

## CLINICAL PROTOCOL

**TITLE:** A Phase 2 Study of Aminolevulinic Acid (ALA) to Enhance Visualization and Resection of Tumors of the Brain

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## 1. OVERVIEW

### **Title**

A Phase 2 Study of Aminolevulinic Acid (ALA) to Enhance Visualization and Resection of Tumors of the Brain

### **Primary Objective**

To determine if the use of 5-aminolevulinic acid (ALA) helps distinguish tumor cells from normal cells leading to an increase in the amount of tumor removal during neurosurgical resection of glioma.

### **Secondary Objective**

Determine the safety of a single dose of 5-aminolevulinic acid (ALA) administered preoperatively at a dose of 20 mg/kg body weight.

### **Study product and route of administration**

5-Aminolevulinic Acid (ALA) will be given orally at a dose of 20 mg/kg body weight.

### **Participants**

300 individuals (age 18 to 72 years) with diagnosed glioma (WHO GI-IV) eligible for surgery will be entered into the trial.

50 participants will be enrolled into each of the following subgroups:

- 1) Newly diagnosed GBM
- 2) Newly diagnosed grade II glioma (astrocytic, oligodendroglial, and mixed)
- 3) Recurrent GBM
- 4) Recurrent grade III glioma (astrocytic, oligodendroglial, and mixed)
- 5) Newly diagnosed low grade glioma (astrocytic, oligodendroglial, ganglioglial, and mixed)
- 6) Recurrent low grade glioma (astrocytic, oligodendroglial, ganglioglial, and mixed)

### **Design**

Single center open label phase 2 trial

### **Estimated total study duration**

Twenty four months

### **Investigational New Drug (IND) sponsor**

Mitchel S. Berger, M.D., Director, Brain Tumor Research Center  
University of California Medical Center, San Francisco

**Study drug provider**

DUSA Pharmaceuticals/Sun Pharma, Tarrytown, NY

**Statistical and data management**

UCSF Department of Neurological Surgery

**Safety Monitoring**

Adverse events will be collected from the time of drug administration. NCI Common Terminology Criteria for adverse events (CTCAE) version 3.0 will be utilized for adverse event reporting.

**2. OBJECTIVES****2.1 Primary Objective:**

To determine if the use of 5-aminolevulinic acid (ALA) helps distinguish tumor cells from normal cells leading to an increase in the amount of tumor removal during neurosurgical resection of glioma.

**2.2 Secondary Objective:**

Determine the safety of a single dose of 5-aminolevulinic acid (ALA) administered preoperatively at a dose of 20 mg/kg body weight.

**3. BACKGROUND**

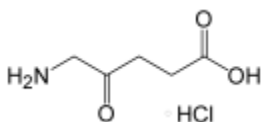
Gliomas are locally invasive tumors in the brain that are very infiltrative and are associated with a poor prognosis. Currently there is no adequate way to properly identify these tumors during the course of surgical resection other than by gross visualization. A number of studies have shown that the extent of resection definitely affects outcome in terms of survival and progression-free survival (1-6). Nevertheless, surgical studies (2-7) have indicated that complete resection of contrast-enhancing tumor is achieved in only about 20-30% of patients and can be considered likely due to difficulty in determining the overall marginal area of the tumor (2). Therefore, it is considered important to achieve the greatest extent of resection possible, reducing the mass of the tumor, prior to administered therapy. Several technical surgery supports have been explored that include magnetic resonance imaging (MRI) (8), neuronavigation (9), and ultrasound (10) but little data from prospective randomized trials exists. The standard mode being used, with few exceptions, still relies on preoperative imaging studies to guide the course of resection, as well as the use of clinical acumen.

