

Protocol Title: A Phase 3 Multicenter Study of Gleolan (Aminolevulinic Acid Hydrochloride) to Enhance Visualization of Tumor in Patients with Newly Diagnosed or Recurrent Meningiomas	
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PROTOCOL SIGNATURE PAGE

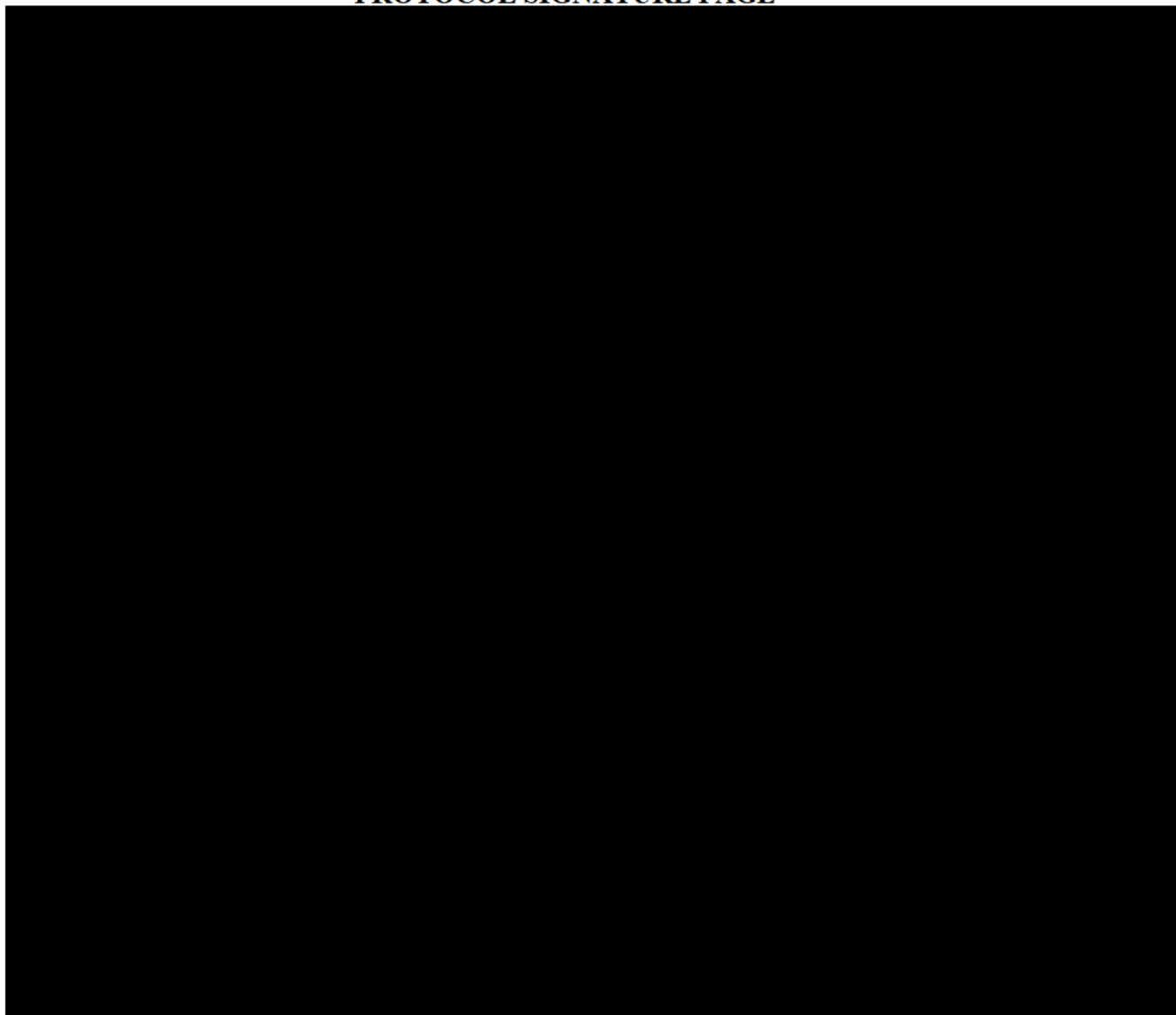


Table of Contents

Table of Contents	3
List of Tables.....	5
List of Figures	5
List of Abbreviations.....	6
1 Protocol Synopsis.....	8
1.1 Schema	13
1.2 Schedule of Events	14
2 Introduction	15
2.1 Scientific Rationale	15
2.2 Summary of Nonclinical Testing of ALA.....	17
2.3 Background on ALA in Meningioma Resection.....	17
2.4 Benefit/Risk Assessment.....	18
2.4.1 Known Potential Risks.....	18
2.4.2 Known Potential Benefits.....	19
2.4.3 Overall Benefit: Risk Conclusion.....	19
3 Objectives, Endpoints and Study Terminology.....	20
4 Study Design	23
4.1 Justification for Dose	23
4.2 Distribution and Biotransformation.....	24
4.3 Elimination	24
4.4 Surgical Treatment and Tissue Assessment and Sample Collection Plan.....	24
4.4.1 Surgical Treatment	24
4.4.2 Tissue Identification and Sample Collection.....	25
4.4.3 Procedures for Bulk Tumor Tissue Identification and Sample Collection.....	25
4.4.4 Procedures for Indeterminate Tissue Identification and Sample Collection Under White Light	26
4.4.5 Procedures for End of Surgery Tissue with Unexpected Fluorescence Identification and Sample Collection	27
4.4.6 Tissue Identification and Collection Flow-Chart	28
4.5 Blinded Histopathological Analysis.....	30
4.6 End of Study Definition	30
4.7 Biopsy Review Panel and Bias Minimization	30
4.7.1 Biopsy Review Panel Data Evaluation and Assessment Output.....	31
4.8 Criteria for Inclusion in the Blinded Biopsy Population and Biopsy Efficacy Analysis Population	33
4.8.1 Indeterminate Tissues in Biopsy Efficacy Analysis Population.....	33
4.8.2 Unexpected Fluorescent EOS Tissues in the Blinded Biopsy Population and Biopsy Efficacy Analysis Population	35
5 Study Population	36
5.1 Inclusion Criteria.....	36
5.2 Exclusion Criteria.....	37
5.3 Screen Failures	38
5.4 Long Term Follow-up.....	38
6 Study Drug	38
6.1 Study Drug(s) Administered	39

6.2	Preparation/Handling/Storage/Accountability	39
6.2.1	Drug Accountability	39
6.2.2	Drug Reconstitution and Administration.....	39
6.3	Gleolan Dose Modifications.....	41
6.4	Study Drug Compliance	41
6.5	Concomitant Medications	41
6.6	Drug Interactions.....	41
6.7	Management of Select Toxicities	41
6.7.1	Photosensitivity Reactions	41
6.7.2	Liver Enzyme Elevations and Hyperbilirubinemia	42
6.7.3	Anti-Emetic Therapy.....	42
6.7.4	Other Anticancer or Experimental Drugs.....	42
6.7.5	Palliative and Supportive Care.....	42
6.7.6	Retreatment Criteria	42
7	Discontinuation of Study Participation	42
7.1	Participant Discontinuation/Withdrawal from the Study	43
7.2	Lost to Follow Up	43
8	Study Assessments and Procedures.....	43
8.1	Efficacy Assessments.....	43
8.2	Safety Assessments	48
8.2.1	Physical Examinations, Vital signs, and Medical History	49
8.2.2	Neurological Exams	49
8.2.3	Clinical Safety Laboratory Assessments.....	49
8.3	Adverse Events (AE) and Serious Adverse Events (SAE)	50
8.3.1	Definitions.....	50
8.3.2	Time Period and Frequency for Collecting AE and SAE Information.....	52
8.3.3	AEs Reportable per protocol.....	52
8.3.4	Relationship to Study Drug	52
8.3.5	Method of Detecting AEs and SAEs.....	53
8.3.6	Recording and Follow-Up of AE and/or SAE	53
8.3.7	Regulatory Reporting Requirements for AEs and SAEs.....	55
8.3.8	Pregnancy Reporting.....	56
8.3.9	Disease-Related Events and/or Disease-Related Outcomes.....	56
8.4	Study Drug abuse, misuse, overdose, and other special situations	57
8.5	Pharmacokinetic and Pharmacodynamic Measurements	57
9	Statistical Considerations	57
9.1	Sample Size Determination	57
9.2	Analysis Populations	58
9.3	Statistical Analyses	59
9.3.1	General Considerations	59
9.3.2	Primary Efficacy Analysis	59
9.3.3	Secondary Efficacy Analyses.....	59
9.3.4	Exploratory Efficacy	61
9.3.5	Safety Analyses.....	61
9.4	Interim Analyses	62
9.5	Handling of Missing Intra-Operative Tissue Location Data.....	62

9.6	Multiple Testing Procedures	62
9.7	Data Safety Monitoring Board	62
10	Supporting Documentation and Operational Considerations	63
10.1	Regulatory and Ethical Considerations	63
10.2	Financial Disclosure	63
10.3	Study and Site Start and Closure	63
10.4	Document and Data Retention	64
10.5	Dissemination of Clinical Study Data	65
10.6	Publication Policy	65
10.7	Clinical Trial Insurance	65
10.8	Contact Information for Trial Sites	65
10.9	Investigator's Responsibilities	65
11	References	68
12	Appendices	69

List of Tables

Table 1.	Schedule of Events	14
Table 2.	Definitions and Terminology for this Protocol	20
Table 3.	Bulk Tumor Tissue Location Identification and Sample Collection Process	26
Table 4.	Indeterminate Tissue Identification and Sample Collection Process	27
Table 5.	Unexpected Fluorescent End of White Light Surgery Tissue Identification and Sample Collection Process	28
Table 6	Biopsy Review Sessions	31
Table 7.	Efficacy Objectives and Endpoints	44
Table 8.	Key Secondary Efficacy Endpoints	44
Table 9.	Other Secondary Efficacy Endpoints	46
Table 10.	Exploratory Objectives and Endpoints	47
Table 11.	Adverse Event Attribution Categories	53
Table 12.	Sample Size Determination Modeling Assumptions	57
Table 13.	Analysis Populations	58

List of Figures

Figure 1.	Overall Study Schema	13
Figure 2.	Tissue Identification and Sample Collection	25
Figure 3.	Flow-Chart of Tissue Location Identification and Tissue Sample Collection	29
Figure 4.	Criteria for Inclusion of an Indeterminate Tissue in the Biopsy Efficacy Analysis Population	34
Figure 5.	Criteria for Inclusion of an Unexpected Fluorescent EOS Tissue Location in the Biopsy Efficacy Analysis Population	36

List of Abbreviations

λ	Wavelength
AE	Adverse Event
ALA	Aminolevulinic Acid
ALA HCl	Aminolevulinic Acid Hydrochloride
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
BIL	Bilirubin
BL	Blue Light
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CFR	Code of Federal Regulations
CIOMS	Council for International Organizations of Medical Sciences
C _{max}	Maximum Concentration
CMP	Complete Metabolic Panel
CRF	Case Report Form
CTCAE	Common Terminology for Adverse Events
DRE	Disease Related Events
DSMB	Data Safety Monitoring Board
DSUR	Development Safety Update Report
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EMA	European Medicines Evaluation Agency
EOS	End of Surgery
FDA	Food and Drug Administration
FNBL	False Negative Blue Light
FNWL	False Negative White Light
FPBL	False Positive Blue Light
FPWL	False Positive White Light
FSH	Follicle-Stimulating Hormone
GCP	Good Clinical Practices
GI	Gastrointestinal
H&E	Hematoxylin and Eosin
HRT	Hormone Replacement Therapy
IB	Investigator's Brochure
ICF	Informed Consent Form
ICG	Indocyanine Green
ICH	International Conference for Harmonisation
IEC	Independent Ethics Committees
IND	Investigational New Drug Application
IRB	Institutional Review Boards
IUD	Intrauterine Device
IUS	Intrauterine System

LTFU	Lost to Follow Up
MedDRA	Medical Dictionary for Regulatory Activities
MOPS	Manual of Procedures
MR	Magnetic Resonance
MRI	Magnetic Resonance Imaging
NCI	National Cancer Institute
NXDC	NX Development Corporation
NPV	Negative Predictive Value
PD	Pharmacodynamics
PK	Pharmacokinetics
p.o.	Oral
PpIX	Protoporphyrin IX
PPV	Positive Predictive Value
PV	Pharmacovigilance
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SGOT	Serum Glutamic Oxaloacetic Transaminase
SGPT	Serum Glutamic Pyruvic Transaminase
SOC	Standard of Care
SUSAR	Suspected Unexpected Serious Adverse Reactions
TNBL	True Negative Blue Light
TNWL	True Negative White Light
TPBL	True Positive Blue Light
TPWL	True Positive White Light
ULN	Upper Limit of Normal
U.S.	United States
WHO	World Health Organization
WL	White Light
WOCBP	Women of Childbearing Potential

1 Protocol Synopsis

Protocol Title

A Phase 3 Multicenter Study of Gleolan (Aminolevulinic Acid Hydrochloride) to Enhance Visualization of Tumor in Patients with Newly Diagnosed or Recurrent Meningiomas

Short Title

MEN-301

Rationale

This Phase 3 open-label single-arm study is designed to investigate the safety, diagnostic performance, and clinical usefulness of the imaging agent Gleolan® (Aminolevulinic Acid Hydrochloride, ALA HCl, ALA, 5-ALA), an orally administered imaging agent for the real time detection and visualization of meningiomas during tumor resection surgery. ALA is a prodrug that is metabolized intracellularly to form the fluorescent molecule Protoporphyrin IX (PpIX). The exogenous application of ALA leads to a highly selective accumulation of PpIX in tumor cells. Following excitation with blue light (BL) ($\lambda = 375 - 440 \text{ nm}$), the PpIX, which has accumulated selectively in tumor tissue, emits a red-violet light. This phenomenon allows for the real-time visualization of tumor tissue during resection surgery.

