Case Comprehensive Cancer Center

STUDY NO: CASE 1305

TITLE: FLUORESCENCE-GUIDED DETECTION OF MALIGNANT GLIOMAS: A DOSE RANGING STUDY USING 5-AMINOLEVULINIC ACID (ALA) INDUCED PROTOPORPHYRIN IX (PpIX) IN A PHASE II CLINICAL TRIAL.

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GCRC Approval: 5/3/06 (now DCRC)

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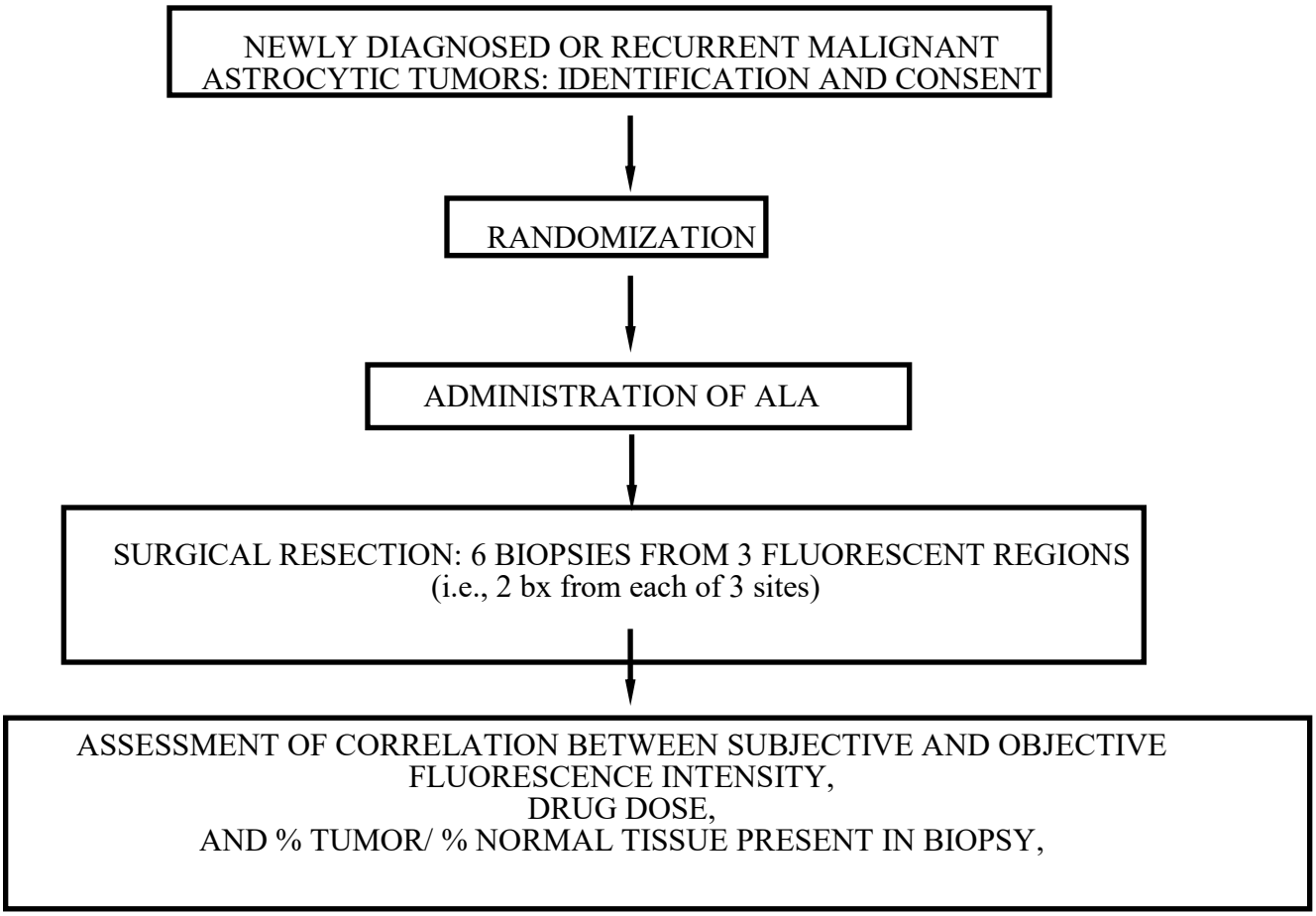
SCHEMA:

Two arm randomized trial of newly diagnosed or recurrent supratentorial malignant astrocytic tumors.



1.0 INTRODUCTION

The treatment of patients with high grade gliomas remains a challenge for modern therapy. The prognosis for these patients is poor; the median survival time after diagnosis and treatment is less than 1 year [1, 2] and may be linked to the completeness of tumor removal. [3-6]. A recent study of 416 patients with glioblastoma multiforme (GBM) indicates that resection of 89% of the tumor volume is necessary to improve survival and a significant survival advantage of 4.2 months was associated with resection of 98% or more of the tumor volume, compared with resection of less than 98% [7]. However, the highest possible degree of tumor resection is often limited by the surgeon's ability to distinguish residual tumor tissue from surrounding brain tissue under conventional white light surgical microscope illumination [8]. For these reasons, methods enabling better intraoperative discrimination of viable tumor borders may be valuable.

Fluorescence imaging may, potentially, enhance the contrast of viable tumor borders. Although administration of fluorescent markers to enhance contrast of malignant gliomas is not new [9, 10, 11], marking tumors with 5-amino levulinic acid (ALA) is conceptually different from other investigations. ALA is not itself fluorescent but is metabolized into the strongly fluorescent proto-porphyrin IX (PpIX) by a number of malignant tumors *in situ* through enzymes of the heme-biosynthesis pathway [12]. This may avoid pitfalls when a fluorescent marker is administered directly, such as leakage from the tumor into surrounding normal brain tissue [13].

ALA-PpIX fluorescence imaging has previously been investigated at St. Michael's Hospital and the University Health Network, Toronto Cancer Institute [Drs. N. Marcon and B. Wilson]. These studies examined the utility of ALA for detection and photodynamic therapy (PDT) of dysplasia in patients with Barrett's disease of the esophagus.

Recently, ALA-PpIX fluorescence imaging was introduced by a German group for intraoperative detection and resection of residual malignant glioma [14, 15]. In the latter study [15], 52 patients were investigated using an operating room-adapted microscope for fluorescence imaging. ALA was administered orally at a dose of 20 mg/kg 3 hours before induction of anesthesia. These workers found that leaving "solidly fluorescing" tissue unresected had a negative influence on survival, whereas the prognosis of patients with residual "vague fluorescence" was not significantly different from that of patients in whom all fluorescent tissue was removed completely. Histologically, solidly fluorescing tissue was mostly characterized by coalescent tumor cells, whereas vaguely fluorescing tissue usually represented infiltrating tumor of intermediate or low cellular density. Analysis of tumor biopsy specimens revealed infiltrating tumor also to be present beyond vaguely fluorescing tissue portions. Recent data suggest that the sensitivity of this dose and administration timing for 5-ALA is 75% while specificity is only 71% [16]. While encouraging, this study may be considered subjective as it involved only one neurosurgeon and was not quantitative in determining the levels of PpIX fluorescence in the tumor and normal tissue. The definitions of "solidly fluorescing…" and "vaguely fluorescining are unclear and imprecise. In addition, all published studies have been performed using the 20 mg/kg 5-ALA dose and 3 hr pre-operative administration.