Over the last several years, 5-aminolevulinic acid (ALA) has been used in Europe to illuminate the tumor and its infiltrative margin and to compare it to white light-based surgery (11) and has been granted marketing approval by the European Medicines Evaluation Agency for use in the European community in adult patients for visualization of malignant tissue during

surgery for malignant glioma (World Health Organization Grades III and IV). ALA is a natural biochemical precursor of hemoglobin that elicits synthesis and accumulation of fluorescent porphyrins in various epithelia and cancerous tissue (12). ALA has been shown in experimental and clinical studies to be taken up by malignant glioma cells, where it is converted into fluorescing porphyrins (13). Strummer, et al. 2006, reports that fluorescence-guided resection by use of 5-aminolevulinic acid (ALA) is easy to do, and does not interrupt the operation (11). Such resection relies on specific synthesis and accumulation by 5-aminolevulinic acid of fluorescent porphyrins in malignant glioma tissue (14, 15). The technique differs from earlier attempts to use a fluorescent agent, such as fluorescein (16), in which the fluorescing agent is in the plasma and reaches the tumor through the disrupted blood-brain barrier, limiting specificity. The European data has been very supportive in terms of facilitating the extent of resection and improving 6-month progression-free survival (11).

### 3.1 5-Aminolevulinic Acid (ALA)

C<sub>5</sub>H<sub>9</sub>NO<sub>3</sub> MW 167.6



ALA is a naturally occurring substance that is found in all organisms, including humans, and is necessary for part of the basic metabolic life processes. Approximately 350 mg of 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). ALA (and other porphyrins) and Pp IX are excreted in the urine and the stool (via bile), respectively. DUSA Pharmaceuticals/Sun Pharma is located in Tarrytown, NY and will be the provider of the product. DUSA Pharmaceuticals/Sun Pharma has investigated the use of ALA and studied its pharmacology, pharmacokinetics, and toxicity.

#### *Pharmacology*

The metabolism of aminolevulinic acid is the first step in the biochemical pathway resulting in heme synthesis. ALA is not a photosensitizer, but rather a metabolic precursor of Pp IX, which is the photosensitizer. Synthesis of ALA is normally controlled by feedback inhibition of the enzyme, ALA synthetase, presumably by intracellular heme levels. ALA, when provided to the cell, bypasses this control point and results in the accumulation of Pp IX, which is converted into heme by ferrochelatase through the addition of iron to the Pp IX nucleus.

#### *Pharmacokinetics*

ALA and Pp IX were measured in plasma in a human pharmacokinetic study using a 128 mg dose of sterile intravenous ALA HCl and oral ALA HCl (equivalent to 100 mg ALA). The mean half-life of ALA was  $0.70 \pm 0.18$  hour after the oral dose and  $0.83 \pm 0.05$  hour after the intravenous dose. The oral bioavailability of ALA was 50-60% with a mean C<sub>max</sub> of  $4.65 \pm 0.94$  µg/mL. Pp IX concentrations were low and were detectable only in 42% of the plasma samples. Pp IX concentrations in plasma were quite low relative to ALA plasma concentrations, and were below the level of detection (10 ng/mL) after 10 to 12 hours.

#### *Toxicity*

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*In vivo* pharmacological and toxicological effects that may be associated with elevated systemic levels of 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 ALA and neurological abnormalities is observed in Acute Intermittent Porphyria (AIP). However, attempts at clarifying the association between elevated ALA and the neurological symptoms of AIP have led to inconsistent results and contradictory conclusions. Most studies have shown that ALA and Porphobilinogen (PBG equals condensation of 2 molecules of ALA) are not neurotoxic *in vivo* when administered in large amounts to animals and humans. Concentration of these precursors in the brain or cerebrospinal fluid (CSF) of porphyric patients is substantially below that required for demonstrable toxicity *in vitro*. Litman and Correia, 1985, hypothesized that depletion of hepatic heme, as seen in AIP, leads to elevated tryptophan content and 5-hydroxytryptamine turnover in the brain, and is responsible for the neurological symptoms of AIP (17). On this basis then, administration of exogenous 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 Pp IX, is a typically finding in Erythropoietic Protoporphyrinemia (EPP) which is associated with decreased activity of ferrochelatase, the enzyme that converts Pp IX to heme. EPP is entirely cutaneous, with none of the hemolytic or neurological problems of other porphyrias.

Mild transient nausea and occasional vomiting have been observed following oral administration of high doses of ALA. Webber et al. 1997, observed that almost one-quarter of all patients who retained ALA had at least one abnormality in liver function tests (18). Nausea and vomiting were seen in 20% of the patients but no other symptoms of porphyria.