The primary objective of protocol NXDC-MEN-301, is to determine the proportion of participants for which Gleolan-induced PpIX fluorescence allows the surgeon to visually obtain correct information as to the presence or absence of tumor in tissue where there is uncertainty regarding that tissue's tumor status based on white light (WL) visualization alone. Types of tissue encountered during surgery for which Gleolan-induced fluorescence may provide real-time utility to the surgeon are:

- Tissue viewed under WL that the surgeon believes is unlikely to contain tumor but fluoresces under BL and is found to contain tumor on pathological examination;
- Tissue viewed under WL that the surgeon believes is likely to contain tumor but does not fluoresce and does not contain tumor on pathological examination, and;
- Tissue unexpectedly exhibiting fluorescence after the surgeon has completed the Standard of Care (SOC) meningioma resection under WL visualization in a Field of View, and is found to contain tumor on pathological examination.

These scenarios represent instances where the use of Gleolan might enhance a surgeon's ability to make a correct decision to remove or leave behind tissue during meningioma resection. The goal is to create a framework for surgeons to make better-informed decisions during meningioma tumor surgery through the use of Gleolan as a visual aid.

Objectives and Endpoints

Efficacy Objective	Endpoint/Estimand
Primary (Clinical Usefulness)	
To determine the percentage of participants for which Gleolan-induced PpIX fluorescence status allows the surgeon to visually obtain correct information as to the presence or absence of tumor in tissue where there is uncertainty regarding that tissue's tumor status based on white light WL visualization alone.	<p><i>Per Protocol Population (Primary)</i> <i>Intent-to-Treat Population (Supportive)</i></p> <p>The percentage of participants who have at least one indeterminate tissue or unexpected fluorescent End of Surgery (EOS) tissue where Gleolan-induced PpIX fluorescence status is consistent with histology.</p> <p><i>Null Hypothesis: Percentage $\leq 30\%$</i> <i>Alternative Hypothesis: Percentage $> 30\%$</i></p> <p><i>Expected Response: Percentage $\geq 50\%$</i></p>
Key Secondary Efficacy Objectives	
To determine the biopsy-level PPV of Gleolan for the real-time visualization of tissue locations on the tumor margin in newly diagnosed or recurrent meningioma during resection surgery.	<p><i>Biopsy Efficacy Analysis Population (Primary)</i> <i>All Biopsy Population (Supportive)</i></p> <p>Positive Predictive Value (PPV) of Gleolan-induced PpIX fluorescence status of biopsied tissue locations at the margin of the tumor.</p> <p>$PPV = TP_{BL} / (TP_{BL} + FP_{BL})$</p> <p><i>Null Hypothesis: PPV $\leq 60\%$</i> <i>Alternative Hypothesis: PPV $> 60\%$</i></p> <p><i>Expected Response: PPV = 80%</i></p>
To determine the biopsy-level NPV of Gleolan for the real-time visualization of tissue locations on the tumor margin in newly diagnosed or recurrent meningioma during resection surgery.	<p><i>Biopsy Efficacy Analysis Population (Primary)</i> <i>All Biopsy Population (Supportive)</i></p> <p>Negative Predictive Value (NPV) of Gleolan-induced PpIX fluorescence status of biopsied tissue location at the margin of the tumor.</p> <p>$NPV = TN_{BL} / (TN_{BL} + FN_{BL})$</p> <p><i>Null Hypothesis: NPV $\leq 40\%$</i> <i>Alternative Hypothesis: PPV $> 40\%$</i></p> <p><i>Expected Response: NPV = 60%</i></p>

Efficacy Objective	Endpoint/Estimand
Key Secondary Efficacy Objectives (continued)	
<p>To determine the participant-level PPV of Gleolan for the real-time visualization of bulk tumor in newly diagnosed or recurrent meningioma during resection surgery.</p>	<p><i>Per Protocol Population (Primary)</i> <i>Intent-to-Treat Population (Supportive)</i></p> <p>Positive Predictive Value (PPV) of Gleolan-induced PpIX fluorescence status of the single bulk tumor biopsied tissue location obtained from each study participant</p> $PPV = TP_{BL} / (TP_{BL} + FP_{BL})$ <p>Null Hypothesis: $PPV \leq 60\%$ Alternative Hypothesis: $PPV > 60\%$</p> <p>Expected Response: $PPV = 80\%$</p>
<p>To determine the biopsy-level diagnostic accuracy of meningioma identification with (i) Gleolan-induced PpIX fluorescence status under BL vs. (ii) visualization under WL, among indeterminate tissue and unexpected fluorescent EOS tissue locations, as assessed by the operating surgeon.</p>	<p><i>Biopsy Efficacy Analysis Population (Primary)</i> <i>All Biopsy Population (Supportive)</i></p> <p>Diagnostic accuracy of Gleolan-induced PpIX fluorescence status among indeterminate tissue and unexpected fluorescent EOS tissue locations is at least 20% greater than the diagnostic accuracy of the surgeons' assessment of indeterminate tissue and unexpected fluorescent EOS tissue locations under WL:</p> $Diagnostic\ Accuracy_{BL} - Diagnostic\ Accuracy_{WL} \geq 20\%$ $\left[\frac{(TP_{BL} + TN_{BL})}{(TP_{BL} + TN_{BL} + FP_{BL} + FN_{BL})} \right] * 100 - \left[\frac{(TP_{WL} + TN_{WL})}{(TP_{WL} + TN_{WL} + FP_{WL} + FN_{WL})} \right] * 100 \geq 20\%$ <p>Null Hypothesis: $DA_{BL} - DA_{WL} \leq 20\%$ Alternative Hypothesis: $DA_{BL} - DA_{WL} > 20\%$</p> <p>Expected Response $\geq 30\%$</p>

Other Secondary Efficacy Objectives	Endpoint/Estimand
To further determine the biopsy-level diagnostic performance of Gleolan-induced PpIX fluorescence status for the real-time visualization of meningioma during resection surgery.	<p><i>Biopsy Efficacy Analysis Population (Primary)</i> <i>All Biopsy Population (Supportive)</i></p> <p>Diagnostic performance of Gleolan-induced PpIX fluorescence status will be computed for indeterminate tissue biopsies and unexpected fluorescent EOS tissue biopsies (combined).</p> <ul style="list-style-type: none"> • Biopsy-level sensitivity • Biopsy-level specificity <p><i>Expected Responses > 70%</i></p>
To demonstrate that the biopsy-level concordance between the Biopsy Review Panel with the operating surgeon is better with BL visualization of Gleolan-induced PpIX fluorescence status than with WL visualization.	<p><i>Biopsy Efficacy Analysis Population</i></p> <p>The concordance between the Surgeon and Biopsy Review Panelists' assessment of white light (WL) visualization to identify tissue as likely or unlikely to be meningioma among <u>indeterminate tissues</u>.</p> <p>Concordance_{WL} of (Majority Biopsy Review Panelists) and (Surgeon) - Fleiss Kappa < 0.4</p> <p><i>Expected Response < 0.4 (Fleiss Kappa)</i></p>
	<p><i>Biopsy Efficacy Analysis Population</i></p> <p>The concordance between the Surgeon and Biopsy Review Panelists' assessment of blue light (BL) visualization to identify fluorescence status of <u>indeterminate tissues</u>.</p> <p>Concordance_{BL} of (Majority Biopsy Review Panelists) and (Surgeon) - Fleiss Kappa > 0.6</p> <p><i>Expected Response > 0.6 (Fleiss Kappa)</i></p>

Overall Design

This Phase 3 open-label single-arm study is designed to investigate the safety, diagnostic performance, and clinical usefulness of Gleolan for the real time detection and visualization of meningiomas during tumor resection surgery. The study is planned to run for approximately 15 months with individual study participation lasting for approximately 2 months (to follow up through 6 weeks post-surgery).

Patients about to undergo resection for suspected meningioma [World Health Organization (WHO) Grade I, II, III] will sign informed consent and will be screened to assess study eligibility. Eligible

study participants will receive an oral solution of Gleolan (20 mg/kg body weight) ~3 hours, (target range 2-4 hours) prior to anesthesia, and then undergo surgery for meningioma resection. During the surgery, the surgeon will use a microscope equipped with WL and BL for visualization of Gleolan-induced PpIX fluorescence for the selection of protocol-driven tissue locations and to assess fluorescence status (for further detail see Definitions and Terms for this Protocol in [Table 2](#)).

It is not expected that the level of fluorescence will vary by tumor grade, and there will be no plan to enroll a proportion of patients across tumor grades.

Study participants will be evaluated within 48 hours post procedure, 2 weeks post procedure, and 6 weeks post procedure for study safety assessment.

Number of Participants

Planned enrollment in the study is 100 participants in the Per Protocol Population. The primary efficacy endpoint is the proportion of participants who have at least one indeterminate tissue or unexpected fluorescent EOS tissue where Gleolan-induced PpIX fluorescence status is consistent with histology as determined by the central histology neuropathologist. This endpoint will be derived by counting the number of participants in the Per Protocol Population who have at least one true positive or true negative result (a success) with respect to the histology result. The primary efficacy analysis will be based on the Per Protocol Population (see [Table 13](#) for description of Analysis Populations).

NXDC believes that if a minimum of 30% of study participants achieve the primary efficacy endpoint, this proportion is clinically meaningful. This means that at least 30% of the study participants will have at least one indeterminate or unexpected fluorescent EOS tissue where Gleolan-induced PpIX fluorescence status is consistent with histological assessment of meningioma. With 100 participants included in the Per Protocol Population the lower bound of a 95% confidence interval of the success rate will be 40.4% using a Wilson (score) confidence interval. This lower bound is considered clinically meaningful in this population.

Intervention Groups and Duration

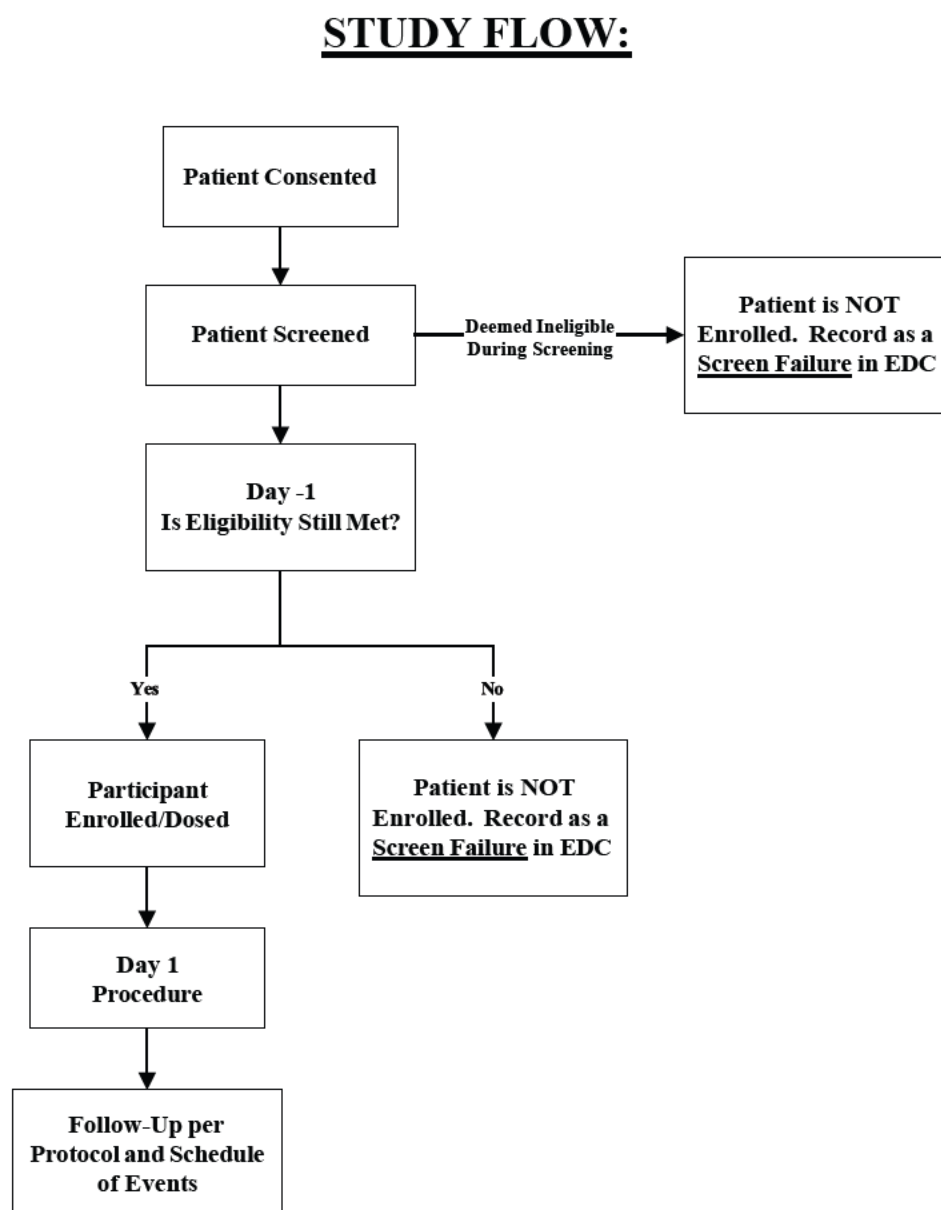
This is a single-arm study; all participants will receive Gleolan. Participants will be evaluated up to 6 weeks post-surgery for study safety assessment.

