The Role of 5-Aminolevulinic Acid Fluorescence-Guided Surgery in Head and Neck Cancers: a Pilot Trial
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Study Drug: 5-Aminolevulinic Acid, Gleolan®

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Revision History:

Version		
Number	**Date**	**Summary of Revisions Made:**
0.0	11/12/2018	Initial version
1.0	11/19/2019	BDW Approved
2.0	8/20/2021	Modified Co-Investigators Updated Data Safety Monitoring Team
3.0	9.7.2021	Data retention period modified
4.0	12.13.2023	Extend study completion date Expand the protocol to include all upper aerodigestive head and neck cancers in addition to squamous cell carcinoma

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Signature Page

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable U.S. federal regulations and ICH guidelines.

Principal Invesigator (PI) Name: **Alfred-Marc Iloreta, MD**

PI Signature:

Alfred-Marc Iloreta

Date:

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LIST OF ABBREVIATIONS

5-ALA	5-Aminolevulinic Acid
AE	Adverse Event
ALT	Alanine Aminotransferase
ALC	Absolute Lymphocyte Count
AST	Aspartate Aminotransferase
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CMP	Comprehensive Metabolic Panel
CR	Complete Response
CT	Computed Tomography
CTCAE	Common Terminology Criteria for Adverse Events
DLT	Dose Limiting Toxicity
DSMB	Data and Safety Monitoring Board
ECOG	Eastern Cooperative Oncology Group
FGS	Fluorescence-Guided Surgery
H&P	History & Physical Exam
HRPP	Human Research Protections Program
IV (or iv)	Intravenously
MRI	Magnetic Resonance Imaging
MTD	Maximum Tolerated Dose
NCI	National Cancer Institute
ORR	Overall Response Rate
OS	Overall Survival
p.o.	per os/by mouth/orally
PPV	Positive Predictive Value
PR	Partial Response
SAE	Serious Adverse Event
SD	Stable Disease
SGOT	Serum Glutamic Oxaloacetic Transaminase
SPGT	Serum Glutamic Pyruvic Transaminase
WBC	White Blood Cells

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STUDY SCHEMA

STUDY SUMMARY

Title	**The Role of 5-Aminolevulinic Acid Fluorescence-Guided Surgery in Head and Neck Cancers: a Pilot Trial**
Short Title	5-ALA FGS Pilot Trial for Head and Neck Cancer
Protocol Number	The standard protocol number used to identify this study- N/A
Methodology	Open label single institution pilot study
Study Duration	$2^{1}/_{2}$ years
Study Center(s)	Single center
Objectives	The objectives of this study are to determine the feasibility of using 5-ALA in fluorescence-guided surgery of head and neck and skull base malignancies.
Number of Subjects	23
Diagnosis and Main Inclusion Criteria	• Resectable head and neck or skull base malignancy • Age 18-80 • Karnofsky score >60%, adequate marrow and organ function • Not pregnant • Able to give consent
Study Product(s), Dose, Route, Regimen	Single dose administration of oral Gleolan® (5-ALA) (20mg/kg) 3-5h prior to surgery
Duration of administration	Day of surgery
Reference therapy	N/A
Statistical Methodology	Assuming a prevalence of 75% and a sample sensitivity of 85%, the

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	sample size needed to estimate a two-sided 95% sensitivity confidence interval with a width of at most 35% (± 17.5% precision assuming asymptotic with continuity correction method) is at least 23). The sample size calculation is performed conservatively assuming only 1 measure per patient, but there will be typically 4 per patient which will improve precision of estimates. We expect to enroll the necessary patients in 6 months at a rate of approximately 4 patients per month. This accrual rate is based on historical monthly rates of surgery in this patient population at the Mount Sinai hospital. **Analysis of the primary endpoint** To assess the performance of 5-ALA as a feasible method to detect tumor tissues, the point estimate of sensitivity will be computed according to the following definition: **Sensitivity**: proportion of tumor tissues that tested positive on 5-ALA induced fluorescence. The definition of a positive test is the fluorescence score equal to or greater than 1. For the assessment of sensitivity and specificity, histological diagnoses of any upper aerodigestive head and neck cancer in addition to squamous cell carcinoma, carcinoma in situ, and severe and moderate dysplasia were classified as malignant, whereas light dysplasia and normal tissue will be classified as benign by pathologists. Each patient is expected to contribute multiple samples and the pathologists will determine presence of malignancy in each sample. A contiguous table (fluorescence scores vs. pathological results) that presents the frequency and percentage of each cell will be prepared. A generalized estimating equation (GEE) model accounting for repeated measure within each patient will be used to estimate the sensitivity, specificity, PPV, and NPV. For sensitivity and specificity, the dependent variable is the PPIX fluorescence test result (positive vs. negative) and the independent variable is presence of tumor cells (tumor vs. normal). **Analysis of the secondary endpoints** For specificity, the point estimate will be computed according to the following definition: **Specificity**: proportion of normal tissues that tested negative on 5-ALA induced fluorescence. The definition of a negative test is a fluorescence score of 0. For sensitivity and specificity, the dependent variable is the PPIX fluorescence test result (positive vs. negative) and the independent variable is presence of tumor cells (tumor vs. normal). For PPV and NPV, the dependent variable is presence of tumor and the independent variable is the test result. The point estimate along with a corresponding two-sided 95% confidence interval will be provided using PROC GENMOD. The 5-ALA related adverse events will be recorded using standardized toxicity criteria and known side effect profile. Frequency of each observed adverse event will be calculated at each monitor time point. The rate of each adverse effect will be estimated as the number of adverse events within 6 weeks after surgery divided by the number of person days followed. The point estimate of the incidence rate along with a corresponding two-sided 95% confidence interval will be provided using PROC GENMOD. A generalized estimating equation (GEE) model accounting for repeated measure within each patient will be used to estimate the sensitivity, specificity, PPV, and NPV.

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1.0 BACKGROUND AND RATIONALE

1.1 Background

5-ALA has been used in the treatment of malignant gliomas in neurosurgical interventions. FGS permits the intraoperative visualization of malignant tissue in real time guidance for differentiating tumor from normal brain tissue that is independent of neuronavigation. Tissue fluorescence after oral administration of 5-ALA has high sensitivity, specificity, and positive predictive values for identifying malignant glioma tumor tissue. 5-ALA-induced tumor fluorescence in diffusely infiltrating gliomas with non-significant magnetic resonance imaging contrast-enhancement permits intraoperative identification of anaplastic foci and establishment of an accurate histopathological diagnosis for proper adjuvant treatment. We conducted an extensive literature review and noted no current applications to sinonasal skull base tumors or head and neck tumors.

5-ALA is a hemoglobin metabolic pathway metabolite that can be orally administered. It accumulates within tumor tissue and surrounding infiltrative cancer cells and is metabolized into protoporphyrin IX (PpIX), a fluorescent metabolite that that can be visualized after activation with 405nm blue light. As factors associated with 5-ALA fluorescence include cellular density, tumor cell proliferative activity, and neovascularization, elevated PpIX production and tissue fluorescence is a highly sensitive, specific, and positive predictor marker for malignant tumor tissue.[3] While other fluorescent markers such as fluorescein sodium have been demonstrated to be efficacious for FGS as well, one study found 5-ALA to be better for detecting tumor cells,[4] and another found it more cost-effective.[5] Therefore 5-ALA was chosen as the target of this investigation.