The most common complaint, due to Pp IX photosensitization, is 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/Sun Pharma), which lasts from approximately 24 to 48 hours following ALA administration.

### **3.2 Invasive Brain Tumor**

The tumor may take on a variety of appearances, depending on the amount of hemorrhage, necrosis, or its age. An MRI will usually show a nonhomogeneous mass with a hypodense center and a variable ring of enhancement surrounded by edema. Mass effect from the tumor and edema may compress the ventricles and cause hydrocephalus. Cancer cells with stem cell-like properties have been found in glioblastoma (this may be a cause of their resistance to conventional treatments, and high re-occurrence rate (19).

The current treatment of patients with brain tumors is traditionally divided into surgical, radiotherapeutic, chemotherapeutic, and experimental treatments. Surgery is usually performed to either fully resect or debulk the gross portion of the tumor (contrast-enhancing portion). 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 shown some efficacy. 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.

The median survival of a patient undergoing biopsy of GBM without surgical resection or further treatment is ~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 37 weeks; 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 within 2cm. 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%.

Lower grade tumors offer better prognosis. Median survival is 5.9 years when treated with biopsy alone. However, early resection of low grade tumors improves 5-year survival to 74% compared to 60% for biopsy and watchful waiting (19). As in malignant glioma, survival rates are age dependent. The ten-year survival rates are 86% for age 0-20, 53% for age 20 to 64, and 20% for age >64. Maximal tumor resection may improve both symptoms and survival rates (6, 20).

### **3.3 Rationale for ALA**

5-aminolevulinic acid (ALA) is a naturally occurring amino acid and a precursor in heme biosynthesis. ALA is produced at the cytosolic surface of the mitochondrial membrane and then transported to the cellular cytosol for heme biosynthesis. The availability of 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 ALA (the rate-limiting precursor) to malignant glial cells leads to an increase in the intracellular accumulation of Protoporphyrin IX, a fluorescent intermediary in heme biosynthesis. As a result, malignant glial cells fluoresce red relative to dark normal brain tissue, when viewed under ultraviolet light (300 nm). This effect enables more complete surgical removal of malignant glial tumor from within normal brain tissue.

### **3.4 Rationale for the Intraoperative Fluorescence/Reflectance Probe**

Although ALA results in visible concentrations of Pp IX in high grade malignant gliomas, detection in lower grade gliomas is limited due a relatively low intensity of fluorescence signals, resulting in incomplete tumor detection. However, Pp IX does accumulate to diagnostically significant levels in low grade gliomas even when not visibly evident to the



surgeon (21). To aid in the quantitative detection of subvisual concentrations of Pp IX in low grade gliomas, an intraoperative fluorescence/reflectance probe was custom designed under NIH funding. The intraoperative probe is a 1.25 mm semi-rigid, blunt end device that is placed in gentle contact with brain tissue by the surgeon for 3-5 seconds to make a spectroscopic measurement. The probe delivers a very small amount of blue light and white light to measure the concentration of Pp IX.

The probe meets all of the criteria necessary for classification as a non-significant risk device according to FDA guidelines. The device is non-invasive with respect to the participant and does not present a serious risk to the health, safety, or welfare of a subject. The device is not used to diagnose, cure, mitigate, or treat disease without concordance from additional data made available through practices which meet or exceed standard-of-care. The light exposure levels are below those known to cause any temperature rise or other significant tissue damaging effects, even in conjunction with ALA which can be phototoxic but at much higher light exposure thresholds.

## **4. PATIENT SELECTION**

### **4.1 Eligibility Criteria**

4.1.1 Patients entering into this study will have the presumptive diagnosis of high grade or low grade glioma based on imaging studies, or will have recurrent high-grade or low grade gliomas that have previously undergone diagnosis (astrocytoma, oligodendroglioma, mixed oligo-astrocytoma, anaplastic astrocytoma, and glioblastoma multiforme). Both of these groups will be undergoing craniotomy for tumor resection.