Data Safety Monitoring Board

A Data Safety Monitoring Board (DSMB) will be utilized for this study (see [Section 9.7](#)).

1.1 Schema

Figure 1. Overall Study Schema



1.2 Schedule of Events

Table 1. Schedule of Events

Study Assessment or Procedure	Baseline	Day -1 ^a	Day 1	Day 1	Day 1	Day 3	Week 2	Week 6 End of Study
	≤ 30 days before procedure	≤ 24hrs pre-procedure	Pre-Gleolan dose	Pre-procedure DOSING	Post-Gleolan dose through-post-procedure	48 hours post-procedure (± 24 hours)	14 days post-procedure (± 4 days)	43 days post-procedure (± 7 days)
Informed Consent ^b	X							
Eligibility Confirmation	X	X						
Demographics and Medical History	X							
Medical History Follow-up						X	X	X
Adverse Event Evaluation	X	X	X	X	X	X	X	X
Concomitant meds	X	X		X		X	X	X
Physical Exam	X					X	X	
Neurological Exam	X	X				X	X	X ^c
Vitals ^d	X	X	X		X	X	X	X ^c
CBC ^m , CMP ^{e, m}	X ^m	X ^m				X	X	X
Brain Magnetic Resonance Imaging (MRI) ^f	X ^f					X ^f		
Pregnancy Test ^g	X	X						
Gleolan ^{h,i,j}				X ^{h,i}				
Tissue Sample Collection(s) ^{k,l}					X			
Karnofsky Performance Scale ^j	X	X						X

- a. If Baseline assessment/procedure has been completed in ≤ 24hrs pre-procedure, that assessment/procedure does not need to be redone at Day -1
- b. Informed consent must be obtained prior to any study-related screening procedures
- c. Not required if visit completed remotely
- d. Height is only collected at screening visit. The weight for dose calculation can be collected on Day -1/Day 1, does not need to be repeated on the Day 1, if collected on Day -1
- e. CBC= Complete Blood Count, CMP= Complete Metabolic Panel (including glucose, calcium, BUN= Blood urea nitrogen, sodium, creatinine, potassium, chloride, CO₂ (Optional), total protein, albumin, AST=aspartate aminotransferase, ALT=alanine transaminase, BIL= Bilirubin, alkaline phosphatase)
- f. Baseline MRI is SOC and will be used to determine eligibility for participation in study. Please note, window for Baseline MRI is ≤90 days pre-procedure. Day 3 post procedure MRI only collected if site SOC, not protocol mandated
- g. Urine or blood pregnancy test for Women of Childbearing Potential (WOCBP). Type of test dependent on site's SOC
- h. Gleolan is dosed as 20 mg/kg. Oral administration is one time only and based on actual weight on day of surgery or Day -1 weight
- i. Gleolan to be dosed 3 hours (target range 2-4 hours) prior to anesthesia
- j. Performed by delegated study personnel
- k. Maximum of 10 per protocol tissues per participant (Up to 6 indeterminate, up to 3 unexpected fluorescent EOS and 1 bulk tumor)
- l. Performed by study surgeon
- m. Baseline CBC and CMP are to be collected ≤ 30 days prior to procedure and will be used to determine eligibility for participation in study. Day -1 CBC and CMP will only be collected per Investigator discretion if deemed necessary for SOC.

2 Introduction

This Phase 3 open-label single-arm study is designed to investigate the safety, diagnostic performance, and clinical usefulness of the imaging agent Gleolan™ (Aminolevulinic Acid Hydrochloride, ALA HCl, ALA, 5-ALA), an orally administered optical imaging for the real time detection and visualization of meningiomas during tumor resection surgery. ALA is a prodrug that is metabolized intracellularly to form the fluorescent molecule PpIX. The exogenous application of ALA leads to a highly selective accumulation of PpIX in tumor cells. Following excitation with BL ($\lambda = 375 - 440$ nm), the PpIX, which has accumulated selectively in tumor tissue, emits a red-violet light. This phenomenon allows for the real-time visualization of tumor tissue during resection surgery.

NX Development Corp (NXDC) is the Sponsor of this protocol for the investigation of Gleolan for use with meningioma resection. Gleolan is approved for marketing in the United States (U.S.) as an optical imaging agent for use in patients with glioma (suspected WHO Grades III or IV on preoperative imaging) as an adjunct for the visualization of malignant tissue during surgery [1]. Gleolan is the same as the authorized medicinal product Gliolan®, initially authorized by the European centralized procedure in September 2007 (EMA/H/C/744). Gliolan is indicated in adults for visualization of malignant tissue during surgery for malignant glioma (WHO grade III and IV) [2].

2.1 Scientific Rationale

Data from the scientific literature support that Gleolan may be safe and efficacious for the intraoperative visualization of newly diagnosed and recurrent meningioma of all histological grades [3-7]. Meningiomas primarily are benign tumors, frequently with defined borders and often enabling complete surgical removal, which offers the best chance for a cure. In SOC surgery, the neurosurgeon opens the skull through a craniotomy to enable full access to the meningioma. The goal of surgery is to remove the meningioma completely, including the tumor extensions that attach it to the meningeal coverings of the brain and bone provided that the resection can be performed safely. In some cases, complete removal can carry potential risks that may be significant, especially when the tumor has invaded brain tissue or surrounding blood vessels[8]. During a typical resection, the neurosurgical microscope is placed for viewing an area of tissue (e.g., a field of view) that the surgeon then resects as completely as possible before repositioning the neurosurgical microscope to another field of view. Most surgeons employ a process for meningioma tumor removal that generally entails 4 stages:

- Stage 1: Detaching the tumor from the dural attachments;
- Stage 2: Removing the bulk tumor (e.g., remove gross tumor);
- Stage 3: Dissecting the tumor capsule from adjacent neurovascular elements; and
- Stage 4: Evaluation of each field of view to ensure that all tumor has been removed.

Preoperative imaging often cannot fully distinguish areas of tumor infiltration. Preoperative magnetic resonance imaging (MRI) images of meningiomas often show changes to the dural tail where areas of gadolinium enhancement taper into surrounding dura but, the nature of these changes

are not always clear to the operating surgeon. Sometimes these changes are due to tumor infiltration, and other times are due to inflammation and/or hyperemia [9].

Tumor tissue that has infiltrated or attached to critical neurologic or vascular structures presents an extreme challenge during resection. Examples include tumor in the cranial nerve foramina such as the trigeminal and optic canal, as well as cranial fossae, sinuses, and vasculature. The use of Gleolan-induced fluorescence may enable the surgeon to more clearly determine if tissue should be removed or not and to make more well-informed decisions. Several publications discussed in [Section 2.3](#) detail examples of clinical usefulness, authored by surgeons who have evaluated Gleolan for meningioma in previous studies.

The situation is more confounding in recurrent meningiomas, for example, because post-surgical and post radiation scar tissue can be difficult to discriminate from recurrent tumor. It may not always be in the patient's best interest to remove scar tissue and to resect it away from attached brain tissue. Misinterpretation of scar or other adjacent tissue not invaded by meningioma cells may lead to over- or under resection under conventional WL surgery.

The primary objective of protocol NXDC-MEN-301, is to determine the proportion of participants for which Gleolan-induced PpIX fluorescence allows the surgeon to visually obtain correct information as to the presence or absence tumor in tissue where there is uncertainty regarding that tissue's tumor status based on WL visualization alone. Types of tissue encountered during surgery for which Gleolan-induced fluorescence may be useful to the surgeon are described below.

- Tissue viewed under WL that the surgeon believes is unlikely to contain tumor but fluoresces under BL and is found to contain tumor on pathological examination;
- Tissue viewed under WL that the surgeon believes is likely to contain tumor but does not fluoresce and does not contain tumor on pathological examination, and;
- Tissue unexpectedly exhibiting fluorescence after the surgeon has completed the SOC meningioma resection under WL visualization in a Field of View, and is found to contain tumor on pathological examination.

These scenarios represent instances where the use of Gleolan might enhance a surgeon's ability to make a correct decision during meningioma resection. The goal is to create a framework for surgeons to make better-informed decisions during brain tumor surgery through the use of Gleolan-induced fluorescence as a visual aid.

Real-time visualization of Gleolan-induced fluorescence provides clinical usefulness by helping the surgeon more accurately discriminate tumor from adjacent non-tumor non-central nervous system tissues or scar tissues more reliably than conventional WL intraoperative assessment. Fluorescence information is helpful at the end of conventional WL surgery to identify residual tumor that was undetected and can be safely resected once visualized, or that is inappropriate for surgical resection but could be treated by adjuvant postsurgical therapies (e.g., radiosurgery). Real-time visualization of Gleolan-induced fluorescence can also be helpful throughout the course of surgery to distinguish tumor from non-tumor.

2.2 Summary of Nonclinical Testing of ALA

Numerous nonclinical studies were performed during ALA development to characterize the pharmacodynamics (PD), pharmacokinetics (PK), and toxicological profiles of ALA to support its approved use as a single-dose, oral imaging agent for malignant glioma [10]. Preclinical evaluation of systemically administered ALA revealed the liver to be the target organ, with hepatotoxic effects present after repeated dosing. There are potential risks with regard to gastrointestinal (GI), cardiovascular and renal function, as well as a significant but short-term photosensitization. The existing nonclinical database on ALA has clearly characterized the risk associated with its use [1, 2], and demonstrates a reasonable safety profile to support its intended single use at a dose of 20 mg/kg dose for the visualization of meningioma during resection surgery. Light-protective measures for skin, however, are recommended for 48 hours after ALA dosing. Additional detail on the available nonclinical data is described in the Investigator's Brochure (IB) [10].

2.3 Background on ALA in Meningioma Resection

The sensitivity of Gleolan-induced fluorescence to visualize meningioma is described in the literature as ranging from 77% to 96%. In most studies there is no difference in sensitivity of Gleolan-induced fluorescence between patients with tumors of different grades (WHO I to III). The majority of cases studied were Grade I meningiomas, which are the most frequently occurring. Potapov et al. (2018) conducted a meta-analysis that included data on meningioma visualization from Marbacher et al. (2014), Millesi et al. (2016) and data from their own study [5, 11, 12]. In total, data from 581 meningioma patients was reviewed, and demonstrated patient level sensitivity of Gleolan-induced fluorescence.

Three peer-reviewed publications provide data on the PPV at the biopsy level in meningioma patients after ALA administration. These studies show that if there is fluorescence under BL following oral administration of ALA, there is corresponding meningioma tumor defined by histopathology in at least a single biopsy in greater than 95% of cases [3, 6]. Two of the studies evaluate the PPV of tissue fluorescence in meningioma [3, 7] and both report a very high PPV (95-100%). The study by Della Puppa (2014) concerns fluorescence of infiltrating meningioma in the bone, and, also in this case, fluorescence indicates the presence of tumor cells with a very high accuracy (100%) [6].

As summarized in the scientific literature, meningiomas are often relatively well circumscribed and the primary treatment modality is tumor resection. However, tumor recurrence can occur and is associated with incomplete resection, bone invasion, involvement of the dura, and the presence of peritumoral edema [4]. ALA is an important tool not only for intraoperative real time visualization of the primary tumor but also of any remnants of tumor, of any satellite lesions, and of any infiltrating tissue in the meninges, sinuses and brain parenchyma. ALA use in meningioma fluorescence-guided surgery helps discriminate tumor from non-tumor cranial and intracranial noncerebral tissue [e.g., bone, scar, dura (e.g., the tissue with which meningioma is associated)]. The use of Gleolan allows the surgeon to have greater confidence that tumor tissue has been effectively removed.