Unpublished reports suggest 2 mg/kg was not detectable (Stummer personal communication),_but values between 2 and 20 mg/kg from a clinical trial setting have not been published.

Our group is developing point-probe systems for intra-operative quantitative PpIX concentration measurement. A 1st generation instrument has been developed and used in another clinical trial [16, 17].

2.0 OBJECTIVES

2.1 PRIMARY AIM

Our primary *long-term goal* is to improve the completeness of surgical resection of malignant brain tumor through image- guided fluorescence localization. We hypothesize that the use of qualitative fluorescence imaging and point PpIX concentration quantification will enable more complete tumor resection than normal direct (i.e., white light) visualization, and thereby improve patient survival [7].

2.2 PRIMARY OBJECTIVE

Primary objective: As a first step, we will assess 2 doses of 5-ALA, 10mg/kg and 20 mg/kg, to determine the optimum ALA dose in terms of both sensitivity and specificity for residual tumor. We will compare residual tumor detection by both *in vivo* qualitative and quantitative fluorescence imaging using histology of the biopsied tissue as the gold standard.

2.3 SECONDARY OBJECTIVE

Secondary Objective: We will assess the correlatation between the recorded *in vivo* qualitative assessment of fluorescence signal from the neurosurgeon with the post-surgical (i.e., *ex vivo*) absolute PpIX concentration detected both intraoperatively and in *ex vivo* tissue biopsies.

2.4 TERTIARY OBJECTIVE

Tertiary Objective: To determine the association between the presence of fluorescence in the surgical cavity and the post-operative image enhancement on MRI. This includes the relationship between the amount and location of residual tumor detected by fluorescence, PpIX concentration, and intra-operative frameless stereotaxy following maximal resection versus the amount and location of tumor imaged post-operatively via CT and/or MRI.

3.0 ELIGIBILITY CRITERIA

3.1 PATIENT ELIGIBILITY CRITERIA

Patients will be eligible for this study if they meet all of the following criteria:

Table 1: Inclusion Criteria

Age	18 years or older
Tumor Pathology	Suspected or confirmed newly diagnosed or recurrent malignant gliomas WHO grade IV
Location	Supratentorial
Resection	Tumor must be judged suitable for resection on the basis of imaging studies.
Consent	Patients must be able to give written, informed consent as approved by the local IRB
Newly Diagnosed Tumors	Patients with newly diagnosed suspected Grade IV glioma who have had not been previously treated with cranial radiation therapy
Recurrent Tumors	Patients with recurrent Grade IV gliomas who have failed cranial radiation therapy

It is the policy of the Case Comprehensive Cancer Center to strive for gender and minority patient participation that represents the population of Northeast Ohio in all clinical investigations. Both men and women of all races and ethnic groups are eligible for this trial.

Grade IV gliomas will likely have a different PpIX uptake and thus fluoresce differently compared to Grade III gliomas [15] therefore, this study will only focus on Grade IV gliomas. However, the grade of the glioma is not certain until surgical resection of the glioma at which point biopsies are taken for histopathological determination of the glioma grade. As a result some patients with Grade III gliomas may be enrolled in this study, but these patients will be excluded from primary analysis. The data obtained from patients with Grade III gliomas will be used to explore differences in fluorescence between Grade III and Grade IV brain tumors. We do not expect that this will result in a statistically significant difference, but only that generate hypothesis for future studies.

3.2 EXCLUSION CRITERIA

Patients will be excluded from participation in the study if they meet any of the following criteria:

Table 2: Exclusion Criteria

Pregnant women or those who are breast feeding
Individuals with history of cutaneous photosensitivity, porphyria, hypersensitivity to porphyrins, photodermatosis, exfoliative dermatitis
Individuals with history of liver disease in last 12 months
Individuals with AST, ALT, ALP, or bilirubin >2.5x normal upper limit any time during the previous 2 months
Individuals with plasma creatinine>180 μmol/L
Individuals who are unable to comply with photosensitivity precautions
Individuals without a probable or expected grade IV glioma

A baseline blood sample will be drawn for creatinine, AST, ALT, ALP, and bilirubin to verify whether or not this exclusion criterion is met.

4.0 RESEARCH DESIGN

4.1 SUMMARY OF TRIAL DESIGN

Summary of Trial Design: This study will enroll evaluable patients undergoing surgical resection of malignant, grade IV gliomas in both of two groups: those with newly diagnosed GBM and those with recurrent GBM. Patient, in each group (primary vs recurrent GBM) will be randomized to one of 2 levels of ALA dose (10 and20 mg/kg) to be given orally at 3 hours prior to anticipated midpoint of surgery.

4.2 RANDOMIZATION

Patients who have consented to this protocol will be randomly assigned to one of two ALA dose groups (Table 3). Randomization will be stratified by whether the tumor is newly diagnosed (i.e. *de novo)* or recurrent. The data center will prepare sealed, opaque envelopes with the randomized assignment to ALA dose and administration time and notify the pharmacy of the trial site so that so that the correct ALA dose can be prepared.

Neurosurgeons and investigators responsible for determining pathologic characteristics, for quantifying fluorescence images, for extracting chemical PpIX, for co-localizing fluorescence images with MR images will be blinded to the ALA dose and administration time. In case of emergency, such as toxicity or allergic reaction attributable to the drug, the research pharmacist on call will break the blind and inform the PI and the physician responsible for clinical management.

A safety monitoring board has been developed at the Case Comprehensive Cancer Center at Case Western Reserve University. Also, forms will be filled out by the surgeon postoperatively on a daily basis, the patient's surgeon will report any adverse effects. In addition to specific signs and symptoms, liver function tests will be ascertained at 48

hours and, if abnormal, these tests will be continued on a twice-weekly basis until they peak and then at weekly intervals thereafter to resolution. In the case of any adverse event, the patient will be examined by the study physician and appropriate medical action taken.

4.3 DSMB AND DSTC

4.3.1. Data Safety Monitoring Board (DSMB)

The Data Safety Monitoring Board for this study is:

The Standing Data Safety Monitoring Board of the Case Comprehensive Cancer Center, Case Western Reserve University, School of Medicine.