4.1.2 Patient age 18 to 72 years.

4.1.3 Karnofsky performance of 60% or greater, see Appendix B.

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

Leukocytes	>3,000/ $\mu$ L
Absolute neutrophil count	>1,500/ $\mu$ L
Platelets	>100,000/ $\mu$ L
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	$\geq 60$ mL/min/1.73 m <sup>2</sup> for patients with creatinine levels above institutional normal.

4.1.5 The effects of Aminolevulinic Acid (ALA) on the developing human fetus are unknown. Therefore, 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.

- 4.16 Patient must have the ability to understand and the willingness to sign a written informed consent document or have a parent or guardian with the ability to understand and the willingness to sign the written informed consent.

## 4.2 Exclusion Criteria

- 4.2.1 History of allergic reactions attributed to compounds of similar chemical or biologic composition to aminolevulinic acid (ALA).
- 4.2.2 Personal or family history of Porphyrrias.
- 4.2.3 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.
- 4.2.4 Pregnancy since aminolevulinic acid (ALA) is of unknown teratogenic or abortifacient effects.

## 4.3 Inclusion of Women and Minorities

Both men and women and members of all ethnic groups are eligible for this study.

## 4.4 Study Calendar

Baseline evaluations are conducted 3 weeks prior to start of protocol.

	Pre-Study	Day 1	Day 2-3	Day 14 (+/- 5days)
ALA <sup>a</sup>		X		
Informed consent	X			
Demographics	X			
Medical history	X			
Concurrent meds	X			X
Physical exam	X		X	X
Vital signs	X		X	X
Height	X			
Weight	X			X
Performance Status	X			X
CBC, w/diff, platelets	X		X	X
Serum chemistry <sup>b</sup>	X		X	X
EKG (as indicated)	X			
Adverse event evaluation	X		X	X

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MRI Brain + gadolinium	X		X <sup>c</sup>	
B-HCG	X <sup>d</sup>			

- a: ALA dose of 20 mg/kg oral administration one time.
- b: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, SGOT(AST), SGPT (ALT), sodium.
- c: MRI immediately post-operation (24-48 hrs post surgery).
- d: Serum pregnancy test (women of childbearing potential).

## 5 TREATMENT PLAN

The study will consist of 300 patients with high-grade or low-grade tumors that meet the eligibility criteria provided in section 4.1. Each patient will be evaluated and found to have a high-grade or low-grade glioma by means of history and recent imaging studies (MRI) and deemed a surgical candidate based on current neurosurgical standards of care. The study will have the University of California San Francisco (UCSF) ethics committee (CHR) approval. Informed consent will be obtained from patients meeting enrollment criteria. The study will be done according to the guidelines of the International Conference on Harmonization - Good Clinical Practice.

### 5.1 Pre-surgical Procedure

A one-time single administration of aminolevulinic acid (ALA), at a dose of 20 mg/kg body weight, will be given orally. ALA will be dissolved in ~100 mL of drinking water or juice and given to the patient approximately 3 hours before anesthesia.

### 5.2 Surgical Procedure

ALA will be mixed in the minimum volume of sterile water or juice immediately before use and given as a single bolus orally approximately 3 hours prior to scheduled anesthesia. Image-guided microsurgical resection of the tumor will be undertaken. Following the standard tumor resection under light microscopy, the residual tumor bed will be illuminated with blue light via a high pass optical filter in the microscopes light chain. The surgeon will examine the tumor bed with a barrier filter in the optical chain of the surgical microscope. The surgeon will quantitatively measure the concentration of Pp IX by placing the intraoperative fluorescence/reflectance probe in gentle contact with brain tissue by the surgeon for 3-5 seconds to make a recording. If fluorescence is present, and if safe, small biopsies will be taken from those areas of fluorescence as well as from non-fluorescing regions in the tumor resection cavity. When possible, the intent would be to collect a number of biopsies with a range of fluorescence intensities from each patient (at least one biopsy from normal-appearing tissues and at least one from the most fluorescent area). All biopsy specimens, including those taken from the bulk tumor, will be reviewed by a neuropathologist to assess tumor content. The expectation is that residual fluorescent tissue in the tumor cavity will be surgically removed (if deemed safe by the

neurosurgeon), potentially leading to more complete tumor resection. Pathologic confirmation of tumor type will be made by neuropathology.

