study and take whatever steps are necessary to protect the safety and well-being of research subjects until the IRB can evaluate the DSMC's report.

Study personnel will be expected to immediately alert the DSMC to any unanticipated problems or serious adverse events. Unanticipated problems and adverse events will also be reported to SCRIHS in accordance with institutional policy (http://www.siumed.edu/adrfa/SCRIHS/AdverseEvent.pdf) and to the FDA as required. Reports generated by the DSMC will be provided to SCRIHS at the time of continuing review.

To ensure the integrity of the data collected from study participants several procedures will be implemented. All study personnel involved in data collection will be thoroughly trained in the assessment methods thus ensuring consistent applications of procedures and measurement consistency across study subjects. All data will be automatically saved to a computer hard drive and stored on a secure server (i.e., password protected) which is backed up daily. All informed consent documents will be kept at the study site in a separate locked file cabinet with restricted access. Issues related to data integrity will be discussed as a recurring agenda item in the bi-monthly study team meetings.

The study coordinator is responsible for data security. Data management activities include: using double data entry and monitoring outliers. Data security measures include keeping hard copies of data double-locked at all times with access limited to investigators and approved study personnel using password protection and a secure server.

Reporting Adverse Events
We will follow 1996 and 2000 International Conferences on Harmonization, Sections E2 and E6 Good Clinical Practice and FDA regulations. An adverse event (AE) is

defined as any untoward medical occurrence that does not necessarily have a causal relationship with this treatment. A Serious Adverse Event (SAE) is defined as any untoward medical occurrence that results in death, is life-threatening, requires hospitalization or prolongation of hospitalization, results in persistent or significant disability/incapacity, or is a congenital anomaly or birth defect.

We will use the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0 for AE reporting. All participating sites will have access to the CTCAE guide and it can be downloaded at http://ctep.cancer.gov

Expectedness - Adverse Events can be 'Unexpected' or 'Expected' for expedited reporting purposes only.

Attribution of the Adverse Event

1	Definite	AE is clearly related to the study treatment
2	Probable	AE is likely related to the study treatment
3	Possible	AE may be related to the study treatment
4	Unlikely	AE is doubtfully related to the study treatment
5	Unrelated	AE is clearly not related to the study treatment

Expedited Adverse Event Reporting – Phase 1 studies with investigational agents (Including hospitalization below):

- For Hospitalization only – Any medical event equivalent to CTC grade 3, 4, or 5 which precipitated hospitalization (or prolongation of existing hospitalization) must be reported regardless of designation as expected or unexpected and attribution.
- The FDA safety information and adverse event reporting system, Medwatch, FDA form 3500A will be used for reporting within 24 hours.

Expedited Reporting Guidelines

- Medwatch Reporting Requirements for Adverse Events that Occur within 30 Days of the Last Dose of the Investigational Agent on Phase 1 Trials.

Phase 1 Trials

	Grade 1	Grade 2	Grade 2	Grade 3		Grade 3		Grades 4 & 5[2]
				Unexpected		Expected		
	Unexpected and Expected	Unexpected	Expected	with Hospitalization	without Hospitalization	with Hospitalization	without Hospitalization	Unexpected and Expected
Unrelated Unlikely	Not Required	Not Required	Not Required	10 Calendar Days	Not Required	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days
Possible Probable Definite	Not Required	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days	24-Hour; 5 Calendar Days	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days

[1] **Adverse events with attribution of possible, probable, or definite that occur greater than 30 days after the last dose of treatment with an agent under a CTEP IND require reporting as follows:**
AdEERS 24-hour notification followed by complete report within 5 calendar days for:

- Grade 3 unexpected events with hospitalization or prolongation of hospitalization
- Grade 4 unexpected events
- Grade 5 expected events and unexpected events

[2] Although an AdEERS 24-hour notification is not required for death clearly related to progressive disease, a full report

IRB NUMBER: 12-135
IRB APPROVAL DATE: 01/22/2017

Note: All deaths on study require both routine and expedited reporting regardless of causality. Attribution to treatment or other cause must be provided.