2.4 Benefit/Risk Assessment

More detailed information about the known and expected benefits and risks and reasonably expected adverse events (AEs) of Gleolan-induced fluorescence guided surgery may be found in the IB, Gleolan package insert and Gliolan Summary of Product Characteristics [1, 2].

In general, risks associated with the use of Gleolan arise in two different ways. Administration of Gleolan itself might induce adverse toxicological reactions, including photosensitivity and elevated liver enzymes following single dosage. It is possible that Gleolan-induced fluorescence might lead the surgeon to perform tissue resections in regions that could induce temporary or permanent neurological deficit. However, additional or more precise knowledge about whether or not tissue is likely to be tumor can help the surgeon make more informed decision beyond what can be achieved with WL only.

2.4.1 Known Potential Risks

The clinical development program which lead to marketing authorization of ALA included 527 high-grade glioma patients. These data demonstrated that ALA does not cause clinically meaningful side effects after a single oral dose of 20 mg/kg. Adverse reactions that occurred in >1% of patients in the week following surgery were pyrexia, hypotension, nausea, and vomiting. Adverse reactions occurring in the first 6 weeks after surgery in <1% of patients were chills, photosensitivity reaction, solar dermatitis, hypotension, abnormal liver function test, and diarrhea [1, 2].

The toxicological safety of Gleolan for preoperative administration to meningioma patients at a dose of 20 mg/kg is supported by safety data that led to the approval of Gleolan for the intraoperative visualization of glioma, by post marketing surveillance data from over 100,000 patients who received the drug worldwide for the visualization of high grade glioma, and from data presented in peer-reviewed publications of clinical investigations of the use of ALA to visualize meningioma tumor tissue.

Safety assessments in this protocol using Gleolan in meningioma patients will focus on the AEs and warnings established in marketed ALA [1, 2] with special attention to any differences in meningioma patients compared to high grade glioma patients.

As is true for the use of ALA in glioma patients, assessments of the safety of Gleolan administration must be differentiated from AEs associated with the resection procedures. Use of Gleolan-induced fluorescence during surgery may result in resection of sensitive regions which could lead to an increased risk of additional deficits. The real time decision to resect this fluorescent tissue is complex and involves the surgeons' best medical judgement for each patient. This sound professional judgement is paramount to mere suspicion of seeing tumor and includes the surgeon's knowledge of anatomy and the function of structures. Additional technical adjuncts, such as neuromonitoring, the use of the Microdoppler, fluorescence-angiography with Indocyanine Green (ICG) and neuronavigation, the latter being used for assessing the proximity to sensitive structures, provided that the registration accuracy is high enough, will help with the complex decision of whether to resect or not. Fluorescence must be regarded as an additional aid in this multifactorial decision.

2.4.2 Known Potential Benefits

ALA guided fluorescence is the only intraoperative imaging method that provides real-time information to the surgeon during resection; all others require interrupting the surgery to acquire information. Switching from the standard WL to the BL fluorescent mode on the microscope is performed with the push of a button, minimizing disruption to the workflow of surgery. Evidence from the published literature supports the hypothesis ALA can be used in the visualization of meningioma (see [Section 2.1](#) and IB) [10]. This is not the only possible advantage. During SOC WL surgery, the surgeon makes multiple decisions regarding whether tissue is likely or unlikely to be tumor at a transition zone between tumor and adjacent normal tissue, which further directs the surgery. The addition of Gleolan-induced fluorescence may enhance a surgeon's ability to determine that tissue is likely or unlikely to be tumor. Without the use of Gleolan, ambiguous or indeterminate tissues result in potential over- or under-resection at each point and inhibit the workflow of surgeons.

As summarized in the scientific literature, meningiomas are often relatively well circumscribed and the primary treatment modality is tumor resection. However, tumor recurrence can occur and is associated with incomplete resection, bone invasion, involvement of the dura, and the presence of peritumoral edema [4]. ALA is an important tool not only for intraoperative real time visualization of the primary tumor, but also for enhanced visualization of any remnants of tumor, of any satellite lesions, and of any infiltrating tissue in the meninges, sinuses and brain parenchyma. ALA use in meningioma fluorescence-guided surgery helps discriminate tumor from non-tumor cranial and intracranial noncerebral tissue (e.g., bone, scar, dura [e.g., the tissue with which meningioma is associated]). Even if fluorescing tissue could not be removed due to functional considerations, the knowledge of such residual tumor may help determine whether adjuvant therapies (radiosurgery, radiotherapy) are indicated and where such therapies should be anatomically focused. Residual tumor is not necessarily visible on postoperative MRI. The use of Gleolan allows the surgeon to have greater confidence that tumor tissue has been effectively removed.

2.4.3 Overall Benefit: Risk Conclusion

The existing body of nonclinical and clinical data on the use of ALA indicates that AEs should occur infrequently, however, these will be carefully monitored during the study. This includes phototoxicity for which the participant should follow basic light protection procedures during the postoperative course for 48 hours. Risks related to the surgery will be similar to the risks of surgery without fluorescence. The use of ALA-induced fluorescence can provide the surgeon with information that may influence the decision whether or not to resect a piece of tissue. Even when believing that an indeterminate area of tissue is indeed tumor, the surgeon will first consider the safety of resecting, based on the anatomical location and other technical adjuncts to surgery, as is standard practice. Based on the available scientific literature on the use of ALA-induced fluorescence in meningioma surgery it is expected that an individual patient's possible benefits will outweigh any of the minor known side effects or procedural risks involved.

3 Objectives, Endpoints and Study Terminology

The objectives and endpoints in this study utilize the definitions and terminology described in Table 2 below.

Table 2. Definitions and Terminology for this Protocol

Term	Definition
White light (WL)	Operating with a surgical microscope using WL illumination
Blue light (BL)	Operating with a surgical microscope adapted with a BL emitting light source and ancillary excitation and emission filters to visualize fluorescence excitation in the wavelength of 375 to 440 nm and for observation of porphyrins which fluoresce between 620 and 710 nm. Filters transmit porphyrin fluorescence as red-violet, as well as a fraction of backscattered blue excitation light necessary for distinguishing non-fluorescing tissue.
End of WL surgery microscopic field of view (End of Surgery [EOS] Field of View)	During a typical resection, the neurosurgical microscope is positioned to view an area of tissue (e.g., a field of view). The surgeon resects meningioma in that field of view as completely as possible before repositioning the neurosurgical microscope to another field of view. The EOS Field of View is defined by the surgeon having completed WL resection and declaring the tissue to be free of residual tumor in a particular Field of View. EOS Fields of View can be of the bone flap or tumor resection cavity.
Bulk tumor tissue	A single tissue location from the bulk tumor assessed for fluorescence status and collected for histology. This tissue location should be biopsied prior to removing the bulk tumor.
Indeterminate tissue	Tissue location viewed under WL visualization that the operating surgeon cannot conclusively determine is or is not meningioma tumor. Up to 6 indeterminate tissues may be collected per participant. In this study, surgeons will record if they consider the indeterminate tissue to be likely or unlikely meningioma tumor prior to turning on the BL.
Unexpected fluorescent tissue observed at end-of-WL-surgery (Unexpected Fluorescent EOS)	As the surgeon completes resection under white light in an EOS Field of View, a tissue sample may be taken from a location that unexpectedly exhibits Gleolan-induced PpIX fluorescence after excitation with BL. Unexpected fluorescent EOS tissues may be observed in (a) tumor cavity and/or (b) bone flap, however, unexpected fluorescent EOS tissues do not include tumor tissue visualized under WL that the surgeon could not safely remove.
Photo/Video	A short segment of video, where suction and rinsing may be used to obtain a clear image of a bulk, indeterminate, or unexpected fluorescent EOS tissue location. This is to be recorded under WL and under BL. During video acquisition under WL and BL the neuronavigation probe will be used to indicate the tissue location of interest. Photographs may be captured during video collection or obtained from the video in post-surgical image processing.

Term	Definition
True Positive ^{blue light} (TP _{BL})	Tissue with Gleolan-induced PpIX fluorescence that is confirmed to be meningioma by the central histology neuropathologist.
False Positive ^{blue light} (FP _{BL})	Tissue with Gleolan-induced PpIX fluorescence that is determined by the central histology neuropathologist not to contain meningioma cells (non-neoplastic tissue).
True Negative ^{blue light} (TN _{BL})	Tissue with <i>no</i> Gleolan-induced PpIX fluorescence that is determined by the central histology neuropathologist to contain no meningioma cells (non-neoplastic tissue).
False Negative ^{blue light} (FN _{BL})	Tissue with <i>no</i> Gleolan-induced PpIX fluorescence that is confirmed to be meningioma by the central histology neuropathologist.
True Positive ^{white light} (TP _{WL})	Tissue identified as meningioma under WL visualization that is confirmed to be meningioma by the central histology neuropathologist.
False Positive ^{white light} (FP _{WL})	Tissue identified as meningioma under WL visualization that is determined by the central histology neuropathologist to contain no meningioma cells (non-neoplastic tissue).
True Negative ^{white light} (TN _{WL})	Tissue identified as non-tumor under WL visualization that is determined to contain no meningioma cells (e.g., non-tumor tissue).
False Negative ^{white light} (FN _{WL})	Tissue identified as non-tumor under WL visualization that is confirmed to be meningioma by the central histology neuropathologist.
Positive Predictive Value (PPV)	$TP_{BL}/(TP_{BL}+FP_{BL})$ Probability that tissue exhibiting Gleolan-induced PpIX fluorescence is confirmed meningioma by the central histology neuropathologist.
Negative Predictive Value (NPV)	$TN_{BL}/(TN_{BL}+FN_{BL})$ Probability that tissue not exhibiting Gleolan-induced PpIX fluorescence is not meningioma as confirmed by the central histology neuropathologist.
Sensitivity	$TP_{BL}/(TP_{BL}+FN_{BL})$ Proportion of biopsies that are meningiomas by the central histology neuropathologist that exhibit Gleolan-induced PpIX fluorescence.
Specificity	$TN_{BL}/(TN_{BL}+FP_{BL})$ Proportion of biopsies that are non-neoplastic by the central histology neuropathologist that do not exhibit Gleolan-induced PpIX fluorescence.
Biopsy-Level PPV	Analysis performed at the biopsy level.

Term	Definition
Diagnostic Accuracy _{BL} of Gleolan-induced PpIX Fluorescence Status to Identify Meningioma Tumor Tissue	$(TP_{BL}+TN_{BL})/(TP_{BL}+TN_{BL}+FP_{BL}+FN_{BL})$ <p>Proportion of biopsies correctly classified by Gleolan-induced PpIX fluorescence among all biopsies evaluated.</p>
Diagnostic Accuracy _{WL} of Meningioma Tumor Tissue Identification Under WL Visualization	$(TP_{WL}+TN_{WL})/(TP_{WL}+TN_{WL}+FP_{WL}+FN_{WL})$ <p>Proportion of biopsies correctly classified by visualization under WL among all biopsies evaluated.</p>
Biopsy Review Panel	The role of the Biopsy Review Panel is to minimize bias that could arise if surgeons choose to biopsy tissue that is <i>obviously</i> likely to be tumor or <i>obviously</i> unlikely to be tumor under WL and confirming the location of the collection of the biopsy of the tissue location. The Biopsy Review Panel is composed of independent neurosurgeons who are not participating as investigators in a study investigator's institution. Biopsy Reviewers will independently review a blinded WL and BL photo and WL video of indeterminate tissue and unexpected fluorescent EOS tissue locations. Each Biopsy Reviewer's post-surgical assessments will be compared to the operating surgeons' real-time assessments, Gleolan-induced PpIX fluorescence status, and central histopathology in defining the Biopsy Efficacy Analysis Population for demonstration of Clinical Usefulness and the Blinded Biopsy Population.
Safety Analysis Population	Participants who meet the eligibility criteria for the study and receive any amount of Gleolan.
Intent-to-Treat (ITT) Population	Participants who meet the eligibility criteria for the study and have at least one tissue included in the All Biopsy Population.
Per Protocol Population	Participants who are dosed with Gleolan who undergo tumor resection, have a central histologically confirmed meningioma (WHO Grade I, II, or III), and have at least one tissue included in the Biopsy Efficacy Analysis Population. This population will be used for determining the primary study endpoint at the participant level.
All Biopsy Population	All Biopsy Population includes all biopsies (bulk, indeterminate, or unexpected fluorescent End of Surgery (EOS) tissues), regardless of acceptance into the Biopsy Efficacy Analysis Population.
Biopsy Efficacy Analysis Population	The Biopsy Efficacy Analysis Population will be made up of indeterminate tissue biopsies that meet the criteria defined in Section 4.8.1 and of unexpected fluorescent EOS tissue biopsies that meet the criteria in Section 4.8.2 .