The utility of intraoperative 5-ALA has been demonstrated through its development for neurosurgical resection of glioma,[5-14] glioblastoma multiforme,[15,16] medulloblastoma,[17] meningioma and other intracranial tumors,[12] as well as spinal tumors.[18] It has been shown to be safe for intraoperative use,[19] to increase gross total resection rates,[5] and to yield high positive predictive value for tumor tissue.[20] Similarly, 5-ALA has been shown to be more sensitive than contrast MRI or PET scan in glioblastoma resection,[20,21] and to yield a synergistic effect in improving glioma resection outcomes when combined with intraoperative MRI.[22]

In recent years, many surgical subspecialties have increasingly recognized the utility of 5-ALA at accurately visualizing precancerous and cancerous tissue. Its use has expanded to photodynamic diagnosis and phototherapy of urothelial,[23] gastric,[24] prostate,[25] and ovarian cancers.[26] In particular, 5-ALA has also shown utility in identifying oral cancers,[27] and in endoscopic identification of laryngeal malignancies in conjunction with autofluorescence bronchoscopy.[28-32] Similarly, trials with autofluorescence have successfully demonstrated the effectiveness of fluorescence-based technologies at improving head and neck cancer detection and increasing successful surgical margins of oral squamous cell carcinoma excision.[33,34] While 5-ALA FGS and other fluorescence-based techniques appear promising for potential use in head and neck malignancies,[35] no clinical trials have yet assessed intraoperative use of 5-ALA for improving resection of head and neck cancers and skull base tumors.

1.2 Study Agent(s) Background and Associated Known Toxicities

1.2 5-Aminolevulinic Acid

5-ALA is a naturally occurring substance that is found in all organisms, including humans, and is a necessary part of many basic metabolic life processes.

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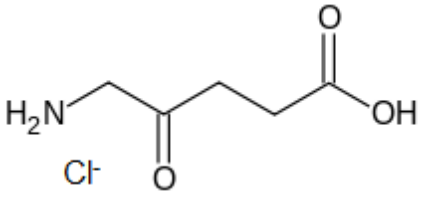
5-Aminolevulinic Acid HCl

$C_5H_9NO_3$ MW 167.6



Approximately 350 mg of 5-ALA is synthesized in humans each day for heme production. Administration of exogenous 5-ALA, results in the production of high intracellular (mitochondrial) concentrations of (PPIX). 5-ALA and PPIX (and other porphyrins) are respectively excreted in the urine and the stool.

1.2.1 Pharmacology

Aminolevulinic acid (ALA) is the first step in the biochemical synthesis of hemoglobin in animals. Aminolevulinic acid is a metabolic precursor of protoporphyrin IX (PPIX), which is a photosensitizer. The metabolism of 5-ALA is normally tightly controlled by feedback inhibition reflecting likely the intracellular levels of hemoglobin. The feedback control is through the enzyme, 5-ALA synthetase. Exogenous oral 5-ALA, bypasses this feedback inhibition and PPIX accumulates to be converted into heme. Excess 5-ALA and PPIX are thus excreted.

1.2.2 Pharmacokinetics

General characteristics

5-ALA is soluble in water and well absorbed through the GI tract. After ingestion, 5-ALA dosing at 20mg/kg, is metabolized to fluorescent porphyrins, predominantly protoporphyrin IX (PPIX).

Absorption

5-ALA HCl as an oral solution is rapidly and completely absorbed and peak plasma levels of 5-ALA are reached 0.5-2 hours after oral administration of 20 mg/kg body weight. With a terminal half-life of 45 minutes, plasma levels return to baseline values 24 hours after administration of an oral dose of 20 mg/kg/ body weight. 5-ALA is generally given on an empty stomach prior to induction of anesthesia.

Distribution and Biotransformation

Oral 5-ALA is metabolized by the liver, kidney, endothelial cells, skin, as well as by malignant gliomas (WHO grade III and IV) and metabolized to fluorescent PPIX. The plasma protein binding of 5-ALA is 12%. Four hours after oral administration of 20 mg/kg/body weight 5-ALA HCl, the maximum PPIX plasma level is reached. PPIX plasma levels rapidly decline and are not detectable 48 hours after administration. In a human pharmacokinetic study (N= 6) using a 128 mg dose of sterile intravenous 5-ALA HCl and oral 5-ALA HCl (equivalent to 100 mg 5-ALA) in which plasma 5-ALA and PPIX were measured, the mean half-life of 5-ALA was 0.70 ± 0.18 h after the oral dose and 0.83 ± 0.05 h after the intravenous dose. The oral bioavailability of 5- ALA was 50-60% with a mean Cmax of 4.65 ± 0.94 µg/mL. PPIX concentrations were low and were detectable only in 42% of the plasma samples. PPIX concentrations in plasma were quite low relative to 5-ALA plasma concentrations, and were below the level of detection (10 ng/mL) after 10 to 12 hours.

At the oral 5-ALA dose of 20 mg/kg/body weight, tumor:normal brain fluorescence ratios are usually high and provide for ‘blue light’ visualization of fluorescent tumor tissue as red-violet for at least 9 hours. Besides tumor tissue, faint fluorescence of the choroid plexus has been reported. 5-ALA is also taken up and metabolized to PPIX by other tissues, e.g. liver, kidneys or skin (see section 4.4).

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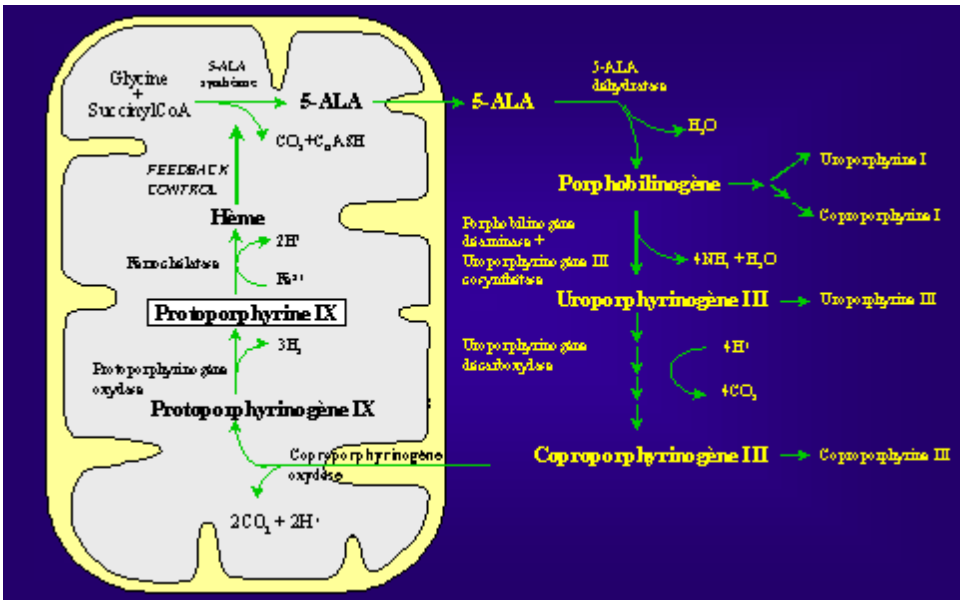
Elimination
5-ALA is eliminated quickly. Approximately 30% of an orally administered dose of 20 mg/kg/body weight is excreted unchanged in urine within 12 hours.

Linearity/non-linearity
There is dose proportionality between AUC0-inf. of 5-ALA values and different oral doses of this medicinal product. The oral dose (20mg/kg body weight) provides an AUC (mg x h/L) of 26.91 (mean) and 27.14 (median).

Patients with renal or hepatic impairment
The pharmacokinetics of 5-ALA in patients with renal or liver impairment has not been investigated. Renal toxicity of the material has been described in association with acute intermittent porphyria and lead exposure.



1.2.3 Toxicity

In vivo toxicological effects associated with elevated levels of 5-ALA, PPIX, and intermediates of porphyrin biosynthesis are well known. These characterize metabolic diseases known as porphyrias. High systemic concentrations of 5-ALA are associated with neurological abnormalities in Acute Intermittent Porphyria (AIP). The relationship is not well understood. 5- ALA and Porphobilinogen (PBG = condensation of 2 molecules of 5-ALA) are not neurotoxic when administered in large amounts to animals and humans. In porphyric patients, the resulting brain or spinal fluid concentration of these precursors is below that required to cause toxicity using in vitro models. It is thought that patients with AIP have depletion of hepatic heme and neurologic symptoms related to elevated brain concentrations of tryptophan and 5- hydroxytryptamine. Thus oral 5-ALA would not deplete hepatic heme, and would not be expected to produce neurological symptoms of AIP. Erythropoietic Protoporphyria (EPP) with reduced conversion of PPIX to heme is associated with photosensitivity, reflecting high systemic and skin levels of PPIX. Patients with EPP experience no hemolytic or neurological problems.