4.3.2. Data Safety Toxicity Committee (DSTC)

Case Comprehensive Cancer Center Data Safety and Toxicity Committee

Anjali Advani, M.D., Chair, DSTC
Paolo Caimi, M.D., Co-Chair, DSTC
Katarzyna Karelus., Administrative Director Case Comprehensive Cancer Center Clinical Research Office

In addition, the Case Comprehensive Cancer Center Data and Safety Toxicity Committee will review all serious adverse events and toxicity reports as well as annual reviews.

4.3.3 Site for surgical resection and fluorescence imaging

Surgical resection and fluorescence imaging will be performed by neurosurgeons at University Hospitals Cleveland Medical Center.

Image processing for quantification of the fluorescence signal and clinical support, training and maintenance for the fluorescence imaging devices will be provided by Dr. Wilson's lab.

In addition, a dedicated person will be assigned that is responsible for on-site clinical support, data/sample management, data/sample transfer to Dr. Wilson's lab, and on-site maintenance and storage of the fluorescent imaging device.

4.3.4 Sites for tissue analysis

Tissue samples will be sent to the study neurohistopathologist for assessment:

Mark Cohen, MD, Neuropathologist Department of Pathology
University Hospitals of Cleveland
Case Western Reserve University
11100 Euclid Avenue
Cleveland, OH 44106
216-844-8928

Frozen tissue samples will be sent to Dr. Wilson's lab for fluorescence microscopy and PpIX extraction.

Brian Wilson, Ph.D.
Div. of Biophysics & Bioimaging
University Health Network
610 University Ave., 7416
Toronto, M52M9 Canada
Wilson@uhmres.utoronto.ca
416 946-2952

4.4 PRE-OPERATIVE PROCEDURES

4.4.1 PATIENT INFORMED CONSENT

Patients will enter this study on a voluntary basis. Enrolled patients receive an information package that includes an informed consent form. This package will inform the patient about the objectives of the study, the procedures that will be used, the potential risks involved, and the procedures that they should undertake to avoid possible photosensitivity reactions. At the time of obtaining consent for surgery, the attending surgeon or their delegate will obtain informed consent for this trial.

4.4.2 LIGHT SENSITIVITY PRECAUTIONS

Before ALA administration, patients will be given verbal and written instructions for protection from sunlight by the staff surgeon or his delegate (see Section 4.7.1). It should be noted that these patients are hospitalized following surgery, so that there is in practice a very small possibility of their being exposed to bright sunlight outdoors. Nursing staff in the recovery room and on the ward will be instructed regarding the need to avoid accidental exposure to bright light in the first 24 hours.

4.4.3 .PATIENT GROUPS AND NUMBERS AND ALA DOSE

This clinical trial has ALA dose at 2 levels (10 and 20 mg/kg) and ALA administration time at 1 time point (3h). Consenting patients scheduled for surgery will be assigned to primary or recurrent arm based on clinical presentation, are then randomly assigned to one of two groups (for dose assignment) within each of the two arms of the study. Sample sizes of the 4 patient groups with two ALA dose levels are listed in the table below:

Table 3: Study Design and Sample Size

	Administration Time	
ALA dose	3 hours New D_x	3 hours Recurrent D_x
10 mg/kg	30	30
20 mg/kg	30	30

For both patients with new and recurrent disease, biopsies will be taken at up to six sites identified by the surgeon: in the tumor center, tumor edge, area surrounding the tumor (if safe), areas seen to fluoresce intraoperatively and an area with MR enhancement outside

the tumor region (if this can be accomplished safely). Prior to collecting these biopsies readings will be taken at the biopsied location with the PpIX point probe by the surgeon. For each of the 6 biopsies, they will be divided into 3 parts and distributed for further analysis as follows: one portion will be sent to the pathologist for assessment of tumor percentage, one portion will be evaluated by the Division of Biophysics at the University of Toronto for PpIX concentration and the other for assessment of fluorescence.

4.4 SURGICAL PROCEDURES

4.4.1 PRECAUTIONS FOR PHOTOSENSITIVITY DURING SURGERY

During surgery, light-opaque drapes will be used to cover all skin, including the area around the surgical site. The patient's eye lids will be closed with tape and covered with goggles that block the appropriate light wavelengths that might damage the retina. The patient will receive instructions on how to prevent exposure to sunlight and bright indoor lighting for a period of 24 hours post surgery (see Appendix I: Section 12).

4.4.2 SURGICAL TREATMENT

During surgery, the intraoperative fluorescence observations and PpIX concentration measurements will be taken by the surgeon and recorded by research personalle. The surgical site will be illuminated with UV/blue light in the wavelength range of 350-490nm with an irradiance of 20-40 $mWcm^{-2}$. A fluorescence image will be taken using a commercially available operating room microscope. A white-light image will also be taken using the same microscope in non-fluorescence mode. Each tissue biopsy will be taken at the discretion of the operating surgeon (the biopsy location on the fluorescence image will be recorded) and divided in 3 parts that will be analyzed by histopathology, fluorometry, and fluorescence microscopy. At each site, the position of the biopsy in the tumor bed will be determined by frameless stereotaxy using an Optical Tracking System (OTS) for correlation with pre- and post-operative CT and/or MRI scans. Biopsies of 50mg or less are taken with biopsy forceps .

4.4.3 INTRA-OPERATIVE MEASUREMENTS

The procedures for performing fluorescence imaging and spectroscopy during and following resection are well established from our prior experience.

4.4.3.1 BULK TUMOR MEASUREMENTS

1. Under white light illumination, the surgeon will identify biopsy sites of tumor and normal tissue with a probe and corresponding white-light and fluorescence images will be taken.
2. A biopsy will be taken of each of the sites identified under white light illumination and under fluorescence imaging.

Additionally, the location of the biopsies will be determined with an Optical Tracking System for post-operative correlation with pre- and post-operative CT and/or MR-scans.

The exact step-by step operating room procedures for these intra-operative measurements are described in Appendix III, Section 12.

4.4.4 SPECIMEN HANDLING

Each biopsy will be divided immediately by the pathologist using a scalpel into 3 parts: 1 will be placed in formalin for standard histopathological assessment. The other 2 parts will be protected from light exposure, snap frozen in liquid nitrogen and will be kept at -80 ºC in a light protected environment until further use, that is analysis by spectrofluorimetry and confocal fluorescence microscopy (see below for procedures).

The intra-operative fluorescence imaging information will not be used in this trial to modify or extend the surgical resection.

4.5 TISSUE AND DATA ANALYSIS

4.5.1 TISSUE ANALYSIS

Each single biopsy, taken for the purposes of this single-center study, is divided in 3 parts to perform 3 different types of analyses. All of these tissue samples will be analyzed according to standard operating procedures for each analysis. . These biopsies will not be used as part of patient care.