Expedited Adverse Event Reporting Timelines (Definitions)

- 24 hours; 5 calendar days – The investigator must initially report the AE via Medwatch within 24 hours of learning of the event followed by a complete Medwatch report within 5 calendar days of the initial 24-hour report.
- 10 calendar days - A complete Medwatch report on the AE must be submitted within 10 calendar days of the investigator learning of the event.
- Any medical event equivalent to CTCAE grade 3, 4, or 5 that precipitates hospitalization (or prolongation of existing hospitalization) must be reported regardless of attribution and designation as expected or unexpected with the exception of any events identified as protocol-specific expedited adverse event reporting exclusions.

Routine Adverse Event Reporting

All Adverse Events must be reported in routine study data submissions. AEs reported through Medwatch must also be reported in routine study data submissions.

11.6 Investigator Responsibilities (21CFR312.50 & 21CFR312.53)

The investigator is responsible for ensuring that the investigation is conducted in accordance with the general investigational plan and protocols, maintaining an effective IND with respect to the investigation, and ensuring that FDA and all participating investigators are promptly informed of significant new adverse effects or risks with respect to the drug.

The investigator will maintain a current signed investigator statement (FDA 1572), a current Curriculum Vitae (CV), and a current clinical protocol.

12. STATISTICAL CONSIDERATIONS

12.1 Study Design/Primary Endpoints

12.1.1 Establish a safe dose for oral 5-ALA administration

NCI Common Toxicity Criteria will be used to quantify toxicity following 5-ALA administration. Special attention will be paid to rash, pruritis, hepatic transaminitis, nausea and vomiting. Based on previously reported German trial, a 30 mg/kg dose is anticipated to be as well-tolerated as 10 and 20 mg/kg doses.

12.1.2 Determine the sensitivity and specificity of the Phase 1 recommended dose in discriminating between normal and malignant tissue intraoperatively.

Four methods are being employed to assess discrimination of tumor from normal brain tissue.

- The neurosurgeon will assess the ability to distinguish tumor from normal brain tissue under ultraviolet illumination and grade the discrimination from 0 to +++ (3+) (See Section 8.1.1). Although arguably the most relevant measure, this is also the most biased and difficult to corroborate measure.
- Under white light the neurosurgeon will assess and biopsy an area of obvious tumor and an area of obviously normal (but possibly edematous) non-eloquent brain. The neurosurgeon will then assess whether these

areas correspond to areas of strong, weak or no fluorescence seen under blue light.

- Under blue light, the neurosurgeon will assess the areas of fluorescence in the tumor cavity and will biopsy areas of strong fluorescence, weak fluorescence and no fluorescence. It may not be necessary to repeat some of these biopsies if the area has already been biopsied under white light. The pathologist will score tumor involvement in each biopsy specimen.

These data will be placed in the following grids – first a 3x3 grid as follows for an analysis of correlation, specifically a Cohen's kappa will be calculated to assess the strength of agreement between fluorescence and tumor density:

Fluorescence vs. Tumor Density	Solid Tumor	Infiltrating tumor/normal brain	No tumor (edematous peritumoral normal brain)
High Fluorescence	(Number of areas identified)	(Number of areas identified)	(Number of areas identified)
Weak Fluorescence	(Number of areas identified)	(Number of areas identified)	(Number of areas identified)
No Fluorescence	(Number of areas identified)	(Number of areas identified)	(Number of areas identified)

Second, these data will also be placed in a simpler 2x2 grid for fluorescence sensitivity/specificity analysis:

Fluorescence vs. Tumor Density	Tumor	No Tumor
Fluorescence	a (Number of areas identified)	b (Number of areas identified)
No Fluorescence	c (Number of areas identified)	d (Number of areas identified)

Sensitivity=a/(a+c); specificity=d/(b+d)

Third, the following grid will be used to examine the sensitivity/specificity of white light examination of the tumor bed and tumor density:

White Light Examination vs. Tumor Density	Tumor	No Tumor
White Light Obvious Tumor	a (Number of areas identified)	b (Number of areas identified)
White Light Obvious No Tumor	c (Number of areas identified)	d (Number of areas identified)

Sensitivity=a/(a+c); specificity=d/(b+d)

Fourth, the following grid will be used to examine the relationship of the neurosurgeon's intraoperative fluoroscopic aided estimate of the extent of

IRB NUMBER: 12-135
IRB APPROVAL DATE: 01/22/2017

resection vs. the actual extent of resection as measured by post-operative MR imaging techniques (See definitions in section 10.1.1), specifically a Cohen's kappa will be calculated to assess the strength of agreement between operative EOR and MRI EOR:

Operative EOR vs. MRI EOR	MRI GTR	MRI NTR	MRI IR
Operative GTR	(Number of areas identified)	(Number of areas identified)	(Number of areas identified)
Operative NTR	(Number of areas identified)	(Number of areas identified)	(Number of areas identified)
Operative IR	(Number of areas identified)	(Number of areas identified)	(Number of areas identified)

12.2 Sample Size/Accrual Rate

It is anticipated that this trial will accrue a mimimum of 3 patients and a maximum of **18* patients in the phase 1 portion of this protocol and 16 patients in the phase 2 portion of the trial. *(*Due to four patients receiving 40mg/kg rather than three, 19 total patients will be studied in phase 1. Protocol deviation approved by IRB on 17 Oct 2013.)*

Total accrual of a maximum of 34 patients in 48 months is expected.

Assuming that the estimated success rate of resection is 0.70, with a sample size of 34, we will have 80% power to detect a moderate kappa of 0.490 from the null hypothesis of zero intraclass kappa, using a large sample 0.050 level two-sided test.

Descriptive statistics will be presented as mean and standard deviation for continuous variables, as frequency and percentage for categorical variables. SAS 9.1 will be used to perform the statistical analyses, type I error (significance level) will be set as 0.05.

12.3 Stratification Factors

No stratification will be performed in this protocol.

IRB NUMBER: 12-135
IRB APPROVAL DATE: 01/22/2017

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IRB NUMBER: 12-135
IRB APPROVAL DATE: 01/22/2017

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APPENDIX A

Performance Status Criteria

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms 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).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
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.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

A-1

IRB NUMBER: 12-135
IRB APPROVAL DATE: 01/22/2017

APPENDIX B

Ultraviolet-Illuminated Operating Room Microscope

Tumor Dye Project – Technical Aspects

5-aminolevulinic acid (5-ALA) – a naturally occurring non-fluorescing metabolic precursor, induces synthesis of protoporphyin IX (PpIX). PpIX fluoresces when exposed to the excitation light of blue-violet (wavelength 375-400nm.) It gives off a light with characteristic peaks 635 and 704nm. This is best viewed by filtering (440 long pass filter) out most other wavelengths except a small amount of excitation light.

The excitation light source is a 400 W short-arc Xe-lamp and a short pass filter (440nm.) integrated into a standard Leica surgical microscope. This filter can be switched on or off by the surgeon when needed. Light is delivered through a commercially available liquid light guide. The Leica OH series microscope is modified so that these filters can be switched on and off whenever the surgeon wishes to observe the fluorescence.

Two special filters for the 5-ALA fluorescence are used in a standard Leica OH microscope. The filters are a short pass (<440nm) filter which rotates in front of the microscope's standard 400-watt Xe light source. In addition, a special long pass (>440nm) barrier filter in the optical path allows clear viewing of the red fluorescence. Both filters are inserted and removed with one button on the microscope handle. With the push of that one button, the standard Leica OH microscope can then go back to being the neurosurgical and spine microscope it was designed for.

Using these modifications intraoperative detection of tumor fluorescence is possible without disturbing the routine of the surgical procedure. Switching between the two illumination modes does not require significant surgeon optical accommodation and does not interrupt the operation. All of these modifications are readily made to the standard neurosurgical microscope and requires no additional personnel in the operating room.

IRB NUMBER: 12-135
IRB APPROVAL DATE: 01/22/2017

Appendix C
Eligibility Checklist

Clinical/Radiological evidence of a primary malignant brain tumor	
Age ≥ 18 years old	
ECOG Performance Status ≤ 2	
Karnofsky ≥ 60%	
Leukocytes ≥ 3,000/mcL	
Absolute neutrophil count ≥ 1,500/mcL	
Total bilirubin within normal institutional limits	
AST (SGOT)/ALT(SGPT) ≤ 2.5 X institutional upper limit of normal	
Creatinine within normal institutional limits or Creatinine clearance ≥60mL/min/1.73 m^2	
Not pregnant	
Able to understand and sign informed consent	
Not currently treated with other investigational agents	
Not allergic to 5-ALA or similar drugs	
No personal or family history of porphyria	
No uncontrolled intercurrent illness including, but not limited to ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements	