Side effects of 5-ALA administration are uncommon (>=1/1000, <1/100) and include hypotension, nausea, and photosensitivity. Transient nausea and occasional vomiting have been observed following oral administration of high doses of 5-ALA. The incidence of nausea

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following 30 mg/kg and 60 mg/kg (oral) was 7 and 15%, respectively. No vomiting was observed with the lower dose, while 8% of the patients vomited following the higher dose. In all instances, the nausea was mild and short lived (less than15 min.). When vomiting occurred, it was mild and occurred only once in each patient. The nausea or vomiting occurred within 2.5 to 3 hours after receiving the drug which did not interfere with the gut absorption of 5-ALA that occurs within 1 hour of oral ingestion.

Abnormalities in liver function have been observed in patients following ingestion of 5-ALA.

Webber et al. observed that almost one-quarter of all patients who received 5-ALA had at least one abnormality in liver function tests. More patients receiving low doses (< 30 mg/kg) developed abnormalities than those receiving high doses. Significant variations in the time of appearance of the abnormalities were also noted (12 to 120 hr. postoperatively). In all cases, liver function tests returned to normal within 2 months after receiving 5-ALA.

In a single-arm European study including 21 healthy male volunteers, erythema of the skin was provoked by direct exposure to ultraviolet light up to 24 hours after oral application of 5-ALA HCl (20 mg/kg/ body weight). PPIX photosensitization manifests as an 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 which lasts from approximately 24 to 48 hours following 5-ALA administration. **It is for this reason that patients will be kept in subdued light conditions for up to 48 hours following surgery.**

Possible drug-related mild nausea was reported in 1 of 21 volunteers. In another single-center study, 21 patients with malignant glioma received 0.2, 2, or 20 mg/kg body weight 5-ALA HCl followed by fluorescence-guided tumor resection. The only adverse reaction reported in this trial was one case of mild skin erythema occurring in a patient treated with the highest dose.

In a single-arm European study including 36 patients with malignant glioma, drug-related adverse events were reported in 4 patients (one patient: mild diarrhea, one patient: moderate hypesthesia, one patient: moderate chills, and one patient: arterial hypotension) 30 minutes after dosing with 5-ALA HCl20 mg/kg body weight prior to fluorescence-guided resection. Follow-up time was 28 days.

In a comparative, unblinded phase-III European trial (MC-ALS.3/GLI), 201 patients with malignant gliomas received 5-ALA HCl (20 mg/kg body weight). 176 of these underwent fluorescence-guided resection with subsequent radiotherapy. 173 patients received standard resection **(without 5-ALA administration)** and subsequent radiotherapy. With a follow-up of at least 180 days after administration, 2/201 (1.0 %) drug recipients experienced side effects (mild vomiting 48 hours after surgery and mild photosensitivity 48 hours after study surgery). A patient accidentally received an overdose of the medicinal product (3000 mg instead of 1580 mg) which was well tolerated. Respiratory insufficiency resolved completely with ventilation. A more pronounced asymptomatic transient increase of liver enzymes was observed in 5-ALA HCl recipients, peaking between 7 and 14 days. Increased levels of amylase, total bilirubin, and leukocytes, but decreased levels of platelets and erythrocytes were observed, but differences between treatment groups were not statistically significant.

Liver toxicity (from phase III European trial) as elevated liver function tests (LFTs) occurred in over 10% of patients after oral 5-ALA, anesthesia, and fluorescence-guided tumor resection. LFT changes included increased alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma glutamyltransferase (GGT) and blood amylase at 24 h, 7 d, and 6 weeks after 5- ALA ingestion. Approximately, 10% had Grade 3 or 4 elevations in GGT at 7 days; 5% had Grade 3 or 4 elevations in GGT at 6 weeks, and 1% had persistent ALT or AST at 6 weeks. **LFTs returned to normal in all patients.** In both groups of patients from the European phase III trial, (5-ALA and placebo control), 1 patient developed a hepatic serious adverse event (SAE). The 5-ALA patient developed cholelithiasis.

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2.0 STUDY OBJECTIVES

2.1 Primary Objectives

2.1.1 To assess the feasibility of using oral Gleolan® as an adjunct diagnostic imaging tool for malignant tumor tissue fluorescence in a pilot cohort of head and neck and skull base cancer patients.

2.2 Secondary Objectives

2.2.1 To further characterize the diagnostic performance of Gleolan ® (5-ALA)-induced PPIX tissue fluorescence for detecting malignant tumor histopathology in head and neck and skull base cancers.

2.2.2. To determine the predictive values of individual Gleolan® fluorescence scores on detecting malignant tumor histopathology in head and neck and skull base cancers.

2.3 Endpoints

2.3.1 Definition:

Safety: Gleolan®-related adverse events will be recorded using standardized toxicity criteria and known side effect profile up to 6 weeks after surgery. Monitoring for adverse events will take place before and after Gleolan® administration, 48h after surgery, 2 weeks and 6 weeks postoperatively.

2.3.1 Primary endpoint:
The feasibility of using oral Gleolan® as an adjunct diagnostic imaging tool for malignant tumor tissue fluorescence will be primarily assessed by computing sensitivity of intraoperative Gleolan® induced PPIX tissue fluorescence.

PPIX tissue fluorescence will be defined categorically as “no” (score 0), “low” (score 1), “medium” (score 2), and “high” (score 3) by operative surgeon, and images will be recorded. For purposes of computing measures of diagnostic performance a score of 0 will be considered a negative test result and a score of 1, 2 or 3 will be considered a positive test result.

2.3.2 Secondary endpoints:
2.3.2.1 The diagnostic performance of Gleolan® will further be assessed by computing measures of specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of intraoperative Gleolan® induced fluorescence with a score of 0 will be considered a negative test result and a score of 1, 2 or 3 will be considered a positive test result.

2.3.2.2 Incidence of Gleolan®-related adverse events

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3.0 PATIENT ELIGIBILITY

3.1 Inclusion Criteria

3.1.1 Subjects included in this trial must have had documentation of a new or recurrent head and neck or skull base tumor for which surgical resection is indicated and has been planned. These patients will include those with newly diagnosed or recurrent malignancies.

3.1.2 Age 18-80

3.1.3 Karnofsky score >60%

3.1.4 Subjects must have normal organ and marrow function as defined below:

- leukocytes	≥ 3,000/mcL
- absolute neutrophil count	≥ 1,500/mcL
- platelets	≥ 100,000/mcl
- total bilirubin	within normal institutional limits
- AST(SGOT)/ALT(SPGT)	≤ 2.5 X institutional upper limit of normal
- creatinine	within normal institutional limits

OR

Creatinine clearance >60 mL/min/1.73m2 for patients with creatinine levels above institutional normal as defined per institution.

3.1.5 The effects of Gleolan® (5-ALA) on the developing human fetus are unknown. For this reason, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. A pregnancy test will be performed for all women of childbearing ability prior to surgery (see Exclusion Criteria below). Should a woman become pregnant or suspect she is pregnant while participating in this study, she should inform her treating physician immediately.

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

3.2 Exclusion Criteria

3.2.1 Patients with non-resectable tumors or not deemed surgical candidates

3.2.2 History of allergic reactions attributed to compounds of similar chemical/biologic composition to ALA.

3.2.3 Personal or family history of porphyria

3.2.4 Uncontrolled concurrent 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

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3.2.5 Women who are pregnant or become pregnant will be excluded from the trial as it is unknown if ALA is teratogenic or has abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with 5-ALA breastfeeding should be discontinued if the mother is treated with 5-ALA.

3.2.6 Prior history of GI perforation, diverticulitis, and or/peptic ulcer disease

4.0 TREATMENT PLAN

4.1 Treatment Dosage and Administration

4.1.1 Treatment will be administered on an inpatient basis. This does not pose a financial burden to the subject as pre-operative care provides for admission to hospital. One-time, single-dose oral administration of Gleolan® (5-ALA) is planned (20mg/kg). No investigational or commercial agent or therapies other than those described below may be administered with the intent to treat the patient's malignancy prior to surgery and patients will not be part of any other clinical trial at the time of this surgery for anything, not just the identified, malignant tumor.