Note: In case a biopsy is too small to be divided in three parts, the biopsy will be fixed in formalin and given to Dr. Cohen for histopathological analysis only.

4.5.1.1 1ST PART OF BIOPSY: HISTOPATHOLOGICAL ANALYSIS

The first part of each biopsy will be fixed in formalin and given to Dr. Cohen who will prepare standard Hematoxylin & Eosin (H&E) stained sections. This histopathology will be assessed by a single experienced neuropathologist, Dr. Cohen. Grading and assessment of tumor extent will be determined in the usual fashion.

4.5.1.2 2ND PART OF BIOPSY: PpIX QUANTIFICATION

The second part of each biopsy will be protected from light exposure, immediately snap-frozen in liquid nitrogen, and stored at -80 ºC before being sent to Dr. Wilson's laboratory at the University Health Network Hospital in Toronto. The PpIX concentration of that sample will be determined using a procedure developed by Lilge *et al.* [19] which, briefly, is as follows: After thawing, the tissue will first be finely chopped with a scalpel blade, and suspended by placing it in Solvable™ (Dupont, Boston) at 50 °C in a water bath and shaken for 1 hour, then mechanically homogenized and re-incubated in Solvable for another hour. The fluorescence of aliquots of the suspended sample will then be measured in a scanning spectrofluorimeter (PTI, London, ON, Canada). The PpIX concentration will then be determined by spiking the sample with known concentrations of PpIX. This procedure is currently used as the standard technique in the same

investigator-group's NIH-sponsored, multi-center, Photofrin-mediated PDT brain tumor clinical trial. For 100 mg brain tissue samples, it has a lower detection limit of 0.02μg PpIX per gram of tissue. The anticipated concentration range in tumor for the lowest ALA dose is expected to be 100 times this value.

4.5.1.3 3RD PART OF BIOPSY: (CONFOCAL) FLUORESCENCE MICROSCOPY

The third part of each biopsy will be protected from light exposure and immediately snap-frozen in liquid nitrogen. It will then be light protected and stored at -80 ºC in to Dr. Wilson's lab in Toronto. (Confocal) fluorescence microscopy will be used to determine the microdistribution of PpIX. This technique is also in routine use in Dr. Wilson's laboratory [17]. Briefly, serial 5μm frozen sections of each biopsy will be made. Alternate sections will be stained for standard histological comparison with the H&E sections above. The quality of H&E sections made in this way is not adequate for clinical diagnosis, but does allow direct one-to-one identification of areas of tumor involvement when compared with the confocal fluorescence microscopy of the adjacent sections. The fluorescence images will be obtained using a UV/visible Zeiss (Thornwood, NY) 510 confocal fluorescence microscope equipped with multiple detection wavelength channels. Regions of tumor and normal brain tissue will be identified and the relative fluorescence intensity and heterogeneity of the fluorescence will be quantified.

4.6 POST OPERATIVE ACTIVITIES

4.6.1 POST OPERATIVE BLOOD SAMPLE COLLECTION

Blood will be drawn for liver function tests 48 hours post tumor resection. If abnormal (≥ than Grade 3), weekly liver function tests will be drawn for 2 weeks. They will then be monitored on a monthly basis until they normalize (see Section 4.7.3). Subsequent to study completion, patients will have regular surveillance as per the current standard of care.

4.7 REPORTING ADVERSE EVENTS ASSOCIATED WITH SYSTEMIC ALA

There have been over 19 published reports of systemic administration of ALA for PDT in at least 230 patients [14, 15, 20-40] with ALA doses up to 60 mg/kg. No serious adverse events or reactions have been reported; however, skin photosensitivity, nausea and vomiting, liver function abnormalities and some other events (as outlined below have been temporally related to ALA administration).

4.7.1 SKIN PHOTOSENSITIVITY

Since ALA-induced PpIX accumulation occurs mostly in cells of epithelial origin, accumulation in skin and subsequent cutaneous injury after sun exposure has been reported. Four volunteers, who ingested doses of ALA ranging from 12.5 to 35 mg/kg, developed photosensitization of skin (but no sunburns) that persisted for approximately 24 hours [37], and a severe sunburn occurred 8 hours after a 10 mg/kg load in a volunteer who was exposed to bright light [38]. The reactions reported in clinical PDT trials (30-60

mg/kg) were usually mild, consisted mainly of erythema or a "mild photosensitivity reaction" and resolved within 24 to 48 hours. Blister formation and skin necrosis has not been reported [40]. Ocular sensitivity has not been reported with systemic ALA.

Based on this evidence, the patients in our study will receive written and verbal instructions regarding skin exposure to ambient light (see Appendix I). They will be advised to stay indoors for 1 day (24 hours), cover exposed parts of their bodies, and protect their eyes from direct sun rays, strong fluorescent or incandescent lighting (e.g. a dentist's lamp or examining light) or strong residential direct indoor lighting (e.g. direct sunlight through a window for more than for more than a few minutes, direct spotlights, floodlights, etc.) for this period. After 1 day, they may expose a small area of skin to the sun for 15 minutes to test for residual sun sensitivity. There is no proof that sun screens are of any value.

4.7.2 NAUSEA AND VOMITING

Dose-dependent mild nausea and occasional vomiting have been reported. Nausea was seen during the administration of hourly fractionated doses [21,30,31,34] or immediately after bolus administration [24,29,37] (i.e., in approximately 2/3 of patients given 60 mg/kg and 1/3 of patients given 30 mg/kg). Vomiting occurred about 12 hours later in a few cases and resolved by 24 hours. The patients in this study will be evaluated and treated as indicated by the severity of their symptoms.

4.7.3 LIVER FUNCTION TEST ABNORMALITIES

Dose-dependent abnormalities in liver function tests have also been reported. The most common abnormality was a rise in transaminases, usually AST [24,25,26,29,31,35,37]. In two studies up to 2/3 of patients given 60 mg/kg and 1/5 of those given 30 mg/kg had elevated AST levels [21,30]. This rise was small in most other reports, but in these two studies (possibly describing the same series of patients), 3 patients had levels 3 to 5 times above normal. In all studies but one, the abnormalities resolved 72 hours later: 5 of 18 patients' transaminases normalized in 10 days and one patient with a history of excessive alcohol intake had elevated levels at 30 days [34]. Only nine patients in these studies had transient elevations in bilirubin (less than two times normal). No study correlated the liver function test abnormalities with the occurrence of nausea and vomiting in individual patients. Based on this experience, follow-up in our patients will consist of testing of liver function tests at 48 hours post tumor resection and, if $\geq$ than Grade 3, weekly for 2 weeks and then at monthly intervals thereafter to resolution.

4.7.4 OTHER EVENTS

One patient developed a lichenoid reaction a week after oral ALA (60 mg/kg) which resolved after 3 weeks [34]. No abnormalities have been noted in serum electrolytes or hematologic indices.