C-1

IRB NUMBER: 12-135
IRB APPROVAL DATE: 01/22/2017

Appendix C
Treatment Day Data Sheet

	Date	*Time*	*Initials*	*Result*
Pre-op				
5-ALA Administration (dose)				
Intra-op				
Neurosurgeon JC JE DA (circle one)				
Tumor Visualization with UV Illumination				
Photos Done?				
Pathologist				
White Light / Intensity by light meter				
Area of obvious tumor identified				
- Biopsy obtained?				
Biopsy code label				
- Fluorescence grade				
Area of normal brain in cavity identified				
-Biopsy obtained?				
Biopsy code label				
-Fluorescence grade				
Blue Light				
Area of strong fluorescence identified				
- Biopsy obtained?				
Biopsy code label				
- Fluorescence grade				
Area of weak fluorescence identified				
- Biopsy obtained?				
Biopsy code label				
-Fluorescence grade				
Area of no fluorescence identified				
- Biopsy obtained?				
Biopsy code label				
- Fluorescence grade				

C-2

IRB NUMBER: 12-135
IRB APPROVAL DATE: 01/22/2017

Appendix C
Post-operative Data

Post-op MRI	Date
Evaluation Method (Circle one)	dsMRI Manual
Extent of Resection:	Gross total Near Total Incomplete
Liver Function Tests	
AST (SGOT)/ALT(SGPT)	
Bilirubin	

IRB NUMBER: 12-135
IRB APPROVAL DATE: 01/22/2017

Appendix C

Pathologist Report Data

Surgical Pathology Number _____________

Code	Pathology Summary
	☐ Solid tumor ☐ Infiltrating tumor/normal brain ☐ No tumor (edematous peritumoral normal brain) ☐ Other____
	☐ Solid tumor ☐ Infiltrating tumor/normal brain ☐ No tumor (edematous peritumoral normal brain) ☐ Other____
	☐ Solid tumor ☐ Infiltrating tumor/normal brain ☐ No tumor (edematous peritumoral normal brain) ☐ Other____
	☐ Solid tumor ☐ Infiltrating tumor/normal brain ☐ No tumor (edematous peritumoral normal brain) ☐ Other____
	☐ Solid tumor ☐ Infiltrating tumor/normal brain ☐ No tumor (edematous peritumoral normal brain) ☐ Other____
	☐ Solid tumor ☐ Infiltrating tumor/normal brain ☐ No tumor (edematous peritumoral normal brain) ☐ Other____

IRB NUMBER: 12-135
IRB APPROVAL DATE: 01/22/2017

Biopsy/Fluorescence Correlations

Biopsy Results

Code/Type	Strongly Fluorescent	Weakly Fluorescent	No Fluorescence
___________ ☐ WL/T ☐ WL/N ☐ BL	☐ Solid tumor ☐ Infiltrating tumor/normal brain ☐ No tumor (edematous peritumoral normal brain)	☐ Solid tumor ☐ Infiltrating tumor/normal brain ☐ No tumor (edematous peritumoral normal brain)	☐ Solid tumor ☐ Infiltrating tumor/normal brain ☐ No tumor (edematous peritumoral normal brain)
___________ ☐ WL/T ☐ WL/N ☐ BL	☐ Solid tumor ☐ Infiltrating tumor/normal brain ☐ No tumor (edematous peritumoral normal brain)	☐ Solid tumor ☐ Infiltrating tumor/normal brain ☐ No tumor (edematous peritumoral normal brain)	☐ Solid tumor ☐ Infiltrating tumor/normal brain ☐ No tumor (edematous peritumoral normal brain)
___________ ☐ WL/T ☐ WL/N ☐ BL	☐ Solid tumor ☐ Infiltrating tumor/normal brain ☐ No tumor (edematous peritumoral normal brain)	☐ Solid tumor ☐ Infiltrating tumor/normal brain ☐ No tumor (edematous peritumoral normal brain)	☐ Solid tumor ☐ Infiltrating tumor/normal brain ☐ No tumor (edematous peritumoral normal brain)
___________ ☐ WL/T ☐ WL/N ☐ BL	☐ Solid tumor ☐ Infiltrating tumor/normal brain ☐ No tumor (edematous peritumoral normal brain)	☐ Solid tumor ☐ Infiltrating tumor/normal brain ☐ No tumor (edematous peritumoral normal brain)	☐ Solid tumor ☐ Infiltrating tumor/normal brain ☐ No tumor (edematous peritumoral normal brain)
___________ ☐ WL/T ☐ WL/N ☐ BL	☐ Solid tumor ☐ Infiltrating tumor/normal brain ☐ No tumor (edematous peritumoral normal brain)	☐ Solid tumor ☐ Infiltrating tumor/normal brain ☐ No tumor (edematous peritumoral normal brain)	☐ Solid tumor ☐ Infiltrating tumor/normal brain ☐ No tumor (edematous peritumoral normal brain)
___________ ☐ WL/T ☐ WL/N ☐ BL	☐ Solid tumor ☐ Infiltrating tumor/normal brain ☐ No tumor (edematous peritumoral normal brain)	☐ Solid tumor ☐ Infiltrating tumor/normal brain ☐ No tumor (edematous peritumoral normal brain)	☐ Solid tumor ☐ Infiltrating tumor/normal brain ☐ No tumor (edematous peritumoral normal brain)