Biopsies of fluorescent and non-fluorescent tissue within the tumor cavity will be graded by the surgeon at the time of biopsy as 0 to 3 fluorescence. A screen shot of the fluorescent image of the resected tumor illuminated with blue light via a high-pass filter will be documented for each patient with an integrating video camera. The fluorescence intensity and the quantitative measurement obtained with the intraoperative probe corresponding to each area on the screen shot from which biopsy is taken will be recorded by the surgeon in the operating room. The sample will be snap frozen in the operating room and provided to the pathologist for grading the tumor from 0 to 3 based on the degree of cellular infiltration. The scale to be used for grading is as follows:

Grade	Surgeon (intensity of fluorescence)	Pathologist (cell density)
0	No fluorescence	No tumor cell density content
1	Mild brightness	Mildly infiltrating tumor cell density
2	Moderate brightness	Moderately infiltrating cell density
3	Intense brightness	Highly infiltrating cell density

The surgeon is responsible for:

- Identifying at least one fluorescent area within the tumor cavity and performing biopsy and grading that site's fluorescence.
- Identifying at least one non-fluorescent area within the tumor cavity and performing biopsy and grading that site's fluorescence.
- Labeling the areas (i.e. fluorescent or non-fluorescent) from which the biopsies are taken.

The pathologist is responsible for:

- Grading each frozen section biopsy for tumor content on a scale of 0 to 3.
- Labeling of specimens to be certain they correlate with biopsies taken by the surgeon.

### 5.3 Extent of Resection Follow-up

Patients will be assessed for completeness of tumor resection immediately following surgery (within 24 to 48 hours) using gadolinium enhanced brain MRI. Extent of resection will be assessed based on the finding of postoperative enhancement or lack of enhancement, on the postoperative imaging scans and will be evaluated as follows:

- Complete Resection (GTR): Resection of all enhancing disease
- Partial Resection (STR): At least a 50-90% decrease in the sum of the longest diameter (LD) of enhancing disease, taking as reference the baseline pre-operative MRI scan

- Near Complete Resection: Between a 90 and 99% resection of enhancing disease
- Minor Resection: All other situations (< 50% removal)

#### **5.4 Supportive Care**

Patients will be managed according to procedures for standard care established for postoperative brain resection patients. The patient will be kept in a "low light" environment for 72 hours following administration of ALA.

## **6 REGULATORY AND REPORTING REQUIREMENTS**

### **6.1 UCSF Data Safety Monitoring Plan for Phase II or III Institutional Study**

#### **Oversight and Monitoring Plan**

The UCSF-CCC Data Safety Monitoring Committee (DSMC) is responsible for monitoring data quality and patient safety for all UCSF-CCC institutional clinical studies. A summary of DSMC activities for this study includes:

- Review of subject data in each cohort
- Quarterly review for progress and safety
- Review of all serious adverse events
- Minimum of a yearly audit

### **6.2 Monitoring and Reporting Guidelines**

Investigators will conduct continuous review of data and patient safety at monthly study group or site committee meetings where the results of each patient's treatment are discussed and the discussion is documented in the minutes. The discussion will include the number of patients, significant toxicities as described in the protocol, doses adjustments, and observed responses. Quarterly summaries will be submitted to the DSMC for review. All grade 3-5 AE's and SAE's will be entered in the CCC Oncore database.

### **6.3 Review and Oversight Requirements**

#### **6.3.1 Adverse Event Monitoring**

Adverse Events (AEs) will be recorded on the Oncore database, all grade 3-5 expected and unexpected AEs will be recorded and updated at each visit.

##### **6.3.1.1 Serious Adverse Event Reporting**

Serious Adverse Event reporting will be in accordance with the UCSF- Committee on Human Research Regulations and Code of Federal Regulation Title 21 Volume 5 Part 312.32.