4.8 ADVERSE EVENT REPORTING

Patients will be monitored postoperatively for adverse events until 30 days after the study drug administration. Adverse events will be graded using the NCI CTC 3.0 criteria. In the

case of any of the events described above, the patient will be examined by a study physician and appropriate medical action taken. The event will be recorded in the patient's study file with the date of occurrence, date of resolution, severity and the actions taken. Any unexpected or serious adverse events, Grade 3 or above, or drug reaction beyond the scope of what was described above, will be reported on a MedWatch form to the Principal Investigator, the Case Comprehensive Cancer Center Clinical Trial Regulatory Affairs Office, Institutional Review Board, Case Comprehensive Cancer Center Data Safety and Toxicity Committee, Food and Drug Administration, DUSA Pharmaceuticals via Dr. Andrew Sloan.

FDA Reporting

The University Hospitals Cleveland Medical Center Principal Investigator, as holder of the IND, will be responsible for all communication with the FDA. In accordance with 21 CFR 312.32, the University Hospitals Cleveland Medical Center Principal Investigator is responsible for notifying the FDA of SAEs that are serious, unexpected (not listed in the Investigator Brochure or package insert) and judged to be related (i.e., possible, probable, definite) to the study drug. Events meeting the following criteria need to be submitted to the FDA as Expedited IND Safety Reports.

7 Calendar Day IND Safety Report

Any unexpected fatal or life-threatening suspected adverse event represent especially important safety information and, therefore, must be reported more rapidly to FDA (21 CFR 312.32(c)(2)). Any unexpected fatal or life-threatening suspected adverse event must be reported to FDA no later than 7 calendar days after the University Hospitals Cleveland Medical Center/ Principal Investigator initial receipt of the information (21 CFR 312.32(c)(2)). University Hospitals Cleveland Medical Center Principal Investigator will complete a Medwatch Form FDA 3500A and notify the FDA by telephone or facsimile transmission.

15 Calendar Day IND Safety Report

The timeframe for submitting an IND safety report to FDA and all participating investigators is no later than 15 calendar days after the University Hospitals Cleveland Medical Center Principal Investigator determines that the suspected adverse event or other information qualifies for reporting (21 CFR 312.32(c)(1)). This includes any serious, unexpected adverse events considered reasonably or possibly related to the investigational agent. University Hospitals Cleveland Medical Center Principal Investigator will complete a Medwatch Form FDA 3500A and notify the FDA by telephone or facsimile transmission. If FDA requests any additional data or information, the /University Hospitals Cleveland Medical Center Principal Investigator must submit it to FDA as soon as possible, but no later than 15 calendar days after receiving the request (21 CFR 312.32(c)(1)(v)).

Follow-up IND Safety Report

Any relevant additional information that the University Hospitals Cleveland Medical Center Principal Investigator obtains that pertains to a previously submitted IND safety report must be submitted to FDA as a Follow-up IND Safety Report without delay, as soon as the information is available (21 CFR 312.32(d)(2)). The University Hospitals

Cleveland Medical Center Principal Investigator will maintain records of its efforts to obtain additional information.

Reporting Serious Problems to FDA
Medwatch Form FDA 3500A:
http://www.fda.gov/Safety/MedWatch/HowToReport/DownloadForms/default.htm
Telephone: 1-800-332-1088
Fax: 1-800-FDA-0178

5.0 MEASUREMENT OF EFFECT

5.1 ENDPOINTS

Objectives: The endpoints are detection of residual tumor and of normal tissue. The *primary* endpoint will be correspondence between intensity of *in vivo* fluorescence and PpIX concentration and the pathologist's quantification of tumor in biopsy specimens, e.g. percent tumor present. The measures used in the *secondary* objective will be PpIX concentration, μg/mg and intra-operative fluorescence intensity. Measures used in the *third objective* will be the biopsy specimen's fluorescence intensity and the MR image enhancement at the biopsy site's intraoperative stereotactic coordinates as determined by the surgeon.

5.2 DATA ANALYSIS

5.2.1 FLUORESCENCE INTENSITY LEVELS VS. HISTOPATHOLOGY

The data sets will be collated into the 4 study groups (2 ALA doses and 2 study arms--i.e., newly diagnosed and recurrent diagnosis) for analysis purposes.

Histopathology of the 1st part of the biopsies, performed by a single experienced neuropathologist **(i.e., Dr. Mark Cohen)** will be used as the gold standard. The white-light, fluorescence images, and intra-operative PpIX concentration readings will be compared to assess the sensitivity and specificity for biopsy-confirmed visible tumor.

5.2.2 Intra-operative vs. *Ex Vivo* PpIX EXTRACTION ASSAY

The intra-operatively determined PpIX concentration will be compared to the intra-operative imaging, ex vivo PpIX concentration, and MR enhancement images. This analysis will facilitate an assessment of the utility of lower ALA doses for enhancing subjective and/or quantitative contrast of tumor versus background fluorescence and/or PpIX concentration.

5.2.3 CONFOCAL FLUORESCENCE MICROSCOPY VS. HISTOPATHOLOGY

The confocal fluorescence microscopy images and adjacent H&E sections will be compared to determine the relationship between microscopic localization of PpIX fluorescence, concentration, and tumor cells.

6.0 STATISTICAL PARAMETERS

6.1 PATIENT DATA SET

For each patient the following data will be captured for analysis (collection timing in parens):

1. Patient demographic information (at enrollment).
2. Tumor/normal tissue classification for each biopsy by the neurosurgeon (intra-operatively).
3. Histology report on 1st part of biopsies (one to several weeks post-operatively).
4. PpIX extraction assay 2nd part of biopsies (one to several months post-operatively).
5. Confocal microscopy fluorescence images on 3rd part of biopsies (one to several months post-operatively).
6. White light images of biopsy sites (i.e. of the tissue area around the biopsy location; intra-operatively).
7. Fluorescence images of biopsy sites over an area sufficient to clearly demarcate the region of interest (intra-operatively).
8. PpIX concentration at biopsy site.
9. Frameless stereotaxy coordinates of biopsies (intra-operatively).
10. Magnetic Resonance appearance of biopsy at coordinates taken in #8 (one to several weeks post-operatively).
11. Magnetic Resonance image volumes (i.e., pre-, intra-, and post-operative; one to several weeks post-operatively).

7.0 DRUG FORMULATION AND PROCUREMENT

7.1 ALA FORMULATION AND TOXICITIES

7.1.1 ALA Formula

The chemical formula for ALA is:

$$NH_2CH_2COCH_2CH_2COOH \cdot HCl$$

Three common synonyms also used to refer to ALA are:

δ-Aminolevulinic acid Hydrochloride
5-Amino-4-oxopentanoic acid Hydrochloride

5-Aminolaevulinic acid Hydrochloride

7.1.2 ALA PREPARATION

ALA (5-amino levulinic acid) is provided in powder form. Patients receive ALA orally, dissolved in orange juice or sterile water (< 60 ml) [14,15,19-40]. ALA is stored in powder form by the investigational pharmacist. Before use the ALA is weighed by the pharmacist and the surgeon or his delegate. The ALA is then dissolved in orange juice or sterile water maximally 15 min prior to ingestion by the patient.