4.1.2 Gleolan® (5-ALA) 20mg/kg will be reconstituted in the minimum volume of sterile water and may be mixed with a clear liquid or apple fruit juice before being administered in a single oral dose approximately 3 hours (3-5h) prior to scheduled surgery. This one-time single dose administration precludes the need for intra-patient dose modification or delay. In the event of emesis of the product, the dose will not be repeated.

Gleolan® (5-ALA) is presented as a powder for oral solution in 60 ml colorless glass vials. The formulation contains 1.5 g 5-aminolevulinic acid hydrochloride corresponding to 1.17 g of 5- aminolevulinic acid. No other ingredients are used besides the clear liquid or apple juice used to mix with at time of consumption. No impurities have been identified. Details on the manufacturing method, validation of the process as well as in process controls are available and are on file with the FDA. The drug product is manufactured according to a documented, validated process that has given comparable results in the production of both pilot and large scale batches. The specification and release tests for the drug product are in compliance with both Pharmacopia European and current guidelines. Where applicable, analytical validation has been performed.

Limits for impurities and possible degradation products are set according to the results reported for the batches used for relevant toxicological and clinical testing taking into account the proposed route of administration as well as the relevant guidelines. The specifications and the routine release tests are adequate to control the quality of the drug product and are on file with the FDA. Stability test results after 36 months storage justify a shelf-life of 36 months when stored at 25°C ± 2°C. After reconstitution, the in-use shelf-life is 24 hours when the solution is stored at room temperature. In conclusion, the data are complete and satisfactory, ensuring the reproducible manufacturing of a product of good quality.

The specification and release tests for the drug substance are in compliance with Pharmacopia European and current guidelines. The analytical methods are either pharmacological methods or well validated. Stability results after both

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accelerated and long term testing indicate that the drug substance 5-aminolevulinic acid hydrochloride can be considered stable. The stability data support a satisfactory retest period of 3 years when stored at -20 °C in the proposed container. The aseptic manufacturing process of the drug product consists of four steps: compounding, filtration, filling and lyophilisation. The validation of the manufacturing process is satisfactory and relevant in-process controls are performed. The synthesis of the drug substances consists of three stages, for which the potential content of each vial is intended to be reconstituted in 50 ml water or apple juice. One ml of the reconstituted solution contains 23.4 mg of 5-aminolevulinic acid, corresponding to 30 mg 5-aminolevulinic acid hydrochloride. The closure consists of colorless glass vial (Type II, Ph Eur) and bromobutyl-rubber stopper 20 mm (Type I, Ph Eur) and a flip cap top.

The oral solution is intended for single (partial) use. 5-aminolevulinic acid hydrochloride is administered orally to adult patients with head and neck malignancy prior to tumor removal by surgery. 5- Aminolevulinic acid is a prodrug that is metabolized intracellularly to form the fluorescent molecule protoporphyrin (PPIX), which is selectively accumulated in tumor cells and epithelial tissue. Following excitation with blue light (λ = 400 – 410 nm) the PPIX, which has accumulated selectively in the malignant tissue, the tumor emits a red-violet light (red-light fluorescence). Tumor resection is extended as the surgeon is able to identify malignant tissue as differentiated from normal tissue after administration of 5-aminolevulinic acid (Stummer et al. 2006, 2010).

4.2 Toxicities and Dosing Delays/Dose Modifications

Any patient who receives treatment on this protocol will be evaluable for toxicity. Each patient will be assessed for the development of toxicity according to the Time and Events table (See section 5.4). Toxicity will be assessed according to the NCI Common Toxicity Criteria for Adverse Events (CTCAE), version 4.0. Dose adjustments should be made according to the system showing the greatest degree of toxicity.

4.3 Concomitant Medications/Treatments

Compounds of similar chemical/biologic composition to 5-ALA are prohibited. Patients should refrain from use of other phototoxic substances (e.g. tetracyclines, sulfonamides, fluoroquinolones, hypericin extracts) for 72h after administration. Patients should avoid exposure of eyes and skin to strong light sources (e.g. operating illumination, direct sunlight or brightly focused indoor light) for 24h after 5-ALA administration. Patients should not be exposed to any photosensitizing agents up to 2 weeks after 5-ALA administration.

Within 24h after 5-ALA administration, other potentially hepatotoxic medicinal products should be avoided.

In patients with pre-existing cardiovascular disease, concomitant blood pressure medications should be used cautiously as 5-ALA has been shown to decrease systolic and diastolic blood pressure, pulmonary artery systolic and diastolic pressure, and vascular resistance.

4.4 Other Modalities or Procedures

Fluorescence-guided resection will be performed using the standard surgical operating

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microscope. The surgical operating microscope will be adapted with a filter set intended to visualize fluorescence excitation in the wavelength range from 390nm to 440nm and for observation in the 600-700nm range. The adaptation includes standard white light emission source and a combination of excitation and observation low and high pass optical filters with slightly overlapping transmission integrated into their optical configuration. The proposed filter set is commercially available globally. A small fraction of excitation light is remitted from the tissue, which then generates a blue tone contrast from the healthy brain anatomy in contrast to the bright red, 5-ALA induced PPIX fluorescence. Thus, there are two light sources to illuminate the resected field: White light phase and Blue light phase. During the white light illumination, an attenuator reduces irradiance to approximately 50% to minimize the need for illumination adjustment. During the blue light phase, an electromagnetic filter switch is used to introduce a dielectrically coated observation filter with a 600-700nm wavelength into the light paths of the surgical microscope. These filters transmit tumor associated red PPIX fluorescence and a portion of backscattered normal tissue blue excitation light which does not fluoresce. A camera system on the microscope is used to record 5-ALA/PPIX red fluorescence.

Gleolan® (5-ALA) will be reconstituted in the minimum volume of sterile water and may be mixed with a clear liquid or apple fruit juice before being given orally approximately 3 hours (3-5h) prior to scheduled surgery. Image-guided microsurgical resection of the tumor will be undertaken as per operator preference. Following standard surgical entry, further tumor resection will be performed by switching to the microscope fluorescence ‘blue light’ mode.

Photographs will be taken during the ‘blue light’ imaging to provide identification of optical fluorescent tumor. Images will be taken before, during and after resection to capture tumor fluorescence. All tumor specimens will be sent to pathology for frozen examination as per institutional standard of care. Any tissue specimens from the fluorescent areas will be photographed and sent for permanent histopathologic examination and molecular genetic analysis to confirm the presence of malignant tumor. The intraoperative pictures of the resection cavity in the ‘blue light’ mode will be analyzed to provide confirmation of tissue/tumor fluorescence associated with tumor resection. The surgeon will document whether visualization of fluorescence was present with each of the labeled tissue specimens. The remainder of the resection will be performed with the ability to switch between white light and blue light as chose by the surgeon. If no fluorescence is observed in the resection site it will be noted, but will otherwise undergo identical tumor resection, photography and histopathologic examination.

Pathologic confirmation of tumor type will be made by a pathologist who will not be informed of the fluorescence status of the tissue samples. Histopathology will characterize the resected tissue as

(1) Diagnostic tissue or not
(2) Presence of malignant tumor cells
(3) Pathologic grading and tumor type

Genetic testing will be performed in accordance with standard of care at the testing institution for the tumor specimens.

4.5 Duration of Therapy

One-time, single dose administration of oral Gleolan®(5-ALA) (20mg/kg) is planned 3-5h prior to induction of anesthesia. However, surgery can be initiated up to 8 hours after dosing. In the event of allergic reaction, other severe adverse effect, or changes in the patient’s condition prior to surgery rendering the patient unacceptable for further treatment in the judgement of the investigator, or patient decides to withdraw from the study, the therapy protocol will be ended and fluorescent-guided surgery will not proceed.