Term	Definition
Blinded Biopsy Population	This includes all biopsies which meet the requirements for the Biopsy Efficacy Analysis Population, without taking into account biopsy histology. This population will only be used for sample size readjustment and will remain blinded to histology status of biopsies and the diagnostic accuracy of Gleolan-induced PpIX fluorescence. The population will be used to estimate the percentage of participants with 1 or more biopsies in the Biopsy Efficacy Analysis Population, and the average number of biopsies in the Biopsy Efficacy Analysis Population per participant.

4 Study Design

This Phase 3 open-label single-arm study is designed to investigate the safety, diagnostic performance, and clinical usefulness of Gleolan for the real-time detection and visualization of meningiomas during tumor resection surgery. The study is planned to run for approximately 15 months with individual study participation lasting for approximately 2 months (from consent through follow up 6 weeks post-surgery).

Patients who are about to undergo meningioma resection may be consented by the Site PI or designee and subsequently screened for eligibility to participate. Baseline data for these participants will be collected for screening and eligibility including demographics (e.g. age, sex, race, comorbid conditions) as well as data collected during the patient's routine workup. This workup, to be completed throughout the patient's preoperative visits, will include MRI, lab work, physical exam, pregnancy status for women of childbearing potential (WOCBP) and neurological exam. Eligible study participants who have provided informed consent will be reassessed pre-procedure to ensure all requirements are still met prior to dosing of study drug.

Consented eligible study participants will receive an oral solution of Gleolan (ALA; 20mg/kg bodyweight, dissolved in tap water) 3 hours, (target range 2-4 hours) prior to anesthesia, and then undergo surgery. During surgery use of WL, BL, and procedures for data collection per protocol will be followed for selection of bulk tumor tissue, indeterminate tissue(s) and unexpected fluorescent EOS tissue(s) (as defined in [Table 2](#)). Study participants will be evaluated within 48 hours post procedure, 2 weeks post procedure, and 6 weeks post procedure for study safety assessment. During these follow up timepoints, data will be collected per the protocol schedule of events. If early postoperative MRI is SOC for study site and performed within 48 ± 24 hours, these will be collected for exploratory purposes. All participants will receive standard follow-up care and testing, regardless of study participation. A Schedule of Events noting all protocol assessments and procedures is provided in [Section 1.2](#).

All data collected for this study will be sourced at the individual site. Source data will be manually entered or uploaded into the study Electronic Case Report Forms (eCRF) for analysis.

4.1 Justification for Dose

Gleolan intended for the visualization of meningioma is the exact same drug substance and drug product as the Gleolan product Food and Drug Administration (FDA)-approved for the

visualization of high-grade glioma. Gleolan will be dosed orally in meningioma study participants at the exact same dose (20 mg/kg by mouth [p.o.]) as that approved for the visualization of glioma.

Gleolan is soluble in water and well absorbed through the GI tract. After ingestion, Gleolan is metabolized to fluorescent porphyrins, predominantly PpIX. Gleolan as drinking solution is rapidly and completely absorbed and peak plasma levels of Gleolan are reached 0.5–2 hours after oral administration of 20 mg/kg body weight. With a terminal half-life of 45 minutes, ALA plasma levels return to baseline values 24 hours after administration of an oral dose of 20 mg/kg body weight. Tissue levels PpIX lag behind ALA plasma levels and appear to reach a maximum 6-8 hours after dosing [14, 15]. Gleolan is generally given 2-4 hours prior to induction of anesthesia.

4.2 Distribution and Biotransformation

Oral ALA is taken up by the liver, kidney, endothelial cells, and skin as well as by malignant gliomas (WHO grade III and IV) and metabolized to fluorescent PpIX. The plasma protein binding of ALA is 12%. Four hours after oral administration of ALA at 20 mg/kg body weight, 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) (128 mg dose of sterile intravenous ALA vs 100 mg ALA orally) resulted in a mean half-life of 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 ALA was 50-60% with a mean C_{max} of 4.65 ± 0.94 $\mu\text{g/mL}$. Plasma PpIX concentrations were low and were detectable only in 42% of the plasma samples. And were below the level of detection (10 ng/mL) after 10 to 12 hours.

At the oral ALA dose of 20 mg/kg body weight, tumor: normal brain fluorescence ratios are usually high and provide for BL visualization of fluorescent tumor tissue as red-violet for at least 9 hours. Besides tumor tissue, faint fluorescence of the choroid plexus was reported. ALA is also taken up and metabolized to PpIX by other tissues, e.g. liver, kidneys or skin [16].

4.3 Elimination

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

4.4 Surgical Treatment and Tissue Assessment and Sample Collection Plan

4.4.1 Surgical Treatment

Treatment options and recommendations for meningioma depend on several factors, including the type, location and grade of the tumor, possible side effects of surgical or other treatment, and the patient's preferences and overall health. Study participants will be undergoing surgical treatment based on current SOC procedure for meningioma resection. Prior to this procedure the study participant will be dosed with study drug as described in [Section 6.1](#).

After surgery, study participants will be treated and evaluated per site SOC to include physical and neurological exam, MRI if SOC at study site at 48 ± 24 hours post-procedure. Sequential clinical follow up will be performed as per SOC at the treating facility. Data from these SOC follow up

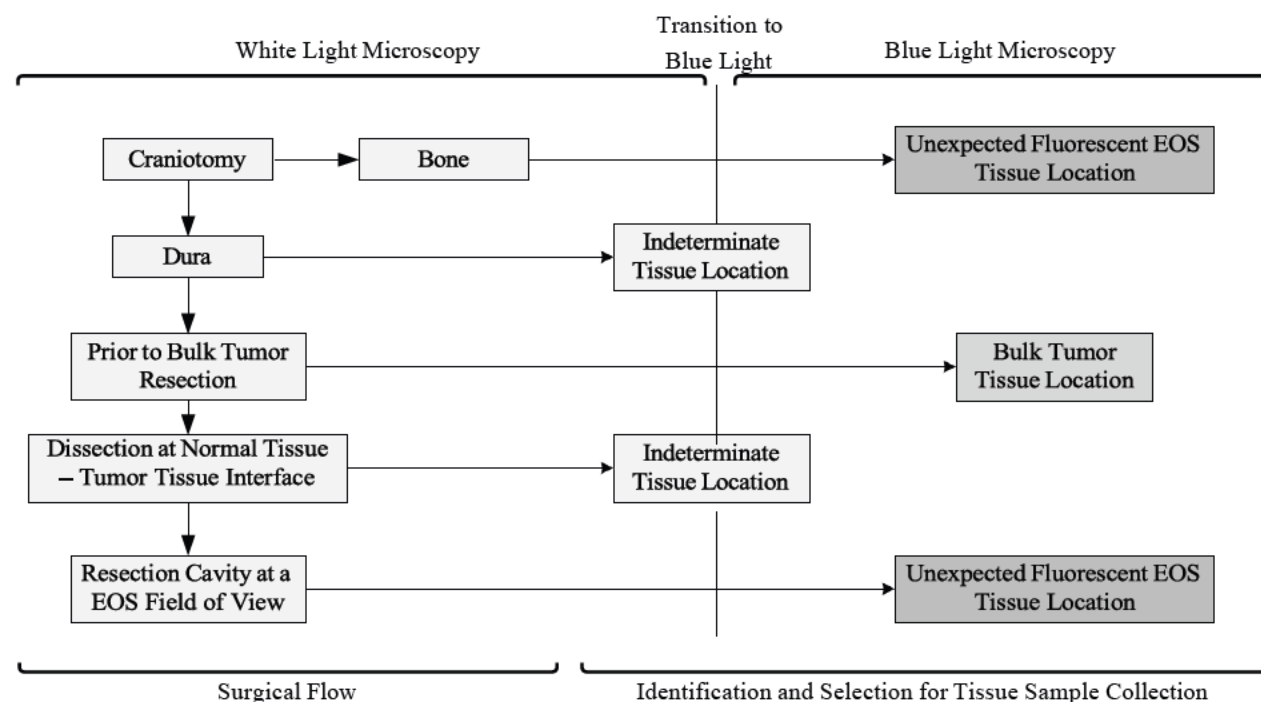
visits will be collected and deidentified to obtain follow up study data. All participants will be followed per the protocol at Week 2 and Week 6 for safety data. After the participant's end of study visit, they will continue to follow up per SOC for the treatment of their meningioma. No restrictions are imposed on subsequent therapies, such as radiotherapy.

4.4.2 Tissue Identification and Sample Collection

Tissues will be identified in the microscope field of view under WL or BL illumination (Figure 2). These tissue locations will be biopsied per protocol and will be numbered with the Pre-Assigned Tissue Location IDs (PATL-ID), which are a 4 digit randomly generated unique identifier, specific to each study participant (e.g., 01-01).

During tissue identification the surgeon will obtain photo/video of the area of interest. Each photo/video recorded will be given a unique PATL-ID to associate it with the tissue location and the time each photo/video is obtained. During the actual process of collection of tissue under WL, video is required.

Figure 2. Tissue Identification and Sample Collection



4.4.3 Procedures for Bulk Tumor Tissue Identification and Sample Collection

Under Blue Light, after removing the dura to expose the bulk tumor under WL, the surgeon will identify a location of the bulk tumor using a surgical pointer, then switch to BL to record the fluorescence status of the bulk tumor. The surgeon should then switch back to WL to take tissue sample from the identified bulk tumor location under video recording. The bulk tumor sample should be obtained for all participants, regardless of fluorescence status. Procedures for bulk tumor sample collection are summarized in [Table 3](#).

Table 3. Bulk Tumor Tissue Location Identification and Sample Collection Process

Visualization	Procedure	CRF Data Recorded
WL	Surgeon removes dura and exposes bulk tumor.	N/A
WL	Surgeon takes photo/video of bulk tumor tissue location	Label and record photo/video ID and time
BL	Surgeon identifies a bulk tumor tissue sample location under BL to assess for Gleolan-induced PpIX fluorescence	Fluorescence status recorded for bulk tumor tissue (positive/negative)
WL	Surgeon collects a tissue sample from bulk tumor under WL video (from fluorescent tissue if fluorescence is present)	Label and record video ID and time Label and record tissue location ID
WL	Surgical removal of bulk tumor continues	N/A

BL = blue light; CRF = case report form; NA = not applicable; WL = white light

4.4.4 Procedures for Indeterminate Tissue Identification and Sample Collection Under White Light

If the surgeon encounters tissue which cannot be confidently interpreted as tumor or non-tumor (e.g., defined as indeterminate tissue for this protocol) the surgeon must follow these procedures ([Table 4](#)).

1. The indeterminate tissue location under consideration, will first be evaluated under WL and the surgeon will record in the CRF if the tissue is likely or unlikely meningioma tissue and if the intention is to leave or resect that tissue. These data are recorded prior to turning on the BL to assess for fluorescence.
2. After viewing the indeterminate tissue location under BL, the surgeon will record the fluorescence status of the tissue and will record whether the intent is to resect or leave the tissue after viewing under BL has changed, and if so, to leave or resect (see Table 4).
3. The surgeon collects a tissue biopsy from the indeterminate tissue location under WL if he/she determines the location is safe for tissue sample collection, regardless of fluorescence status.

When an area of indeterminate tissue is located under WL, the surgeon will record declarations regarding the tissue location in the CRF and obtain a photo/video of the Field of View with the indeterminate tissue location identified with a pointer. The surgeon is to place the pointer (e.g., a surgical instrument) adjacent to the location and video under WL and then, without moving the point of the surgical instrument, switch to BL. Under BL, the surgeon is to record declarations of fluorescence status and surgical decision, then they can switch to WL and complete the tissue biopsy and end the video recording. The indeterminate tissue samples should be obtained, regardless of fluorescence status.

Table 4. Indeterminate Tissue Identification and Sample Collection Process

Visualization	Procedure	CRF Data Recorded
*Up to 6 distinct indeterminate tissue locations may be identified and collected per participant		
WL	Surgeon observes tissue location in the surgical microscope field of view that is indeterminate for meningioma under WL, an indeterminate tissue location is identified.	Location and time of tissue location ID (via neuronavigation) Surgeon assessment of indeterminate tissue location status as likely or unlikely tumor Surgeon's intent to resect or leave indeterminate tissue
WL	Surgeon takes photo/video of the indeterminate tissue location, using a surgical instrument as a pointer.	Label and record photo/video ID and time
BL	Surgeon views indeterminate tissue location, identified by surgical pointer, under BL to assess for Gleolan-induced PpIX fluorescence.	Fluorescence status of indeterminate tissue location is recorded (positive/negative) Surgeon's intent to resect or leave indeterminate tissue location is recorded
WL	Surgeon collects tissue sample from indeterminate tissue location under WL video recording.	Label and record video ID and time Label and record tissue location ID
WL	Surgical resection continues.	