7.2 ALA SUPPLY

ALA is provided by:

DUSA Pharmaceuticals, Inc.
25 Upton Drive
Wilmington, MA 01887
Telephone: (978) 657-7500

7.3 ALA STORAGE

ALA is provided in powder form in 10 g vials.

7.4 ALA PROCRUREMENT

Drug is supplied *gratis* by DUSA Pharmaceuticals, Inc. to our investigational pharmacy.

8.0 STATISTICAL CONSIDERATIONS

8.1 STATISTICAL ANALYSIS PLAN

Primary objective: We will assess 2 doses of 5-ALA, 10mg/kg and 20mg/kg, to determine the optimum ALA dose in terms of both sensitivity and specificity for residual tumor. The endpoint will be correspondence between intensity of *in vivo* fluorescence and PpIX concentration and the pathologist's quantification of tumor in biopsy specimens, e.g. percent tumor present. Receiver operating characteristic (ROC) curves will be calculated for each of the 2 combinations of ALA dose to estimate a level of fluorescence that distinguishes residual tumor from normal tissue with large true positive fraction (TPF) and small false positive fraction (FPF). A joint 95% confidence region for TPF FPF will be calculated, using $\alpha^*=1-\sqrt{1-\alpha} = 0.975$ and univariate confidence limits for the individual probabilities. Having chosen the cut-points for each of the 4 combinations, positive and negative predictive values and diagnostic likelihood ratios will be calculated to describe the characteristics of each [18].

To compare the test characteristics of each ALA dose and to assess how the test characteristics are affected by demographic, disease, and surgical characteristics we will

fit multivariable generalized mixed models to tumor and normal biopsy specimens using robust standard errors to account for the correlation of samples taken from the same individual.

Secondary Objective: We will assess the correlation between the recorded *in vivo* qualitative assessment of flouresence signal from the neurosurgeon with the post-surgical (i.e., *ex vivo*) absolute PpIX concentration detected in *ex vivo* tissue biopsies. The surgeon will subjectively score the intra-operatively observed fluorescence level as follows: 0, no fluorescence, 1, low fluorescence, 2, moderate fluorescence, or 3, high fluorescence [after 16]. We will first graph fluorescence against each PpIX concentration measurement (intraoperative and *ex vivo*) to evaluate the shape of the relationship. Additionally we will calculate a Spearman's correlation coefficient with 95% confidence interval to assess the correlation between fluorescence against each PpIX concentration measurement (intraoperative and *ex vivo*). The sensitivity and specificity of these observations will be determined [after 16], where the sensitivity of observable intraoperative PpIX fluorescence is the probability of observable intraoperative fluorescence given the presence of tumor cells in the tissue and specificity is the probability of no observable intraoperative fluorescence given that there are no tumor cells in the tissue [16]. We will also explore whether dose, patient, and disease characteristics affect the strength of the relationship between these measurements using multivariable generalized mixed models.

Tertiary Objective: To determine the association between the presence of fluorescence in the surgical cavity and the post-operative image enhancement on MRI. This includes the relationship between the amount and location of residual tumor detected by fluorescence, PpIX concentration, and intra-operative frameless stereotaxy following maximal resection versus the amount and location of tumor imaged post-operatively via CT and/or MRI. Assessment of agreement between fluorescence, intra-operative PpIX concentration, and MR intensity will be explored using the radiologist's assessment of MR intensity, measured on an ordinal scale. A multivariable ordinal logistic model will be fitted to assess the strength of the relationship and whether dose, patient or disease characteristics affect the relationship.

All models for all objectives will be fitted with robust standard errors to account for correlation between specimens taken from the same individual. Statistical models will be examined for goodness of fit; residual and influence statistics will be computed to ensure that outlying values do not have a disproportionate effect on the final models.

8.2 SAMPLE SIZE AND STATISTICAL POWER

Our study is powered appropriately for determination of the primary objective which involves the assessment of 2 doses of 5-ALA, 10mg/kg and 20mg/kg, to determine the optimum ALA dose in terms of both sensitivity and specificity for residual tumor. We calculate the total sample size for 80% power at the 0.05 significance level, given that the sensitivity will decrease by 15% with decreasing ALA dose within patient group (i.e. newly diagnosed or recurrent). Therefore our null hypothesis is that the sensitivity of the 10mg/kg dose is less than or equal to 15% different from the 20 mg/kg dose and the

alternative hypothesis is that the sensitivity of the 10mg/kg dose is greater than 15% different from the 20 mg/kg dose. We also take into account having multiple biopsies per individual on study. The total sample size per group per dose is as follows:

The primary objective of this Phase II study is to estimate and compare the combined diagnostic accuracy of in-vivo qualitative assessment of fluorescent signal in detecting residual tumor when used by the neurosurgeon using 2 doses of 5-ALA: 10 mg/kg and 20 mg/kg. The in-vivo qualitative assessment by the neurosurgeon will be coded on an ordinal scale ranging from 0 (no signal) to 3 (highest signal), as was done in a similar study reported by Roberts et al (J Neurosurg 2010 Apr 9, epub ahead of print) using a dose of 20 mg/kg in newly diagnosed gliomas. As in Roberts et al, we assume a score of 1 or higher will be considered positive. In the study by Roberts et al (2010) the reported sensitivity and specificity were 0.75 and 0.71, respectively, using the histopathologic reading as gold standard. In this study, because having high sensitivity and specificity are both important in terms of avoiding false negatives (which would involve not removing residual tissue) and false positives (removing normal tissue), the Youden index, defined as Sensitivity + Specificity -1 will be used as an overall measure of diagnostic accuracy. This study is designed so that if the dose levels of 10 and 20 mg/kg have true Youden indices that differ by 0.10 or more, then the probability is 80% or higher that the observed Youden index will be higher for the group with the higher actual index, i.e., the probability of selecting the correct dose level based on observed Youden indices will be 80% or higher.