IRB NUMBER: 12-135
IRB APPROVAL DATE: 01/22/2017

SCRIHS 10-035 5-ALA
Intra-Operative Data Sheet

Sample Code	Light	Area Biopsied	Fluorescence (0 to +++)
___________________ Time:	☐ White	☐ Obvious Tumor ☐ Obvious Not Tumor	
	☐ Blue	☐ Strongly Fluorescent ☐ Weakly Fluorescent ☐ No Fluorescence	
___________________ Time:	☐ White	☐ Obvious Tumor ☐ Obvious Not Tumor	
	☐ Blue	☐ Strongly Fluorescent ☐ Weakly Fluorescent ☐ No Fluorescence	
___________________ Time:	☐ White	☐ Obvious Tumor ☐ Obvious Not Tumor	
	☐ Blue	☐ Strongly Fluorescent ☐ Weakly Fluorescent ☐ No Fluorescence	
___________________ Time:	☐ White	☐ Obvious Tumor ☐ Obvious Not Tumor	
	☐ Blue	☐ Strongly Fluorescent ☐ Weakly Fluorescent ☐ No Fluorescence	
___________________ Time:	☐ White	☐ Obvious Tumor ☐ Obvious Not Tumor	
	☐ Blue	☐ Strongly Fluorescent ☐ Weakly Fluorescent ☐ No Fluorescence	
___________________ Time:	☐ White	☐ Obvious Tumor ☐ Obvious Not Tumor	
	☐ Blue	☐ Strongly Fluorescent ☐ Weakly Fluorescent ☐ No Fluorescence	

Grade	Definition	
0	No fluorescence. No difference between any of the tissues visualized.	No fluorescence
+	Low fluorescence. Red glow is barely distinguished from surrounding non-fluorescent tissue.	Weak fluorescence
++	Moderate fluorescence. Dull red glow of fluorescent tissue.	
+++	High fluorescence. Bright red glow of fluorescent tissue	Strong fluorescence

IRB NUMBER: 12-135
IRB APPROVAL DATE: 01/22/2017

Estimate of extent of fluorescent tissue resected (%):

Estimate of extent of enhancing tumor resected (%):

C-6

IRB NUMBER: 12-135
IRB APPROVAL DATE: 01/22/2017

APPENDIX D

Dear Dr. Cozzens,

CTEP does not have a CAEPR for this agent at this time.

For any future CAEPR requests, please submit additional details such as the protocol number, who the proposed trial's sponsor will be, where the trial will be held, and who is expected to supply trial agent(s).

Regards,

Yael

Yael R. Vinciguerra
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From: Jeffrey W. Cozzens, M.D. [mailto:jeff.cozzens@gmail.com]
Sent: Monday, March 30, 2009 6:39 PM
To: NCI CTEP PIO with Contractors
Subject: 5-ALA

I am currently writing a proposal utilizing 5-aminolevulinic acid (5-ALA). Please send me the current CAEPR or ASAEL for this drug.

_____

Jeffrey W, Cozzens, M.D., FACS
Neurosurgery
e-mail: jeff.cozzens@gmail.com
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