UCSF CHR website for guidance in reporting serious adverse events  
[http://www.research.ucsf.edu/chr/Guide/Adverse\\_Events\\_Guidelines.pdf](http://www.research.ucsf.edu/chr/Guide/Adverse_Events_Guidelines.pdf)

FDA website for guidance in reporting serious adverse events  
<http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=312.32>

MedWatch forms and information:  
<http://www.fda.gov/medwatch/getforms.htm>

Serious Adverse events will be reported on the MedWatch form. A copy of the MedWatch report and CHR forms must be sent to CCC- DSMC at Box 1297. The date the SAE was sent to all required reporting agencies will be documented and one Oncore, hard copies of the report will be maintained in the regulatory files.

**If the SAE is death and determined to be possibly, probably or definitely related to the investigational drug or any research related procedure, the event must be reported to the DSMC Chair or his designee within 24 business hours. The reporting procedure is by personal communication via phone or in person with written documentation of the 1:1 communication via e-mail with a copy of the e-mail to DSMC Administrator and DSMC Coordinator.**

### **6.3.2 Review of Adverse Event Rates**

If the study has an increase of unexpected or expected Adverse Events, grade 3 or 4 above the rate reported in the Investigational Brochure or package insert, the increase rate of AEs will be reported to the DSMC at the time of Identification. The Chair and PI will discuss the finding and proceed with a written course of action. Each quarterly report will indicate if the AE incidence is within the scope of the investigational brochure or package insert. If at any time the Investigator stops enrollment or stops the study due to safety issues, the DSMC Chair and Administrator must be notified within 24 business hours via e-mail. The DSMC must receive a formal letter within 10 business days and the CHR must be notified.

### **6.3.3 Study Progress – Quarterly Review**

Principal Investigators are required to submit quarterly study progress reports to determine whether accrual projections are being met, to summarize grade 3 and 4 toxicities (expected and unexpected) and SAE reports. This report will also indicate if the rate of all grade

3-5 AE's are above the AE rates documented in the Investigational Brochure or package insert. In addition, a progress report on recruitment and subjects known responses to the investigational therapy must be submitted. At this time also send the committee all external DSMB reports and formal audit reports.

These quarterly reports are reviewed at Data Safety Monitoring Committee meetings. These reports are required: February 1, May 1, August 1, and October 1. Failure to submit such reports may result in trial suspension. Send reports to: DSMC Box 1297

Data Safety Monitoring Committee Contacts:

DSMC Chair: Alan Venook, MD

Phone (415) 353-2745

Email venook@cc.ucsf.edu

Box 1705

#### 6.4 Adverse Event Reporting

An adverse event is any untoward medical occurrence in a patient administered a pharmaceutical product, which does not necessarily have a causal relationship with the treatment. An adverse event can be any unfavorable and unintended sign (e.g. including an abnormal laboratory finding), symptom, or disease temporally associated with the use of the drug, whether or not it is considered to be drug related. This includes any newly occurring event or previous condition that has increased in severity or frequency since the administration of the drug.

A serious adverse event (SAE) is any adverse event, occurring at any dose and regardless of causality that:

- is fatal
- life-threatening Life -threatening means that the patient was at immediate risk of death from a reaction as it occurred (i.e., does not include a reaction which hypothetically might have caused death had it occurred)
- requires inpatient hospitalization or prolongation of existing hospitalization
- results in persistent or significant disability/incapacity
- is a congenital anomaly/birth defect
- is an important medical event, which is defined as an event that may not result in death, be life-threatening, or require hospitalization but may be considered an SAE when, based upon appropriate medical judgment, it may jeopardize the patient and may require medical or surgical intervention to prevent a SAE.

Since potential adverse events related to ALA are known to be transient in nature, it is felt that a 14-day evaluation window is relevant to assess the safety profile of this agent. All adverse events will be collected from the time of drug administration and will continue to be collected until 14 days (+/- 3days) from the time of drug dosing. In addition, serious adverse events (SAE) will be collected beginning from the time the subject signs the informed consent.

The descriptions and grading scales found in NCI Common Terminology Criteria for adverse events (CTCAE) version 3.0 will be utilized for adverse event reporting and are available on line <http://ctep.cancer.gov/reporting/ctc.html>. SAEs and expedited reporting of specific expected AEs will be submitted to the FDA via the FDA safety information and adverse event reporting system MedWatch, FDA form 3500A.