For each patient, samples will be removed from six sites (2 center of tumor, 2 edge of tumor, and 2 adjacent normal tissue). It is assumed that on average, three samples will be tumor positive based on pathology, and three will be tumor negative. Thus, on average, each subject will contribute 3 true positive samples (used in estimating sensitivity), and 3 true negatives (used in estimating specificity). If n subjects per stratum (new or recurrent) are studied at a given dose, and π and θ are the sensitivity and specificity, we assume that the estimated sensitivity and specificity obtained as the proportions of true positives and true negatives are approximately normally distributed with means π and θ and respective variances $\pi(1-\pi)(1+2\rho)/3n$, and $\theta(1-\theta)(1+2\rho)/3n$, where ρ is the within-subject correlation of binary responses obtained for tissues on the same subject. This formula can be derived for example assuming a beta-binomial model to model extra-binomial variability. Here the term $(1+2\rho)$ is the design effect where the coefficient of ρ is m-1, where m is the number of true positive or true negative samples per subject. Given specified values of sensitivity and specificity for the two doses reflecting a scenario where Youden indices differ between doses, the probability that the better dose has the higher observed Youden index can be determined, and the sample size n determined such that this probability is high.

Table 1 below presents required numbers of patients per group in order to have 80% probability of correctly identifying the optimal dose, when the Youden indices of the two doses differ by 0.10, using a range of plausible values for sensitivity and specificity, for intrasubject correlations of ρ=0.1 and 0.2**. Based on these results it can be seen that with sample sizes of 30 glioblastoma multiformes (GBMs) there is a 80% or higher**

chance of correctly identifying the optimal dose when Youden indices differ by 0.10 or more. Assuming sample size n=30 and intrasubject correlation ρ=0.2, the 95% confidence intervals for π=0.75, θ=0.70, and youden index=0.45 are (0.64, 0.86), (0.59, 0.81), and (0.23, 0.67), respectively.

Table 1. Required sample size per group to correctly identify the optimal dose with probability of 80%, when Youden indices differ by 0.10 between doses, for various combinations of sensitivity and specificity and intrac-subject correlation, ρ.

Dose=20 mg/kg			Dose=10 mg/kg			ΔYouden	Required # subjects per group	
Sens	Spec	Youden	Sens	Spec	Youden	(20 – 10)	ρ=0.10	ρ=0.20
0.75	0.70	0.45	0.60	0.75	0.35	0.1	23	27
0.75	0.70	0.45	0.65	0.70	0.35	0.1	24	28
0.70	0.70	0.40	0.60	0.70	0.30	0.1	24	28
0.70	0.70	0.40	0.55	0.75	0.30	0.1	24	28
0.65	0.70	0.35	0.55	0.70	0.25	0.1	25	30
0.65	0.70	0.35	0.50	0.75	0.25	0.1	25	29
0.75	0.70	0.45	0.70	0.85	0.55	-0.1	21	24
0.75	0.70	0.45	0.75	0.80	0.55	-0.1	21	25
0.70	0.70	0.40	0.70	0.80	0.50	-0.1	22	26
0.70	0.70	0.40	0.65	0.85	0.50	-0.1	22	26
0.65	0.75	0.40	0.60	0.90	0.50	-0.1	21	25
0.65	0.70	0.35	0.60	0.85	0.45	-0.1	22	26

However, based on imaging, it is difficult to distinguish Grade IV malignant gliomas (GBMs) from Grade III tumors, otherwise known as anaplastic astrocytomas (AAs) in newly diagnosed patients. Traditionally, AA's account for 10-15% of all malignant gliomas with a rate of 11.1% reported for the US in 2010 (41). Assuming that 15% of all patients with newly diagnosed malignant gliomas have AA rather than GBM, we will need to accrue 35 patients to accrue 30 patients with newly diagnosed GBM. In the case of patients with recurrent malignant gliomas, all patients presenting with histological evidence of glioma who cary a diagnosis of GBM from an earlier surgery are considered to have "recurrent GBM" by convention. Patients carrying a diagnosis of AA who have recurrent have degenerated to GBM approximately half the time. Accordingly, of 33 patients with recurrent malignant glioma, 29 would be expected to have recurrent GBM, while 5 would be expected to have recurrent tumor arising from AA. Of the 5 with AA, two or three would be expected to have progressed to GBM.

Thus, in order to accrue 30 patients with newly diagnosed GBM, we will need to accrue 35 patients with newly diagnosed malignant glioma. In order to accrue 30 patients with recurrent GBM, we will need to accrue 33 patients with recurrent malignant glioma.

There are no planned interim analyses for efficacy because this is not a therapeutic trial. Monitoring of safety and trial progress will be the responsibility of the Data and Safety Toxicity Committee.

9.0 PLAN FOR INCLUSION OF VULNERABLE POPULATIONS

9.1 Plan for Inclusion of Illiterate Individuals:

We may potentially enroll illiterate individuals to this trial. During the consent process, the consent form will be read to the patient by the study coordinator or consenting physician. This consent process will be witnessed by a third party with no interest in this trial who will sign the consent form as a witness.

9.2 Plan for Inclusion of Non-English Speaking Individuals:

We may potentially enroll non-English speaking individuals to this trial. If a short form consent document is available in a particular patient's language we will use the short form consent document to consent the patient. This short form will only be used once. When using the short form consent document, a translator will be present during the discussion (or UHCMC language line will be used). We will not use family members as translators for medical research. The translator will verbally review the full length English informed consent form with the subject and will discuss all parameters of participation. If the subject agrees to participate, the study staff (person obtaining consent), the translator, and the PI will sign the English consent. The subject will sign the short form only. The documents will be kept together in the subject's research file.

For any subsequent non-English speaking patients, we will translate the consent form and obtain IRB approval prior to the consent process. Once the translated consent form has been reviewed and approved by the IRB, we will only then consent the patient with the consent form written in the patient's native language.

9.3 Plan for Inclusion of Pregnant Women and Fetuses:

Pregnant women are excluded from participation in this trial. See exclusion criteria in Section 3.2. If a woman becomes pregnant while participating in this trial, all study treatment will be discontinued. We will continue to follow the patient as outlined in the protocol. Research personnel will not offer the subject any type of inducement (monetary or otherwise) to terminate the pregnancy nor will the research personnel have any part in any decisions regarding timing, method, procedures used to terminate the pregnancy, or any part in determining viability of a neonate.

10.0 RECORDS TO BE KEPT

Patients will be identified by site and study number. Demographic information corresponding to study numbers will be kept in a locked office in the research area of the clinical trial site. However, health records may be given to and inspected by the Case Western Reserve University Institutional Review Board for purposes of monitoring the study. All records will be kept for 3 years after the completion of the trial.

All intraoperative procedures will be carried at University Hospitals of Cleveland (Cleveland, OH). The tissue biopsies required for histopathology will be given to Dr. Dr. Mark Cohen. Tissue biopsies for spectrofluorimetry and confocal fluorescence microscopy will be transported to Dr. Wilson's laboratory at the Ontario Cancer Institute/Princess Margaret Hospital. No identifying information will be transferred; the biopsies will be labeled with the patient study number and the image number. These biopsies are not required for patient care purposes. The tissues will be handled according to institutional safety protocols. These are the same procedures that have been used for our investigator group's preceding glioma Photofrin-PDT trial.