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4.6 Duration of Follow Up

Analysis of progression and correlative studies will be performed as per standard of care for 6 weeks post-surgery. Follow-up will consist of 3 time points: within 48h post-surgery, a 2 week and a 6 week postoperative office visit. Patients removed from treatment for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

4.7 Removal of Patients from Protocol Therapy

Patients will be removed from therapy when any of the criteria listed in Section 5.5 apply. Notify the Principal Investigator, and document the reason for study removal and the date the patient was removed in the Case Report Form. The patient should be followed-up per protocol.

4.8 Patient Replacement

Patients who withdraw from the study will be replaced through further recruitment at clinical sites to meet target recruitment goals.

5.0 STUDY PROCEDURES

5.1 Screening/Baseline Procedures (60-0 days before surgery)

Assessments performed exclusively to determine eligibility for this study will be done only after obtaining informed consent. Assessments performed for clinical indications (not exclusively to determine study eligibility) may be used for baseline values even if the studies were done before informed consent was obtained.

All screening procedures must be performed prior to registration unless otherwise stated. The screening procedures include:

5.1.1 *Informed Consent*

5.1.2 *Medical history*

Complete medical and surgical history, history of infections

5.1.3 *Demographics*

Age, gender, race, ethnicity

5.1.4 *Review subject eligibility criteria*

5.1.5 *Review previous and concomitant medications*

5.1.6 *Physical exam including vital signs, height and weight*

Vital signs (temperature, pulse, respirations, blood pressure), height, weight

5.1.7 *Performance status*

Performance status evaluated prior to study entry according to Appendix A.

5.1.8 *Adverse event assessment*

Baseline adverse events will be assessed. See section 6 for Adverse Event monitoring and reporting.

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5.1.9 *Hematology*

Complete Blood Count (CBC) to include: white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT) , MCV, MCHC, WBC differential, platelet count.

5.1.10 *Serum chemistries*

Comprehensive metabolic panel (CMP) to include: albumin, alkaline phosphatase, ALT/SGPT, AST/SGOT, BUN, creatinine, electrolytes (sodium, potassium, calcium, chloride, bicarbonate), glucose, and total bilirubin.

5.1.11 *Pregnancy test (for females of child bearing potential)*

Will be performed prior to 5-ALA administration preoperatively as standard operating procedure for all surgical patients.

5.1.12 *Tumor assessment*

Standard of care pre-operative imaging will be conducted as part of usual surgical planning.

5.2 Procedures During Treatment

5.2.1 Day 0 (Day of Surgery) 5-ALA Administration

- Physical exam, vital signs
- Administration of 5-ALA 3h prior to surgery- single dose oral 20mg/kg
- Monitoring for adverse events

5.2.2 Day 0 (Day of Surgery) Fluorescence-Guided Surgery

- Intraoperatively, blue light microscopy will be used to visualize tumor.
- Images will be captured during resection, and surgeon will assign subjective fluorescence scores of no brightness (0), mild brightness (1-pink), moderate brightness (2-orange) or robust brightness (3- lava-like orange). Fluorescence homogeneity or heterogeneity will be noted. After excision the tumor will again undergo imaging to characterize 5-ALA fluorescence and homogeneity or heterogeneity.
- Patient vital signs will be monitored and recorded throughout surgery.
- Following pathology analysis, tumor classification will be recorded. Histopathologic examination of tumor specimens will be correlated with fluorescence on intraoperative imaging. Morphological factors such as tissue cellularity, cytological atypia, nuclear hyperchromasia, as well as tumor markers and immunostaining will be examined. Presence of positive or negative surgical margins will be recorded.
- 5-ALA sensitivity, specificity, positive predictive value and negative predictive value will be determined for different tumor types and histopathology based on correlation of intraoperative fluorescence with tumor pathology.

5.2.3 Within 48h after treatment termination

- Physical exam, vital signs
- Monitoring for adverse effects

5.3 Follow-up Procedures

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5.3.1 Patients will be followed at designated time points of within 48h, 2 weeks, and 6 weeks postoperatively for regular post-surgical examinations. Recorded at these visits:

- Physical exam, vital signs
- Concurrent medications
- Adverse events

5.4 Time and Events Table

Study Assessment or Procedure	Baseline (-60 to day 0 before surgery)	Day 0 (At 5-ALA administration)	Day 0 (prior to surgery)	Within 48h of surgery	Week 2	Week 6
Informed Consent	X					
Demographics and Medical History	X			X	X	X
Physical Exam (vitals, weight, Karnofsky status)	X	X		X	X	X
Concurrent Medications	X	X	X	X	X	X
Adverse Events Evaluation	X	X	X	X	X	X
Tumor Imaging	X					
Pregnancy test[d]	X					
Other labs (CMP, CBC, GGT)[a,b,c]	X			X	X	X
Gleolan® (5-ALA)[e]		X				

a. Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, potassium, total protein, SCOT (AST), SCPT (ALT), sodium, gamma glutamyl transpeptidase (GGT)
b. Phosphorus, uric acid, creatinine kinase, and amylase will be added as clinically necessary/indicated.
c. Repeat only those labs that remain abnormal after 6 weeks unless attributed to other therapy
d. Pregnancy test (women of childbearing potential)- part of standard pre-operative protocol
e. Gleolan® (5-ALA) is dosed as 20mg/kg. Oral administration is one-time only and based on actual weight on day of surgery.

5.5 Removal of Subjects from Study

Patients can be taken off the study treatment and/or study at any time at their own request, or they may be withdrawn at the discretion of the investigator for safety, behavioral or administrative reasons. The reason(s) for discontinuation will be documented and may include:

5.5.1 Patient voluntarily withdraws from treatment (follow-up permitted);

5.5.2 Patient withdraws consent (termination of treatment and follow-up);

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5.5.3 Patient is unable to comply with protocol requirements;

5.5.4 Patient demonstrates disease progression (unless continued treatment with study drug is deemed appropriate at the discretion of the investigator);

5.5.5 Patient experiences toxicity that makes continuation in the protocol unsafe;

5.5.6 Treating physician judges continuation on the study would not be in the patient's best interest;

5.5.7 Patient becomes pregnant (pregnancy to be reported along same timelines as a serious adverse event);

5.5.8 Development of second malignancy (except for basal cell carcinoma or squamous cell carcinoma of the skin) that requires treatment, which would interfere with this study;

5.5.9 Lost to follow-up. If a subject cannot be located to document status after a period of 2 months postoperatively, the subject will be considered lost to follow-up. All attempts to contact the subject during this time period will be documented.

6. ADVERSE EVENTS

6.1 Adverse Event Monitoring

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

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

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

6.2 Definitions

6.2.1 Definition of Adverse Event

An adverse event (AE) is any untoward medical occurrence in a patient receiving study treatment and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an experimental intervention, whether or not related to the intervention. The occurrence of an AE may come to the attention of study personnel during study visits and interviews or by a study participant presenting for medical care. Adverse events will be coded in accordance with the CTCAE V4.0. For analysis of adverse events, AEs will be obtained at the time of ingestion of the investigational product before surgery, during the first 48 hours, 2 weeks and 6 weeks after surgery. In addition, serious adverse events (SAE) will be collected beginning from the time the subject signs consent. All AEs must be

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graded for intensity and relationship to study product. The investigator is responsible for recording all AEs that are observed during this study according to the definition.

6.2.2 Severity of Adverse Events

If possible, all AE will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0. The CTCAE v4 is available at http://ctep.cancer.gov/reporting/ctc.html

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

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

6.2.3 Serious Adverse Events

A “serious” adverse event is defined in regulatory terminology as any untoward medical occurrence that:

6.2.3.1 Results in death.

If death results from (progression of) the disease, the disease should be reported as event (SAE) itself.

6.2.3.2 Is life-threatening.

(the patient was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe).

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

6.2.3.4 Results in persistent or significant disability or incapacity.

6.2.3.5 Is a congenital anomaly/birth defect

6.2.3.6 Is an important medical event

Any event that does not meet the above criteria, but that in the judgment of the investigator jeopardizes the patient, may be considered for reporting as a serious adverse event. The event may require medical or surgical intervention to prevent one of the outcomes listed in the definition of “Serious Adverse Event“.
For example: allergic bronchospasm requiring intensive treatment in an emergency room or at home; convulsions that may not result in hospitalization; development of drug abuse or drug dependency.