#### **6.4.1 Probable ALA adverse events to be expected**

Transient side effects have been noted, most commonly, skin photosensitivity, nausea, vomiting, abdominal discomfort, and lowering of blood pressure which can result in lightheadedness. Since ALA may cause nausea and vomiting, patients will be monitored and treated as needed with anti-nausea medication.

Dose-dependent abnormalities in liver function tests have been documented. The most common effect has been a rise in transaminases (typically AST), (23-29). Liver function tests AST and ALT will be performed throughout the study, please see the Study calendar provided in section 4.4.

ALA-induced Pp IX accumulation occurs predominantly in cells of epithelial origin and isolated cases of skin photosensitivity with subsequent cutaneous injury after sun/bright light exposure have been reported (29, 30). Skin reactions in clinical photodynamic therapy trials with doses of 30-60 mg/kg have been mild, consisting primarily of erythema that resolved within 24 to 48 hours. Blister formation and/or skin necrosis have not been reported in these trials and neither have any indications of ocular sensitivity been described with systemic ALA. Precautions will be taken to protect the patient from the effects of skin photosensitivity during surgery. Patients will receive instructions on how to protect themselves and to minimize exposure to sunlight and/or indoor lighting for up to 72 hours. Patients with a history of cutaneous photosensitivity, porphyria, hypersensitivity to porphyrins, photodermatitis, exfoliative dermatitis or an inability to comply with the photosensitivity precautions associated with the study will be excluded from enrollment.

The light dose (due to fluorescence excitation) to the brain during fluorescence image-guided resection can be estimated to be approximately  $216 \text{ J.cm}^{-2}$ , based on data presented in Stummer (11), an irradiance of  $\sim 60 \text{ mW.cm}^{-2}$ , and an approximate illumination time of 1 hour. The risk of complications due to a potential PDT effect are considered minimal since no serious adverse events were reported by Stummer (11, 14), the ALA dose in our trial (20 mg/kg) is equal to that used in these studies and is lower than the ALA doses used for PDT which typically reach 60mg/kg (12, 23-28, 30-38).

#### **6.4.2 Other possible adverse events**

The following more serious expected adverse events that could occur in this study protocol will not require expedited reporting, but the event will be captured in the patient's study file.

- Reaction to anesthesia.
- Balance problem as the results of the tumor itself or from the surgery to correct it.



- Bleeding which may occur as the result of the surgery or from medications.
- Blood clot development, which may occur with any surgery.
- Brain injury that could happen depending on the location of the tumor and removal procedure.
- Cardiac complications since there is a chance the procedure could cause an irregular heartbeat or heart attack.
- Functional loss as there is a possibility of experiencing problems such as opening the mouth, chewing, speech, language, and memory difficulties following surgery.
- Hydrocephalus as it is possible that the normal flow of spinal fluid around the brain may be altered.
- Infection that may occur at the incision site and may also occur within the bone flap. Infection-related risks also include meningitis or a brain abscess.
- Loss of nerve function or paralysis.
- Post-operative pain such as a headache that may persist for a week to a month and sometimes for a longer time period.
- Recurrence of the tumor may return to the same site.
- Respiratory difficulties, which could include breathing difficulties, post-operative pneumonia or a pulmonary embolus.
- Seizure activity.
- Visual disturbance.

## **6.5 Environmental Assessment**

A claim is made for categorical exclusion under 21 CFR 25.31 (e) for this IND. The use of ALA is not considered toxic and the standard procedure for chemotherapy waste disposal will followed.

## **7 STATISTICAL CONSIDERATIONS**

### **7.1 Study Endpoints**

This is a single-center, single arm, open label phase II trial of fluorescence-guided resection by use of 5-aminolevulinic (ALA) at a dose of 20 mg/kg body weight in glioma patients. We hypothesize that surgical removal of the residual fluorescent tissues in the tumor cavity, presumably representing tumorous cells, will result in an increase in the amount of tumor removal during neurosurgical resection. In addition, we hypothesize that for recurrent malignant glioma, that the fluorescent material will reflect active viable tumor and not changes related to treatment such as macrophages or gliosis.