BL = blue light; WL = white light

4.4.5 Procedures for End of Surgery Tissue with Unexpected Fluorescence Identification and Sample Collection

After completion of WL surgery in an EOS Field of View and declaration by the surgeon that all visible tumor has been safely removed, the EOS Field of View is then viewed under BL and the surgeon looks for any fluorescing tissue locations. An unexpected fluorescent EOS tissue sample should only be collected if that tissue can safely be biopsied (in the medical judgement of the surgeon). There may be circumstances when surgeons are unable to safely remove all meningioma and intentionally leave some meningioma tissue unresected. Such tissue intentionally left behind should not be treated as unexpected fluorescent EOS tissue. Unexpected fluorescent EOS tissue locations which cannot be safely resected should be noted on the eCRF.

For unexpected fluorescent EOS tissue locations identified under BL in a EOS Fields of View, if a tissue can safely be biopsied for the unexpected fluorescent tissue, the surgeon should place a surgical instrument adjacent to the unexpected fluorescing tissue as a pointer, and take WL photo/video and BL photo/video (Table 5). The surgeon should declare his/her intent to resect this tissue or leave it behind after viewing it under BL. Subsequent tissue sample collection should be

done under WL video. If an unexpected fluorescent EOS tissue location is identified and a tissue sample cannot be safely taken, a WL and BL video should still be obtained of this location.

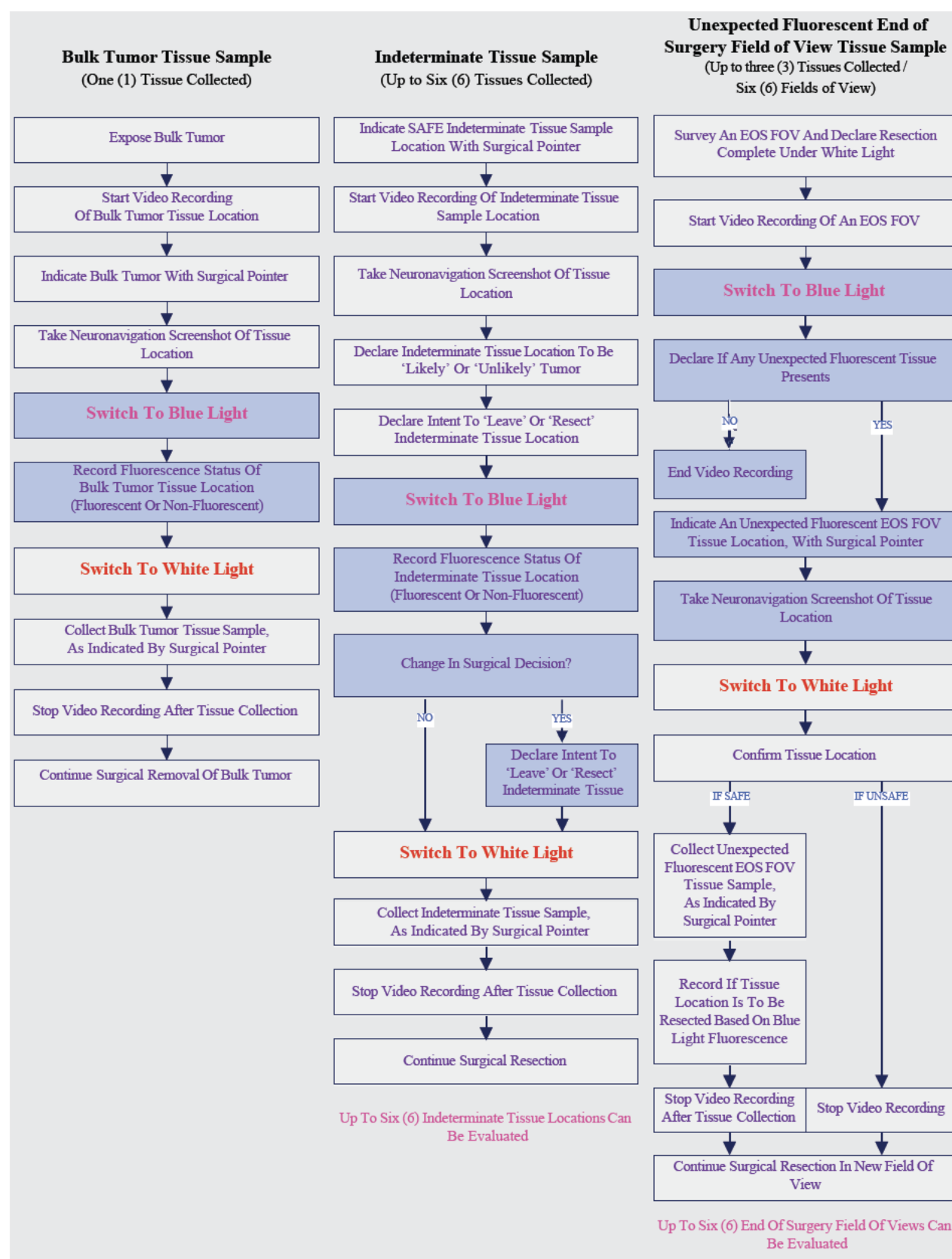
Table 5. Unexpected Fluorescent End of White Light Surgery Tissue Identification and Sample Collection Process

Visualization	Procedure	eCRF Data Recorded
*Up to 6 EOS Fields of View can be evaluated. If there is unexpected fluorescence under BL in any of the 6 EOS Fields of View, then up to 3 distinct unexpected fluorescent EOS tissues from the fluorescence areas may be collected for the study.		
WL	Surgeon surveys EOS Field of View and determines that resection is complete (all tumor that can be safely resected)	Location and time (via neuronavigation) Declare completion of safe resection
WL	Surgeon takes a photo/video of the EOS Field of View	Label and record photo/video ID and time
BL	Surgeon without changing microscope view surveys the EOS Field of View for fluorescence	Fluorescence status recorded (positive/negative)
BL	Surgeon takes a photo/video of the EOS Field of View	Label and record photo/video ID and time
BL	If fluorescence is observed in the EOS Field of View, the surgeon should select an unexpected fluorescent EOS tissue location and record fluorescence status	Fluorescence status recorded (positive/negative)
BL	Surgeon takes photo/video of tissue location, using a surgical instrument as a pointer	Label and record photo/video ID and time
WL	Surgeon takes a photo/video of tissue location, using a surgical instrument as a pointer	Surgeon's intent to resect or leave tissue
WL	Unexpected fluorescent EOS tissue sample is collected under WL video recording and preserved for pathology. (Only if surgeon feels the tissue can be safely collected.)	Label and record video ID and time Label and record tissue location ID if it can be safely collected. If it cannot be safely collected, record the inability to take unexpected fluorescent EOS tissue in the eCRF

BL = blue light; WL = white light

4.4.6 Tissue Identification and Collection Flow-Chart

An overview of the procedures for identification collection of each type of tissue and rules for use of WL and BL are illustrated in [Figure 3](#).

Figure 3. Flow-Chart of Tissue Location Identification and Tissue Sample Collection

4.5 Blinded Histopathological Analysis

Tissues biopsied per protocol will be given unique PATL-ID numbers and sent to local pathology lab for preparation of paraffin blocks. From each paraffin block, the local pathology lab will prepare three uncovered slides for shipment to the study's central neuropathology lab where they will be stained with Hematoxylin and Eosin (H&E). The slides will be read and the assessment of tumor or not tumor will be recorded for each slide in the eCRF. If the central lab neuropathologist cannot confirm the tumor status, that particular biopsy will be excluded from the Biopsy Efficacy Analysis Population. The paraffin block will be retained at the local pathology laboratory (see Manual of Procedures [MOPS] for tissue sample collection and histology processes). Histological assessment (presence or absence of meningioma cells) of each biopsied tissue location obtained per protocol will be used as the "truth standard" for determination of diagnostic performance and diagnostic accuracy of Gleolan-induced PpIX fluorescence.

Histopathological assessments may also occur, dependent on SOC, at a given clinical site. Local histopathology on frozen section(s) collected may also be performed per neurosurgeon preference. These assessments will not use any of the protocol-driven tissue locations.

4.6 End of Study Definition

A participant is considered to have completed the study if he/she has completed all phases of the study including the final study visit, or if the participant or Investigator withdraws him/her from the study prior to final visit. Lost to Follow Up (LTFU) will also be considered end of study for such participants. A participant will be considered LTFU after three documented phone calls have been made and a certified letter is sent. The end of the study date is defined as the date of the final visit per schedule of events or the date withdrawn or LTFU. Day 1 data that is complete from study participants that are LTFU will be used for Per Protocol Population and Biopsy Efficacy Analysis Population endpoints. Participants that are LTFU or withdrawn prior to end of study will not be replaced.

4.7 Biopsy Review Panel and Bias Minimization

The primary role of the Biopsy Reviewers is to minimize bias that could arise due to a surgeon's assessment of indeterminate tissue or unexpected fluorescent EOS tissue under WL. For example, if (a) the surgeon selects an indeterminate tissue and declares it to be likely tumor and (b) all Biopsy Reviewers also assess the tissue to be likely tumor, and (c) the tissue exhibits Gleolan-induced fluorescence, and (d) histology confirms the tissue is tumor.

The secondary role of the Biopsy Reviewers is to confirm the accuracy of the tissue collection process. The Panel will confirm that Investigators took a biopsy from the same location initially identified as indeterminate or unexpected fluorescent EOS tissue. The Biopsy Review Panel will be composed of independent neurosurgeons who are not participating as investigators nor affiliated with any of the study investigators institutions.

The Biopsy Review Panel will be given independent online access to four sequential sessions where photos, videos and minimal data about the participant will be provided for evaluation ([Table 6](#)).

The Biopsy Review Panelists will record their assessments. The purpose of each of the four sessions is described below:

- Session 1 objective: Evaluate the WL visualization of indeterminate tissues and declare whether Biopsy Review Panelists consider each indeterminate tissue to be likely or unlikely tumor. The results of Session 1 will be used in the evaluation criteria for selecting indeterminate tissue for the Biopsy Efficacy Analysis Population (see [Section 4.8.1](#))
- Session 2 objective: Evaluate a WL EOS Field of View image to identify location(s) of likely tumor tissue, if any. Biopsy Review Panelists will be provided a WL image of the field of view. Each panelist will independently identify up to 5 locations, if any, in the Field of View which are likely tumor. The results from Session 2 will be used in the evaluation criteria for selecting unexpected fluorescent EOS tissues for the Biopsy Efficacy Analysis Population (see [Section 4.8.2](#)).
- Session 3 objective: Evaluate the presence or absence of fluorescence in a BL image with an identified indeterminate tissue or unexpected fluorescent EOS tissue location. The data will be used in analysis of exploratory endpoint(s) in the Statistical Analysis Plan (SAP; see [Section 9](#))
- Session 4 objective: Confirm that the tissue location identified in WL photo/video with a pointer by the surgeon is the tissue that was biopsied for histology. Each Biopsy Review Panelist will make an independent assessment which will be used as a criteria in selecting indeterminate tissue (see [Section 4.8.1](#)) or unexpected fluorescent EOS tissue (see [Section 4.8.2](#)).

4.7.1 Biopsy Review Panel Data Evaluation and Assessment Output

Table 6 Biopsy Review Sessions

Session 1	White Light (WL) Indeterminate Session
Information provided to Biopsy Review Panel: <ul style="list-style-type: none"> • WL Image with Tissue Location as Indicated by Surgeon • WL Video of Tissue Location • MRI Neuronavigation Screen Shot of Tissue Location, if available • Anatomical description of tumor location • De novo / recurrence status of tumor • Patient age 	
Action by Biopsy Review Panel: <ul style="list-style-type: none"> • Each tissue location is assessed as likely or unlikely tumor 	Use of this Panel Action for Evaluation of Tissue Location for Inclusion in the Biopsy Efficacy Analysis Population:

	Each Biopsy Review Panelist's independent assessment is one of six criteria for indeterminate tissue inclusion (see Section 4.8.1).
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Session 2	White Light (WL) Unexpected Fluorescent EOS Session	
Information provided to Biopsy Review Panel: <ul style="list-style-type: none">• WL image of each EOS field of view with and without grid overlay• WL Video of EOS Field of View• Neuronavigation Screenshot of associated Bulk tumor location, if available• WL Video of associated Bulk tumor• Anatomical description of tumor location• De novo / recurrence status of tumor• Patient age		
Action by Biopsy Review Panel: <ul style="list-style-type: none">• Each field of view is assessed for coordinates of the grid which likely contain tumor tissue.• If any area of likely tumor is identified, the coordinate(s) of each grid location will be entered into eCRF (up to 5 grid locations which are likely tumor as assessed independently by each Panelist)		Use of this Panel Action for Evaluation of Tissue Location for Inclusion in the Biopsy Efficacy Analysis Population: Each Biopsy Review Panelist’s independent identification of a grid location(s) will be compared with the location where an unexpected fluorescent EOS tissue collected. This paired assessment is one of three criteria for unexpected fluorescent EOS tissue inclusion (See Section 4.8.2).