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6.3 Steps to Determine If an Adverse Event Requires Expedited Reporting

Step 1: Identify the type of adverse event using the NCI Common Terminology Criteria for Adverse Events (CTCAE v4).

Step 2: Grade the adverse event using the NCI CTCAE v4.

Step 3: Determine whether the adverse event is related to the protocol therapy
Attribution categories are as follows:

- Definite – The AE *is clearly related* to the study treatment.
- Probable – The AE *is likely related* to the study treatment.
- Possible – The AE *may be related* to the study treatment.
- Unrelated – The AE is clearly NOT related to the study treatment.

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

Step 4: Determine the prior experience of the adverse event.
Expected events are those that have been previously identified as resulting from administration of the agent. An adverse event is considered unexpected, for expedited reporting purposes only, when either the type of event or the severity of the event is not listed in:

- the current known adverse events listed in the Agent Information Section of this protocol;
- the drug package insert;
- the current Investigator's Brochure

6.4 Reporting Requirements for Adverse Events

6.4.1 Expedited Reporting

- The Principal Investigator must be notified within 24 hours of learning of any serious adverse events, regardless of attribution, occurring during the study or within 30 days of the last administration of the study drug.
- The IRB/PPHS must be notified within 5 business days of "any unanticipated problems involving risk to subjects or others" (UPR/UPIRSO).

  The following events meet the definition of UPR

  a. Any new information that indicates a new or increased risk, or safety issue (e.g., interim analysis, safety monitoring report, publication, updated sponsor safety report), that indicates an unexpected change to the risk/benefit ratio for the research.

  b. An investigator brochure, package insert, or device labeling is revised to indicate an increase or magnitude of a previously known risk, or describes a new risk.

  c. Withdrawal, restriction, or modification of a marketed approval of a drug, device, or biologic used in research protocol

  d. Protocol deviation or violation that harmed subjects or others or that indicated subjects or others might be at increased risk of harm.

  e. Complaint of subject that indicates subjects or others might be at increased risk of harm or at risk of a new harm

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f. Any breach in confidentiality that may involve risk to the subject or others.

g. Any harm experienced by a subject or other individual that in the opinion of the investigator is unexpected and at least probably related to the research procedures.

6.4.2 Routine Reporting

All other adverse events- such as those that are expected, or are unlikely or definitely not related to the study participation- are to be reported annually as part of regular data submission.

Those AEs that do not require expedited reporting are reported in routine study data submissions. Expected AEs such as Grade 1 and 2 Skin Reactions, AST/ALT elevation, Grades 1 and 2, nausea/vomiting will not be reported in an expedited fashion. If the subject has an elevated GGT at the time of study entry, but has normal ALT and AST, reporting will not be required unless the grade has increased by 2 levels.

6.5 Stopping Rules

If patients exhibit respiratory distress or serious allergic reaction following 5-ALA administration, their participation in the study will cease

Stopping rules for trial enrollment have been incorporated into the protocol based on liver function tests (LFTs). The extensive European experience with Gleolan® and fluorescence- guided tumor resection has shown that patients very commonly have a transient increase in their liver enzymes (>=1/10). Peak values occur between 7 and 14 days after administration. However, LFT increases can occur up to 6 weeks after administration. The liver toxicity data available from the Phase III European trial (Stummer 2006) has shown that after 6 weeks, the incidence of Grade 3 and Grade 4 events (>5-10x or >10x upper limit of normal) occur in less than 6% of patients for ALT/GPT, AST/GOT, and Gamma-GT (GGT) liver testing.[36] Approximately 10% had Grade 3 or 4 elevations in GGT at 7 days; 5% had Grade 3 or 4 elevations in GGT at 6 weeks, and 1% had persistent ALT or AST elevations at 6 weeks. **LFTs returned to normal in all patients.** In both groups of patients from the European phase III trial, (Gleolan® and placebo control), 1 patient developed a hepatic serious adverse event (SAE). The Gleolan® patient developed cholelithiasis.

Patients will be continuously monitored for sustained Grade 3 or 4 elevations in their LFTs at six weeks following Gleolan® administration. A comprehensive metabolic serum panel, including albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, potassium, total protein, SGOT[AST], SGPT[ALT], sodium, gamma glutamyltranspeptidase (GGT), will be obtained prior to surgery, within 48 hours of surgery, 2 weeks, and 6 weeks after surgery. These tests will only be repeated after 6 weeks if SGOT[AST], SGPT[ALT], and gamma glutamyltranspeptidase (GGT) remain elevated after 5-ALA dosing.

We will continuously monitor the rate of sustained Grade 3 or 4 elevations in LFTs at 6 weeks following Gleolan® administration and accrual will be halted if there is sufficient evidence that the sustained Grade 3 or 4 LFT elevation rate exceeds the acceptable rate of 5% by more than 5% (unacceptable rate 10%). If the cumulative number of patients that experience grade 3 or 4 elevations in the LFTs at 6 weeks from drug administration is greater than the associated boundary value b_k listed in the table below, among the k patients enrolled in the trial, enrollment in the trial will be halted for safety considerations prompting IRB notification and a DSMB meeting will be requested.

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# of accrued subjects, k	2-9	10-20	21-23
Boundary, b_k	1	2	3

The following table shows the probability of early stopping if the true rates of sustained grade 3 or 4 elevations in the LFTs are at or lower than 10%. For safety of the participants, we would like to have a low probability of early stopping if the sustained LFT rates are low, but high probability of early stopping if the sustained LFT rate is high.

Probability of early stop based on true grade 3 or 4 LFT rate					
True SAE rate	5%	6.2%	7.5%	8.8%	10%
Probability of early stop	0.15	0.21	0.28	0.36	0.44

This stopping rule was computed using the toxbdry function in R and calculations of this function are based on methods described in Chapter 12 of Jennison and Turnbull[39] and in the illustrative paper by Ivanova, Qaquish and Schell[40].

toxbdry(0.05,0.10,c(1:23),cP0=0.15,cP1=0.30,ngrid=5)

7.0 DRUG INFORMATION

7.1 Gleolan®

7.1.1 Description and Composition

Gleolan® is available in colorless glass vials containing 1.5 g 5-aminolevulinic acid hydrochloride (Gleolan®) corresponding to 1.17 g of 5-aminolevulinic acid (5-ALA) No other ingredients are used. The closure consists of colorless glass vial (Type II, Ph Eur) and bromobutyl-rubber stopper ø 20 mm (Type I, Ph Eur) and flip cap. The powder for oral solution is intended for single (partial) use. To prepare the ready-to-use solution the content of one vial is dissolved in 50 ml of water or apple juice. The concentration of the reconstituted solution is 3%. Gleolan® is administered orally to patients prior to tumor removal by surgery (20 mg/kg BW). The reconstituted solution may be prepared up to 24 hours prior to use if protected from light. It should be given hours (range 3-5 hours) prior to planned induction of anesthesia for surgery, although its effect is present for up to 8-12 hours. This step is critical for an optimal visualization of tumor tissue due to pharmacokinetic properties of Gleolan®. Tumor resection is improved since malignant tissue can be differentiated more easily from normal tissue after administration of Gleolan® following excitation with blue light (λ = 400 - 410 nm) in malignant tissue. The rationale for the development of a powder for oral solution is rapid onset of action and individual dosage of the drug product compared to slower onset of action and less flexible dosing of conventional tablets/capsules.

7.1.2 Manufacture

Name and address of the manufacturer responsible for batch release:
IDT Biologika GmbH
Am Pharmapark
Dessau-Rosslau, 06861, Germany (DEU)

Manufacturing process
The manufacturing process briefly consists of the dissolution preparation, sterile filtration (0.45 followed by 0.22 microns) aseptic filling and assembling process and lyophilization. Evaluation of critical manufacturing steps is available together with In-Process Controls and Process Validation Data. These are all considered to be satisfactory.

7.1.3 Control of Excipients

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The only other ingredient (water for injection) conforms to the specification in Ph Eur. The documented certificates of analysis verify conformance of the specified parameters.