The study will commence only after approval by the University Hospitals of Cleveland IRB.

10.1 Electronic Medical Records

This study will access electronic medical records systems to obtain medical information for the subjects enrolled to this study. In order to insure patient safety, investigators and study personnel must have up-to-the-minute health information for subjects enrolled to this study. Therefore, electronic medical records must be utilized to obtain medical information in a timely manner. The following electronic systems will be used: IDX program to access scheduling information; UH Physician Portal to access lab results and physician notes; UHHS Information Network (mainframe) to access lab results and physician notes; PACS to access radiological imagining results; and MySecureCare (Sunrise Clinical Manager) to access some or all of the above information when this application is fully functional. This information will be obtained by the PI, co-investigators, study coordinator, and/or data manager for this study via password-protected login. Access to these systems is required for the life of this research study. Information obtained from electronic systems will be copied (scheduling information) into the Ireland Cancer Center Clinical Trials Unit research chart and/or printed (lab results, physician notes, etc.) and stored in the research chart. Research charts are kept secure and destroyed according to UH policy. All study personnel involved in this research will adhere to the UH policies regarding confidentiality and Protected Health Information. Some study personnel may access the above electronic systems for research purposes only.

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41. http://www.cbtrus.org/2009EighteenStates/Table5.pdf

APPENDICES

12.0 APPENDIX I: SKIN PHOTOSENSITIVITY DUE TO ALA

Patient Information and Instructions

The drug you receive during this study is called ALA (which stands for Aminolevulinic Acid). It collects in all tissues, including the skin. The amount of ALA that will be used is much less than the usual amount given when ALA is used for treatments. However, after receiving the ALA, we recommend the following precautions for 24 hours:

1. No direct sun exposure.

During this time, exposure to sunlight may cause a severe sunburn. This can be prevented by protection from sunlight, either by using thick clothing or by not going outside. Sun protection creams of any strength are not effective. Sunglasses, facemasks, gloves, long sleeves and pants are required during this time if you are out in the sun. Direct sunlight through a window should also be avoided. Brief times (one minute or less) in weak sunlight are safe and may help desensitize you to sunlight.

2. No exposure to strong indoor lights.

During this time, exposure to strong indoor lighting may also cause a severe burn. Examples of such lighting are: tanning booths, a dentist's lamp, a doctor's examining lamp, an operating room lamp, a direct spotlight or floodlight, and strong bathroom vanity lights. Ordinary room lights and reading lamps with shades are safe and will also help to desensitize your skin. You do not need to stay in a completely dark room or to cover your skin and eyes while indoors. In the operating room, appropriate precautions will be taken to avoid strong light exposure.

At the end of the 24-hour period, we recommend that you expose a small area of skin (like the back of your hand) to sunlight for five minutes. If there is no sunburn by the next day, you probably have no more ALA left in your skin. You may then return to normal light exposure.

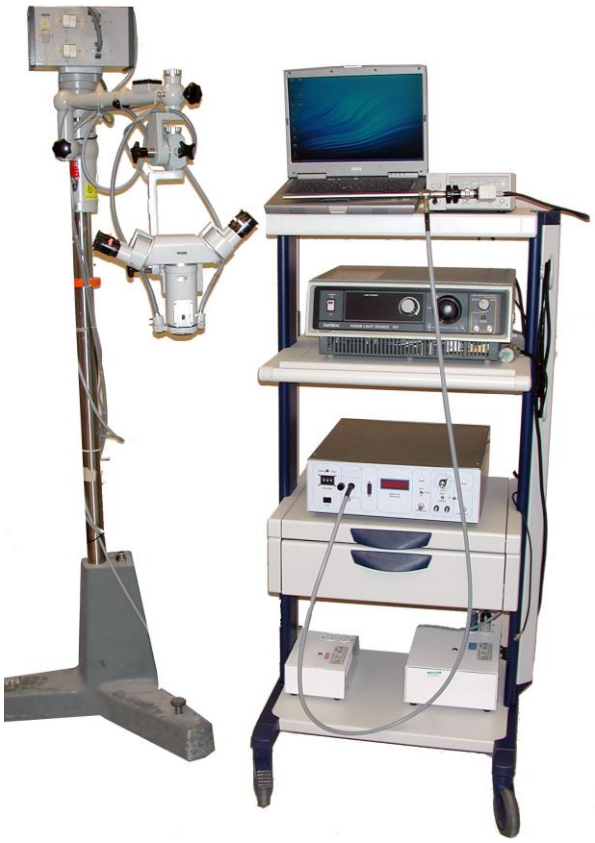
If at any time a prickly or burning feeling or a skin reddening is experienced after exposure to the bright light, please call us at the numbers below and remain in dim light until symptoms have disappeared.

If you experience any other unusual reaction please call the numbers below.

Contact numbers:

Dr. Andrew Sloan phone no: (216) 844-6054
Christopher Murphy, RN phone no: 216-983-3021
resident on call (for night-time and weekend emergencies) phone no: (216) 844-1000, ask for pager number: 30153

APPENDIX II: DESCRIPTION OF THE FLUORESCENCE IMAGING SYSTEM



This fluorescence imaging system is specifically designed for surgical procedures. It consists of a compact surgical camera that has 720x480 pixels resolution and displays 30 frames/sec. It is multispectral. i.e. it can simultaneously detect 3 wavelength bands simultaneously (400-500nm, 500-600nm, and 600-700nm). This system is hand-held and a standard laparoscope is used as entrance optics. The excitation light source is a 300W xenon lamp, similar to lamps used for endoscopy, which has a spinning filter wheel so that is 2 different wavelength bands are used for excitation (440nm, 80nm FWHM (150mW) and 470nm, 60nm FWHM (240mW)). When the illuminated area is 6 cm^2 the maximum irradiance is 40mW.cm^{-2}. Quantitation of the fluorescence

image is achieved by ratiometric image processing algorithms on the different images obtained by multi-excitation and multi detection. Additionally, the images can be quantified by use of a sterile fluorescence standard (i.e. a fluorescent crystal attached to a fiber optic cable) with known fluorescence that will be placed in the surgical cavity to serve as a reference. The computer and software allow to display and store the fluorescence images as well as more quantitative ratio images in real time. In addition, these images can be displayed on the monitors available in the OR for surgical guidance.

The system is has electrical approvals by Canadian Standards Association (CSA) and Underwriter Laboratories Inc. (UL) and is currently being used in a clinical trial for fluorescence guided detection of residual tumor after prostatectomy using ALA-PpIX at University Health Network, Toronto (Dr. J. Trachtenberg and Dr. B.C. Wilson).

This system will be used in this clinical trial.

APPENDIX III: STUDY FLOW CHART

This flow chart details the fluorescence imaging/biopsy procedures.
(WLI: white light imaging; FLI: fluorescence imaging)