Session 3	Blue Light (BL) Session	
Information provided to Biopsy Review Panel: <ul style="list-style-type: none">• BL image with identified indeterminate tissue or unexpected fluorescent EOS tissue location• BL Video of Tissue Location		
Action by Biopsy Review Panel: <ul style="list-style-type: none">• Each indeterminate tissue or unexpected fluorescent EOS tissue location is assessed for fluorescence status (yes/no)		Use of this Panel Action for Evaluation of Exploratory Endpoints in the Statistical Analysis Plan (see Section 9).

Session 4	White Light (WL) Biopsy Location Review
Information provided to Biopsy Review Panel: <ul style="list-style-type: none">• WL Image with an indeterminate tissue or unexpected fluorescent EOS tissue location• WL Video of an indeterminate tissue or unexpected fluorescent EOS tissue collection• BL Image with an indeterminate tissue or unexpected fluorescent EOS tissue location	

Session 4	White Light (WL) Biopsy Location Review
<p>Action by Biopsy Review Panel:</p> <ul style="list-style-type: none"> Confirmation that the tissue collection location from which indeterminate tissue or unexpected fluorescent EOS tissue in the photo/video is the location identified by the surgeon when WL and BL declarations were made. 	<p>Use of this Panel Action for Evaluation of Tissue Location for Inclusion in the Biopsy Efficacy Analysis Population:</p> <p>Confirmation by the majority of the three Biopsy Review Panelists that the tissue location identified in WL photo/video with a pointer by the surgeon is the tissue that was collected for histology</p>

4.8 Criteria for Inclusion in the Blinded Biopsy Population and Biopsy Efficacy Analysis Population

The Biopsy Efficacy Analysis Population will be made up of indeterminate tissues and unexpected fluorescent EOS tissues that meet their criteria described below ([Figure 4](#)).

Participants in the Per Protocol Population will also be included in the Biopsy Efficacy Analysis Population if they have at least one indeterminate or unexpected fluorescent EOS tissue location. The Biopsy Efficacy Analysis Population will be utilized for diagnostic performance and diagnostic accuracy analyses on a biopsy level.

The results of the Biopsy Review Panel are used to determine the inclusion of indeterminate and Unexpected Fluorescent EOS tissue locations in the Blinded Biopsy Population and the Biopsy Efficacy Analysis Population as shown in Figure 4.

4.8.1 Indeterminate Tissues in Biopsy Efficacy Analysis Population

Indeterminate tissues in the Biopsy Efficacy Analysis Population will be defined based on assessments of the operating surgeon, each of the three Biopsy Review Panelists (who are blinded to the surgeon's intraoperative decision), and the central histology neuropathologist. Biopsy Review Panelists will independently perform a blinded post-surgical review of the WL photo and video for each indeterminate tissue. Histological assessment of each indeterminate biopsied tissue location will be performed by central laboratory neuropathologist. For inclusion in the Biopsy Efficacy Analysis Population, an indeterminate tissue location must meet Criteria 1 and either Criteria 2 or Criteria 3:

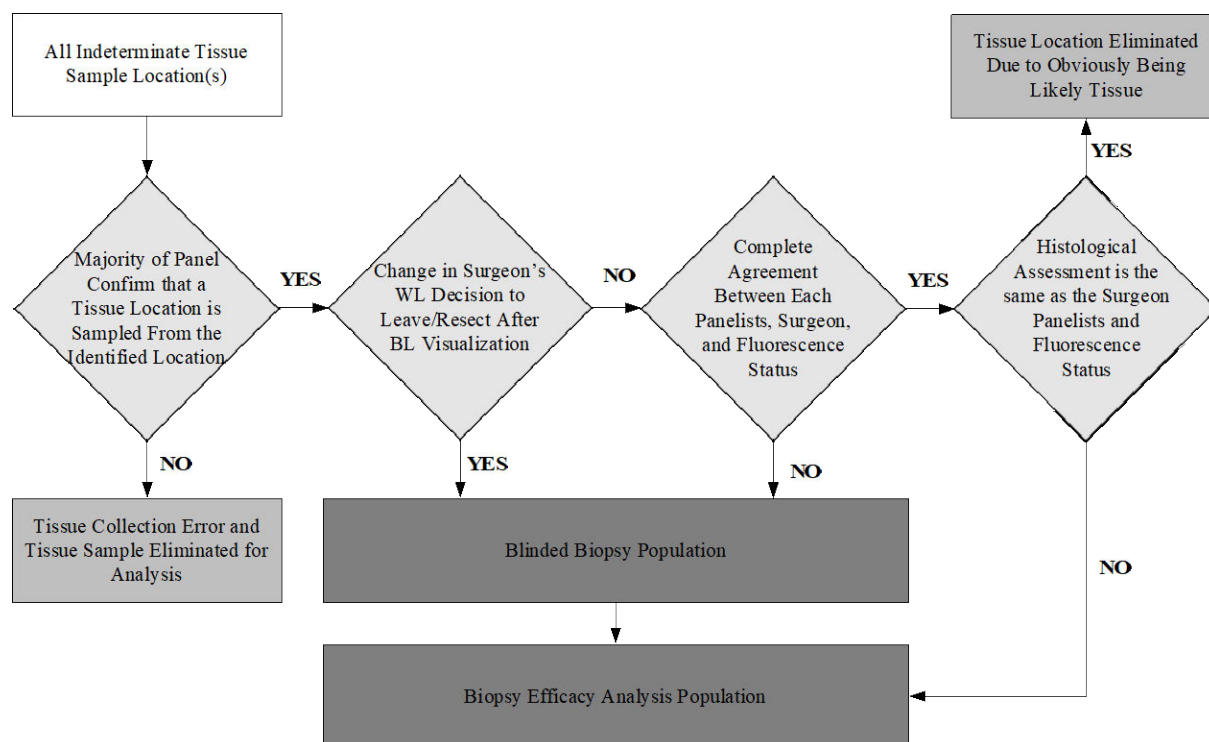
Criteria 1: Confirmation of location by majority of the three Biopsy Review Panelists that the tissue location identified in WL photo/video with a pointer by the surgeon is indeed the tissue that was biopsied for histology (via review of WL video of the tissue sample collection).

Criteria 2: A tissue location where the operating surgeon changed his/her plan made under WL to remove or leave tissue after visualization of fluorescence status of the tissue under BL.

Criteria 3: Any disagreement among the following 6 assessments for each indeterminate tissue location:

- 1) WL Assessment of Biopsy Review Panelist #1
- 2) WL Assessment of Biopsy Review Panelist #2
- 3) WL Assessment of Biopsy Review Panelist #3
- 4) WL Assessment of Operating Surgeon
- 5) Histology of the biopsied tissue location as assessed by the central histology neuropathologist
- 6) BL Assessment of fluorescence status by operating surgeon, assuming positive fluorescence infers tumor presence and negative fluorescence infers tumor absence.

Figure 4. Criteria for Inclusion of an Indeterminate Tissue in the Blinded Biopsy Population and Biopsy Efficacy Analysis Population



Since Gleolan-induced fluorescence is expected to support decision making when there is uncertainty about the identity or presence of tumor in a tissue (indeterminate tissue location), the Biopsy Efficacy Analysis Population excludes the data from biopsies in which there is unanimous agreement between study surgeon, each Biopsy Review Panelist and central histology, independent of the tissue fluorescence status. This is to ensure that surgeons are not declaring obvious locations of tissue as indeterminate [e.g., tissue locations in which fluorescence status would not be necessary

for identifying tissue as meningioma (for instance in a biopsied tissue location taken from the bulk tumor)].

4.8.2 Unexpected Fluorescent EOS Tissues in the Blinded Biopsy Population and Biopsy Efficacy Analysis Population

Unexpected fluorescent EOS tissues are, by definition, areas of tissue for a field of view where the surgeon has declared completion of resection under WL, and that unexpectedly fluoresce when the surgeon switches to BL. Therefore, given the high published PPV and sensitivity of Gleolan-induced fluorescence to indicate meningioma tissue by histology, the unexpected fluorescent EOS tissues in the Biopsy Efficacy Analysis Population will be defined only based on assessments by each of the three Biopsy Review Panelists, who are blinded to the intraoperative decisions.

Three Biopsy Review Panelists will independently review blinded post-surgical WL photos in up to six EOS Fields of View for each participant. Each of these WL photos will be overlaid with a grid for orientation. Using the grid, the Biopsy Review Panelists will identify and record coordinates of tissue in the EOS Field of View photo where they suspect tumor tissue to be present.

For an unexpected fluorescent EOS tissue to be in the Biopsy Efficacy Analysis Population a tissue location must meet Criteria 1 and NOT meet Criteria 2:

Criteria 1: Confirmation by the majority of the three Biopsy Review Panelists that the tissue location identified in WL photo/video with a pointer by the surgeon is indeed the tissue that was biopsied for histology (via review of WL video of the tissue sample collection).

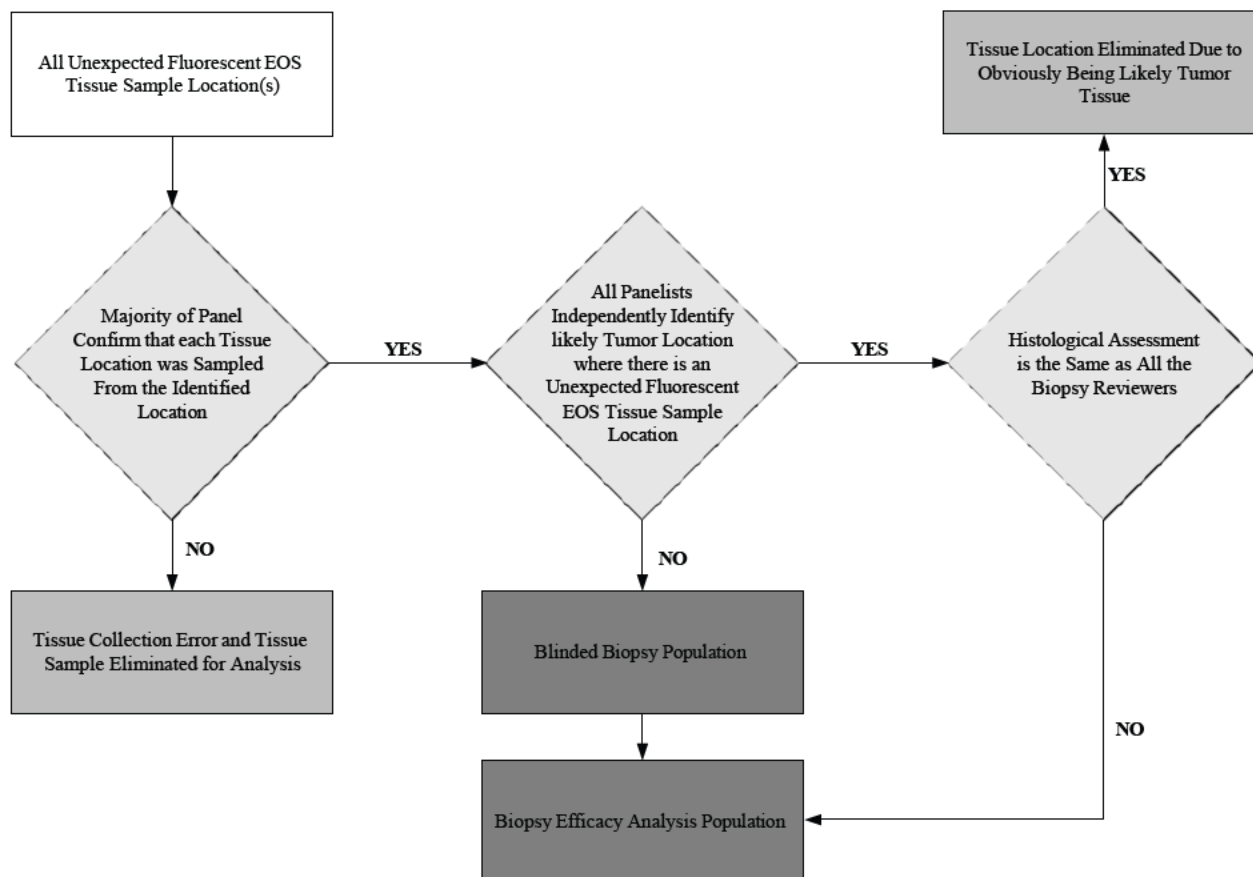
Criteria 2: Unanimous agreement from all Biopsy Review Panelists identify that a location is likely tumor

- 1) WL Assessment of Biopsy Review Panelist #1
- 2) WL Assessment of Biopsy Review Panelist #2
- 3) WL Assessment of Biopsy Review Panelist #3

In this way, the Biopsy Efficacy Analysis Population will exclude tissues when all three Biopsy Review Panelists identify likely tumor presence at the EOS tissue location in the WL photo/video of the EOS Field of View.

The Blinded Biopsy Population will exclude tissue locations where all three Biopsy Reviewers identify likely tumor presence in the WL photo/video of the EOS Field of View at that EOS tissue location.