7.1.4 Product Specification

The product specification is simple and includes relevant tests by validated methods for identity (IR/HPLC), assay (HPLC), clarity, color, purity (degradation products by HPLC), water content (Ph Eur), sterility (Ph Eur), endotoxins, pH, particulate matter, residual solvents (GC), etc. Batch analyses show good uniformity and compliance with the agreed specification. Data is on file with the FDA.

7.1.5 Container Closure System

The product is a sterile lyophilized preparation in colorless glass vials, containing 1.5 g Gleolan® (corresponding to 1.17 g. 5-ALA). The closure consists of colorless glass vial (Type II, Ph Eur) and bromobutyl-rubber stopper ø 20 mm (Type I, Ph Eur) and flip cap. Batch results comply with the respective specifications.

7.1.6 Stability

Stability studies have been performed taking the relevant guidelines into account. Thirty-six (36) months of stability data are provided for lyophilized preparation batches stored at 25ºC/60%RH and six months of stability data are provided for the lyophilized preparation stored at the accelerated condition of 40ºC/75%RH. The results of the stability analyses support the shelf life and storage conditions as defined in the package insert. Furthermore, in use stability has been investigated. The content of one vial was dissolved in water as recommended for the drug product and stored for 24 h. The parameters investigated are identical to the parameters investigated for the lyophilisate. All results obtained meet very well with the specification set for the drug product.

The Investigator, or a responsible party designated by the Investigator, will maintain a careful record of the inventory and disposition of all agents.

7.1.7 Side Effects

Side effects include nausea, GI upset, hypotension, photosensitivity and elevation of liver enzymes. See section 1.2.3 for full side effect and toxicity profile.

8.0 STATISTICAL CONSIDERATIONS

8.1 Study Design/Study Endpoints

This is a single center, single arm pilot study conducted at the Mount Sinai Health System with 23 subjects planned for enrollment. The aim is to examine feasibility of 5-ALA (Gleolan®) use as an adjunct diagnostic imaging tool to detect malignant tumor tissue in head and neck and skull base cancer patients. Eligible patients will take 5-ALA 3h prior to surgery with single oral dose of 20mg/kg. The standard surgical procedure will be conducted accompanied by the intra-operative blue light microscopic image for surgeons to assign PPIX tissue fluorescence scores of no brightness (0), mild brightness (1-pink), moderate brightness (2-orange) or robust brightness (3- lava-like orange). The resected tissues will be examined by pathologists to confirm presence of cancer cells.

8.2 Sample Size and Accrual

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There is no previous publication for applying 5-ALA to head and neck cancer patients. The sample size calculation is based on the experience of 5-ALA in brain cancer. A literature review study summarized the usage of 5-ALA in patients with malignant Gliomas between 2000 and 2015.[3] The sample sizes in the studies included in the review ranged from 11 to 97. The sensitivity ranged from 75% and 95% while the specificity ranged from 53% to 96%. In our study, all tissue samples will be collected from the head and neck cancer patients undergoing standard of care surgical resection. The surgeons will provide the pathologists with the patient resected specimen marked, typically in 4 different regions with 3 regions located within the tumor region and 1 located at the boundary or outer region of the tumor. Each region will be marked according to the associated intensity of the PPIX tissue fluorescence score at that location as no brightness (0), mild brightness (1-pink), moderate brightness (2-orange) or robust brightness (3- lava-like orange). Thus each patient is expected to contribute 1 tissue sample from a normal margin region and 3 tissue samples from the tumor tissue region at a ratio of 1:3 (i.e. the pathologists will examine the samples from each patient histopathologically), which translates to a tumor tissue prevalence of 75% in the overall tissue samples.

The following calculations were performed using PASS 15 Power Analysis and Sample Size Software (2015). NCSS, LLC. Kaysville, Utah, USA, ncss.com/software/pass and using the methods for calculating sample size for co-primary endpoints of sensitivity and specificity.[41-43]

Assuming a prevalence of 75% and a sample sensitivity of 85%, the sample size needed to estimate a two-sided 95% sensitivity confidence interval with a width of at most 35% (± 17.5% precision assuming asymptotic with continuity correction method) is at least 23.We will use a sample size of 23 in this study.

If the prevalence is as low as 50% in our sample then the width of our 75% sensitivity confidence interval will increase to 40.6% (±20.3 precision) which still gives a lower bound for the CI above 50%.

The sample size calculation is performed conservatively assuming only 1 measure per patient, but there will be typically 4 per patient which will improve precision of estimates.

We expect to enroll the necessary patients in 6 months at a rate of approximately 4 patients per month. This accrual rate is based on historical monthly rates of surgery in this patient population at the Mount Sinai hospital.

8.3 Data Analyses Plans

The characteristics of participants and information collected before 5-ALA is administered will be summarized using standard statistics, such as mean, SD, median, 1st and 3rd quartiles for continuous variables, and count, percentage for categorical variables.

Analysis of the primary endpoint

To assess the feasibility of using 5-ALA as an adjunct diagnostic imaging tool to detect malignant tumor tissue, the point estimate of sensitivity will be computed according to the following definition:

Sensitivity: proportion of tumor tissues that tested positive on 5-ALA induced fluorescence.

The definition of a positive test is the fluorescence score equal to or greater than 1 while a negative test is considered a score of 0. For the assessment of sensitivity and

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specificity, histological diagnoses of any upper aerodigestive head and neck cancer in addition to squamous cell carcinoma, carcinoma in situ, and severe and moderate dysplasia were classified as malignant, whereas light dysplasia and normal tissue will be classified as benign by pathologists. The analysis population includes all patients who sign the inform consent regardless of taking 5-ALA or not. Each patient is expected to contribute multiple samples and the pathologists will determine presence of malignancy in each sample. A contiguous table (fluorescence scores vs. pathological results) that presents the frequency and percentage of each cell will be prepared. A generalized estimating equation (GEE) model accounting for repeated measure within each patient will be used to estimate the sensitivity and specificity.[44-45] For sensitivity and specificity, the dependent variable is the PPIX fluorescence test result (positive vs. negative) and the independent variable is presence of tumor cells (tumor vs. normal).

Analysis of the secondary endpoints

To further determine the diagnostic performance of 5-ALA for detecting of malignant tumor tissue, specificity, PPV and NPV will be computed. Specificity will be computed according to the following definition:

Specificity: proportion of normal tissues that tested negative on 5-ALA induced fluorescence.

The definition of a positive test is the fluorescence score equal to or greater than 1 while a negative test is considered a score of 0. For the assessment of sensitivity and specificity, histological diagnoses of any upper aerodigestive head and neck cancer in addition to squamous cell carcinoma, carcinoma in situ, and severe and moderate dysplasia were classified as malignant, whereas light dysplasia and normal tissue will be classified as benign by pathologists. The analysis population includes all patients who sign the inform consent regardless of taking 5-ALA or not. Each patient is expected to contribute multiple samples and the pathologists will determine presence of malignancy in each sample. A contiguous table (fluorescence scores vs. pathological results) that presents the frequency and percentage of each cell will be prepared. A generalized estimating equation (GEE) model accounting for repeated measure within each patient will be used to estimate the sensitivity and specificity.[44-45] For sensitivity and specificity, the dependent variable is the PPIX fluorescence test result (positive vs. negative) and the independent variable is presence of tumor cells (tumor vs. normal).

For PPV and NPV, a generalized estimating equation (GEE) model accounting for repeated measure within each patient will be used. The dependent variable is presence of tumor, and the independent variable is the test result. The point estimate along with a corresponding two-sided 95% confidence interval will be provided using PROC GENMOD.

The 5-ALA related adverse events will be recorded using standardized toxicity criteria and known side effect profile. Frequency of each observed adverse event will be calculated at each monitor time point. The rate of each adverse effect will be estimated as the number of adverse events within 6 weeks after surgery divided by the number of person days followed. The point estimate of the incidence rate along with a corresponding two-sided 95% confidence interval will be provided using PROC GENMOD.[46]

The analyses will be conducted using SAS 9.4 software. Copyright ©[2002-2012] SAS Institute Inc.