The primary analysis will be based on one biopsy taken from the most fluorescent area and one from normal appearing tissues for each patient as judged by the neurosurgeon.

The endpoints of interest in this study are as follows:

- Primary Endpoint: Positive Predictive Value (PPV), defined as the percentage of biopsies taken from the most fluorescent tissues determined by the pathologist as having tumorous content.
- Second Endpoints:
  - Negative Predictive Value (NPV), defined as the percentage of biopsies taken from non-fluorescent tissues determined as having no tumor content by the pathologist.
  - The safety of the use of 5-aminolevulinic (ALA) at a dose of 20 mg/kg body weight for neurosurgical resection in glioma patients
  - The correlation between fluorescent intensity as scored by the neurosurgeon and the tumor content as scored by the pathologist
  - The percentage of patients for whom there is no fluorescent tissue after surgery

## 7.2 Sample Size Determination

There is interest in assessing the correlation of 5-ALA uptake and degree of fluorescence among the different grades and histological subtypes of newly diagnosed high- and low- grade glioma. In addition, once a high-and low- grade glioma has been treated with radiation and chemotherapy, it is well accepted that treatment related changes can be seen histologically. There has been little experience in the assessment of the use of 5-ALA in the recurrent setting and in this study we are interested in the specificity of 5-ALA uptake in tumor cells in the post treated setting. As such, in addition to newly diagnosed glioma patients, there will be a specific subgroup of recurrent glioma who will be studied.

The patients will be allocated to the following subgroups:

- 1) Newly diagnosed GBM
- 2) Newly diagnosed grade III glioma (astrocytic, oligodendroglial and mixed)
- 3) Recurrent GBM
- 4) Recurrent grade III glioma (astrocytic, oligodendroglial and mixed)
- 5) Newly diagnosed low grade glioma (astrocytic, oligodendroglial, ganglioglioma, and mixed)
- 6) Recurrent low grade glioma (astrocytic, oligodendroglial, ganglioglioma, and mixed)

A total of 50 patients per subgroup will be enrolled. The main goal is to determine the intraoperative detection of malignant and low grade glioma by the use of 5-aminolevulinic (ALA), as measured by PPV defined above. In order for this agent to be useful, we would expect PPV to be at least 80%. Therefore, 80% is considered the lower threshold for the hypothesis testing (i.e. under H<sub>0</sub>). The calculation of sample size was based on a targeted PPV of 92%. Fifty patients per cohort will provide approximately 90% power to detect this improvement using a one-sided exact binomial test with alpha of 0.10. The agent will be deemed useful if 44 or more biopsies taken from the most fluorescent tissues are determined by the pathologist as having tumorous content.

### 7.3 Statistical Analysis

The primary analysis will include all patients who are eligible for this study and who undergo planned neurosurgery. PPV and NPV will be estimated and the 95% confidence interval associated with each measure will be provided. Secondary analysis will take advantage of multiple biopsies taken from areas representing a range of fluorescence intensity within each patient. The Spearman's rank correlation coefficient will be calculated to assess the correlation between fluorescent intensity as scored by the neurosurgeon and the tumor content as scored by the pathologist. To account for within-patient correlation, the Cochran-Mantel-Haenszel Chi-Squared test will be used to test the association between fluorescence intensity and tumor content.

All patients who are enrolled and take 5-ALA will be considered evaluable for toxicity. Since potential adverse events related to 5-ALA are known to be transient in nature, it is felt that a 14-day evaluation window is relevant to assess the safety profile of this agent. The formal analysis and final report of toxicities will be performed when all patients have been enrolled and followed for toxicities for 14 days after administration of 5-ALA. Reporting of adverse events is described in Section 6. We intend to monitor adverse events on a monthly basis to determine if there are unacceptable toxicities. A report of all adverse events since the first subject was enrolled will be prepared every month, along with a tabular display of the relative frequency of all grade 2 or higher toxicities considered possibly, probably, or likely related to 5-ALA. This information will be submitted to the UCSF-CCC Data Safety Monitoring Committee (DSMC) who is responsible for monitoring data quality and patient safety for all UCSF-CCC institutional clinical studies.

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