9.0 STUDY MANAGEMENT

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9.1 Conflict of Interest

Any research personnel who has a conflict of interest with this study (patent ownership, intellectual property, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must declare their conflict of interest to the appropriate institutional review bodies. Local institutional conflict of interest policies will be followed for all research personnel associated with the research project.

9.2 Institutional Review Board (IRB) Approval and Consent

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

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

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

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

9.3 Required Documentation

(For Cancer Clinical Trials Office Managed Studies multi-site studies)

Before the study can be initiated at any site, the following documentation must be provided to the CCTO by all sites.

- A copy of the official IRB approval letter for the protocol and informed consent
- IRB membership list
- CVs and medical licensure for the principal investigator and any associate investigators who will be involved in the study
- A copy of the IRB-approved consent form
- CAP and CLIA Laboratory certification numbers and institution lab normal values
- Executed clinical research contract (if applicable)

9.4 Registration Procedures

Treatment Trials utilizing biologics, drugs, or devices must register subjects through the Cancer Clinical Trials Office Subject Central Registration process. Include the following boilerplate language:
All patients must be enrolled onto trial through the Cancer Clinical Trials Office Central Registration process. Prior to registration, a member of the study staff must scan and email the following documents as individual PDF files to the Central Registration Mailbox (central.registration@mssm.edu) with a cc to the Central Registrars.

- Signed Informed Consent(s)

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- Signed CCTO Registration Form
- Signed Eligibility Checklist
- Additional supporting documentation (i.e. lab/scan reports) may be included at the study teams' discretion.

The designated Central Registrar will review all received documents for consistency and completeness.

- If there is any concern or discrepancy noted, the study staff member who originated the central registration request will be contacted immediately for clarification.

If the patient is deemed eligible for study enrollment based on a thorough review by the Central Registrar, the patient will be entered into the CTMS system and a Registration Confirmation Letter is generated and sent to the following individuals:

- Study team
- Treating Physician
- Research Infusion Nurse designee
- Research Pharmacy

9.5 Data Management and Monitoring/Auditing

11.5.1 Elements of a Data and Safety Monitoring Plan

List the name(s) of the individual(s) at the Icahn School of Medicine at Mount Sinai (ISMMS) who will be responsible for data and safety monitoring of this study. For each individual, indicate their role, name, title, and department information.

ISMMS Principal Monitor:
Principal Investigator

Last Name: Iloreta
First Name: Alfred-Marc
Academic Title: Assistant Professor
Department: Otolaryngology
Mailing Address: 1 Gustave L Levy Place
10th Floor
New York, NY 10020
Phone: Office 212-241-9410
E-mail: Alfred-Marc.Iloreta@mountsinai.org

ISMMS Additional Monitors:
Co-Investigator

Last Name: Bederson
First Name: Joshua
Academic Title: Professor and Chair
Department: Neurosurgery
Mailing Address: Mount Sinai Hospital
Annenberg Building
1468 Madison Avenue
Floor 8, Room 28

Phone: Office 212-241-2377
E-mail: joshua.bederson@mountsinai.org

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Last Name: Shrivastava
First Name: Raj
Academic Title: Professor
Department: Neurosurgery
Mailing Address: Mount Sinai Hospital
Annenberg Building
1468 Madison Avenue
Floor 8, Room 35

Phone: Office 212-241-6147
E-mail: raj.shrivastava@mountsinai.org

Dr. Iloreta will be the principal monitor for this study. The majority of study patients will be under his care. He will be best positioned to assess and respond to any adverse events that are reported.

Patients will be monitored for adverse events as described in section 6. All adverse events will be collected from the time of drug administration until 6 weeks after surgery. In addition, SAEs will be collected beginning from the time the subject signs consent. The description and grading scales found in the revised NCI Common Terminology Criteria for adverse events (CTCAE) version 4.0, which will be utilized for adverse event reporting are available online at http://ctep.cancer.gov/reporting/ctc.html. Expected adverse events specific for Gleolan® are listed in section 1.2.

Safety and data information will be reviewed by the monitors every 6 months.

See section 6.6 stopping rules.

As described previously, dose selection for Gleolan® is 20mg/kg based on previous studies.

National Cancer Institute Common Toxicity Criteria will be used to evaluate adverse events as described in section 6.

Data will be recorded in patient binders and secure Mount Sinai redcap storage, ensuring completeness and accuracy through redundancy. Data will also be checked at set intervals by the DMC.

If temporary or permanent suspension of the study occurs, in addition to PPHS the occurrence will be reported to the IRB.

8.5.2 Data Monitoring Committee/Data Safety Monitoring Board (DMC/DSMB)

9.6 Adherence to the Protocol

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

9.6.1 Emergency Modifications

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

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9.6.2 Other Reportable New Information and Protocol Deviations/Violations

In accordance with local IRB requirements, the following information must be reported within five (5) business days.

- Non-compliance with federal regulations governing human research or with the requirements or determinations of the IRB, or an allegation of such non-compliance
- Failure to follow the protocol due to the action or inaction of the investigator or research staff.
- Breach of confidentiality
- Premature suspension or termination of the research by the sponsor or investigator.

9.7 Amendments to the Protocol

Should amendments to the protocol be required, the amendments will be originated and documented by the Principal Investigator. It should also be noted that when an amendment to the protocol substantially alters the study design or the potential risk to the patient, a revised consent form might be required.

The written amendment, and if required the amended consent form, must be sent to the IRB for approval prior to implementation.

9.8 Record Retention

Study documentation includes all Case Report Forms, data correction forms or queries, source documents, Sponsor-Investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

Government agency regulations and directives require that the study investigator must retain all study documentation pertaining to the conduct of a clinical trial. In the case of a study with a drug seeking regulatory approval and marketing, these documents shall be retained for at least two years after the last approval of marketing application in an International Conference on Harmonization (ICH) region. In all other cases, study documents should be kept on file until six years after the completion and final study report of this investigational study.

9.9 Obligations of Investigators

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

The Principal Investigator at each institution or site will be responsible for assuring that all the required data will be collected and entered onto the Case Report Forms. Periodically, monitoring visits will be conducted and the Principal Investigator will provide access to his/her original records to permit verification of proper entry of data. At the completion of the study, all case report forms will be reviewed by the Principal Investigator and will require his/her final signature to verify the accuracy of the data.

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11.0 APPENDICES

APPENDIX A

Performance Status Criteria

Karnofsky performance scale	
Percent	**Description**
100	Normal, no complaints, no evidence of disease.
90	Able to carry on normal activity; minor signs or symptoms of disease.
80	Normal activity with effort; some signs or symptoms of disease.
70	Cares for self, unable to carry on normal activity or to do active work.
60	Requires occasional assistance but is able to care for most of his/her needs.
50	Requires considerable assistance and frequent medical care.
40	Disabled, requires special care and assistance.
30	Severely disabled, hospitalization indicated. Death not imminent.
20	Very sick, hospitalization indicated. Death not imminent.
10	Moribund, fatal processes progressing rapidly.
0	Dead.

APPENDIX B

Ultraviolet-Illuminated Operating Room Microscope

Technical Aspects

5-aminolevulinic acid (Gleolan® – a naturally occurring non-fluorescing metabolic precursor, induces synthesis of protoporphyrin IX (PPIX). PPIX fluoresces when exposed to the excitation light of blue-violet (wavelength 390-440nm.) It gives off a light with characteristic peaks 635 and 704nm. This is best viewed by filtering (450 long pass filter) out most other wavelengths except a small amount of excitation light.

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

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

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The surgical operating microscope is a Class I exempt device that is commercially available in the United States.

The surgical operating microscope will be adapted with a filter set intended to visualize fluorescence excitation in the wavelength range from 390nm to 440nm and for observation in the 450nm range. The adaptation includes a combination of excitation and observation filters with slightly overlapping transmission integrated into their optical configuration. The proposed filter set is commercially available globally with the expectation of the United States, and is the appropriate filter set-up for intraoperative visualization of malignant gliomas after administration of Gleolan®

- Leica OH4 with FL400 accessory
- Leica OH6 with FL400 accessory
- Zeiss Pentero with Blue 400 filter

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