Figure 5. Criteria for Inclusion of an Unexpected Fluorescent EOS Tissue in the Blinded Biopsy Population and Biopsy Efficacy Analysis Population



5 Study Population

Patients with suspected newly diagnosed or recurrent meningioma of all histological grades who are indicated for, and have a planned resection, will be screened for enrollment.

5.1 Inclusion Criteria

1. A pre-operative MRI ≤ 90 days of study enrollment documenting a suspected meningioma or suspected recurrence of a meningioma for which a meningioma resection is indicated and has been planned.
2. Adult age ≥ 18 years.
3. Patient must have normal organ and bone marrow function and be appropriate surgical candidates per site SOC.
4. Patient must have recording of each parameter as defined below:

Bilirubin	Below upper limit of normal
AST (SGOT)	< 2.5 X institutional upper limit of normal
ALT (SGPT)	< 2.5 X institutional upper limit of normal
Creatinine	Below upper limit of normal
	OR
Creatinine clearance	>60 mL/min/1.73 m ² for participants with creatinine levels above institutional normal

5. The patient must demonstrate the ability to understand the informed consent document and the willingness and ability to sign a written informed consent document. The study consent documents will be prepared in English and German and Spanish. Translation for non-English, non-German, or non-Spanish speaking participants will be provided as appropriate by institution, as required.
6. WOCBP and men participating must agree to use highly effective forms of contraception, and men must also agree not to donate sperm for the duration of treatment, and for at least 42 days after the one time use of the study drug (for further definition see [Appendix 3](#)).

5.2 Exclusion Criteria

1. History of allergic reactions attributed to compounds of similar chemical/biologic composition to Gleolan.
2. Known or documented personal or family history of porphyria.
3. Uncontrolled concurrent illness, including but not limited to ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia or psychiatric illness.
4. Patient has had a meningioma resection or radiation treatment within 90 days prior to informed consent.
5. Social or medical situations that would limit compliance with study requirements (e.g. ability to travel for follow-up or inability to obtain appropriate pre-op MRI (e.g. cardiac pacemaker).
6. Women who are pregnant or plan to become pregnant during study participation.
7. Prior history of gastrointestinal perforation, diverticulitis, and/or peptic ulcer disease within 90 days of informed consent.
8. Simultaneous participation in another clinical study or participation in another clinical study in the 30 days directly preceding treatment or within 5 plasma half-lives of the preceding study drug, whatever is longer.

9. Simultaneous use of other potentially phototoxic substances (St. John's wort, griseofulvin, thiazide diuretics, sulfonyleureas, phenothiazines, sulphonamides, quinolones and tetracyclines), and topical preparations containing ALA for 24 hours during the perioperative period (see MOPS for detailed list).
10. Unwillingness by patient to sign consent or return for subsequent visits following surgery.
11. Any condition that in the opinion of the Investigator would exclude the patient as a viable candidate for this study.
12. Patient undergoing planned embolization prior to meningioma resection that is separate from the meningioma resection procedure itself.

5.3 Screen Failures

Screen failures are defined as patients who have provided informed consent and are screened for enrollment but do not meet all inclusion/exclusion criteria and are not dosed with Gleolan. Minimal information will be collected on screen failures from either defined group and may include demographics, screen failure details, and eligibility criteria.

Individuals who do not meet the criteria for participation in this study (screen failures or consented screen failures) may be rescreened in the event of a future resection or recurrence.

5.4 Long Term Follow-up

Gleolan is administered only once to participants prior to surgery. It is SOC to follow surgical participants for a maximum of 6 weeks postoperatively, after which time participants typically do not have any continued interaction with the neurosurgeon. The plan for this study is to follow study participants for six weeks post meningioma resection. If a participant has an ongoing AE, however, he/she will be followed until resolution or stabilization of the AE.

6 Study Drug

Gleolan is manufactured by LYOCONTRACT GmbH Pulverwiese 1, Ilsenburg, 38871, Germany. It is supplied in 1.5 gram units, to be reconstituted in water prior to oral administration (refer to Gleolan Administration, [Section 6.2.2.2](#)).

The investigational study drug (vial and cardboard box) will be labelled in accordance with the FDA for clinical study sites in the U.S., and in accordance with Competent Authorities' labelling requirements for clinical study sites in Germany and Austria. The Investigator or an approved representative (e.g., pharmacist), will ensure that the study drug is stored in a secured area, under recommended storage conditions, and in accordance with applicable local regulatory requirements. Study drug should be stored at controlled room temperature (59°F-77°F/15°C -25°C; excursions up to 86°F/30°C is allowed) and protected from light. The Investigator agrees to use the study drug only within the framework of this clinical study and in accordance with this study protocol.

6.1 Study Drug(s) Administered

Intervention Name	Gleolan (Aminolevulinic Acid Hydrochloride)
Type	Drug
Dose Formulation	One vial contains 1,500 mg ALA HCl lyophilized powder
Unit Dose Strength(s)	Reconstituted ALA HCl solution contains 30 mg per mL
Dosage Level(s)	20 mg/kg
Dosage Regimen	One time - Day 1
Treatment Period	Single use only, Day 1
Route of Administration	Oral
Use	Experimental
Sourcing	NXDC
Packaging and Labeling	Study drug will be provided in a vial. Each vial will be labeled as required per country requirement
Current/Former Name(s) or Alias(es)	Gliolan, ALA, 5-ALA, Aminolevulinic Acid Hydrochloride, Alaglio (Japan)

6.2 Preparation/Handling/Storage/Accountability

Only participants screened for enrollment and meet all inclusion/exclusion criteria may receive study drug and only authorized site staff may supply or administer study drug. All study drug must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the Investigator and authorized site staff.

The Investigator, institution, or the head of the medical institution (where applicable) is responsible for study drug accountability, reconciliation, and record maintenance (e.g., receipt, reconciliation, and final disposition records).

Further guidance and information for the final disposition of unused study drug are provided in the MOPS.

6.2.1 Drug Accountability

Upon receipt at the investigative site, study drug must be stored at room temperature (i.e., 59°F-77°F/15°C-25°C; excursions up to 86°F/30°C are allowed) in the original packaging. The drug should be protected from light and excessive humidity in a monitored, locked, secure area with limited access. Storage area temperature conditions must be monitored and recorded daily. All temperature excursions will be reported to the Sponsor for assessment and authorization for continued use. Accountability for study drug is the responsibility of the Investigator. The study site must maintain accurate records demonstrating dates and amount of study treatment received, to whom dispensed (participant-by-participant accounting), and accounts of any study treatment accidentally or deliberately destroyed. A written explanation must be provided for any discrepancies. The Investigator must return all unused study drug provided.

6.2.2 Drug Reconstitution and Administration

Drug reconstitution and administration of Gleolan for this study are identical to the procedures outlined in the Prescribing Information [1].

Gleolan is a lyophilized drug product that is reconstituted as an oral solution. One vial contains 1,500 mg ALA HCl lyophilized powder, equivalent to 1,170 mg ALA. The entire ALA HCl material is reconstituted by adding 50 mL of water to make a solution contains 30 mg per mL and is clear and colorless to slightly yellow in color. A single dose of Gleolan is administered orally prior to surgery. The recommended reconstituted oral dose of Gleolan is 20 mg/kg. It is administered to the participant orally 3 hours (target range 2-4 hours) before anesthesia. Gleolan must be used with a standard surgical operating microscope adapted with a BL-emitting light source (WL power density 40-80 mW/cm²) and ancillary excitation and emission filters to visualize PpIX-induced fluorescence excitation in the wavelength of 375 to 440 nm and for observation from 620 to 710 nm. Filters transmit porphyrin fluorescence as red-violet, as well as a fraction of backscattered blue excitation light necessary for distinguishing non-fluorescing tissue.

6.2.2.1 Reconstitution of Gleolan

Gleolan powder must be reconstituted prior to administration by a healthcare provider according to the following instructions:

- Determine the total number of vials needed to achieve the intended dose for the study participant according to the equation below (rounded up to the nearest whole vial):
- Completely remove the white cap and aluminum crimp seal from each vial.
- Remove and retain the rubber stopper from the vial.
- Using an appropriate volumetric measuring device (e.g., flask, graduated cylinder, dosing syringe), measure 50 mL of drinking water and add to each vial containing 1,500 mg of Gleolan.
- Gently swirl the vial to completely dissolve the Gleolan.
- The resulting reconstituted solution (30 mg of ALA HCl per mL) is clear and colorless to slightly yellowish.
- If required, replace the stopper and store reconstituted solution for up to 24 hours at room temperature prior to administration.

6.2.2.2 Gleolan Administration

Gleolan is for ORAL USE ONLY. The reconstituted Gleolan solution is administered according to the following steps:

- Calculate the administration volume, in mL, to achieve the intended dose according to the following equation:

$$\frac{\text{AAAAA} \times \text{VVA} \times \text{SSSSSSSS} \times \text{PPPPPPSSPPPPPPPPPPSS}}{\text{BBBBSSSS} \times \text{WWWPPWW} \times \text{SS} \times \text{kkWW} \times 20 \text{ mmWW} / \text{kkWW}} = \frac{\text{mm}}{30 \text{ mmWW} / \text{mmmm}}$$

- Transfer the entire contents of the prepared vial(s) into an appropriate dosing container (e.g., oral medicine bottle); ensure the entire contents of the vials are transferred.
- After transfer, discard the empty vial(s) following site specific procedure for drug disposal.
- Using a disposable volumetric syringe, remove the administration volume of reconstituted Gleolan solution from the dosing container and transfer to a separate oral dosing container.
- Discard unneeded volume of Gleolan solution following site specific procedure for drug disposal.

Each participant will receive the study drug, Gleolan, approximately 3 hours, (target range 2-4 hours) prior to anesthesia for scheduled surgery.

6.3 Gleolan Dose Modifications

Gleolan requires a one time, single-dose administration at 20 mg ALA/kg patient weight. There should be no modification in the dose for study participants. In the event of emesis of the product, the dose will not be repeated, however, the participant remains enrolled and should be followed from Day 1 through End of Study per protocol.

6.4 Study Drug Compliance

Study participants will be dosed at the individual study site and will receive study drug directly from the Investigator or designee, under medical supervision in an inpatient setting. The reconstitution and time, volume (mL), dosage (mg) date and time of each dose administered will be recorded in the source documents and recorded in the eCRF. The dose of study drug and study participant's identification will be confirmed at the time of dosing (oral ingestion) by a member of the study site staff other than the person administering the study drug.

6.5 Concomitant Medications

Any medication (excluding non-prescription medication) that the participant is receiving at the time of enrollment, or receives during their participation in the study, must be recorded. This includes all medication the participant receives pre-procedure, intraoperatively, post-procedure and at discharge.

Medications will be recorded and should include the following:

- Reason for use/Indication
- Dates of administration including start and end dates
- Dosage information including dose and frequency
- Route

The Medical Monitor should be contacted if there are any questions regarding concomitant or prior therapy.

6.6 Drug Interactions

Participants taking Gleolan who are exposed to an additional photosensitizing agent may experience a phototoxic skin reaction (severe sunburn). Due to the risk of possible phototoxic reactions, study participants should avoid administering phototoxic drugs such as St. John's Wort, griseofulvin, thiazide diuretics, sulfonamides, phenothiazines, sulphonamides, quinolones, and tetracyclines, and topical preparations containing ALA for 24 hours before and 2 weeks after administration of Gleolan (see MOPS for full list).

6.7 Management of Select Toxicities

6.7.1 Photosensitivity Reactions

For a grade ≥ 2 photosensitivity reaction that is observed during the 48-hour post procedure monitoring period, consultation or outpatient referral (as appropriate) to dermatology is required. Participants will be educated regarding avoidance of light sources.

6.7.2 Liver Enzyme Elevations and Hyperbilirubinemia

Any liver enzyme elevation meeting the definition of Hy's Law (see below) will be reported as an Adverse Event (AE)/Serious Adverse Event (SAE).

Hy's Law is defined:

ALT or AST $>3 \times$ Upper Limit of Normal (ULN)

AND

Serum total bilirubin $>2 \times$ ULN, without initial findings of cholestasis (elevated serum alkaline phosphatase $> 2 \times$ ULN)

AND

No other plausible reason to explain the combination of increased aminotransferase and total bilirubin elevation, such as viral hepatitis A, B, or C; preexisting or acute liver disease; or another drug capable of causing the observed injury

6.7.3 Anti-Emetic Therapy

Based on previous studies of ALA, significant nausea as a part of this treatment is not expected. Use of anti-emetics is allowed, if indicated. If the participant experiences emesis, no redosing under any circumstance is allowed in the study at any time.

6.7.4 Other Anticancer or Experimental Drugs

Participants shall not be denied SOC therapy following Gleolan dosing and resection surgery. Sites should manage the participants according to their clinical site's particular SOC without any restrictions.

6.7.5 Palliative and Supportive Care

All palliative and supportive care necessary for optimal care of the patient should be provided.

6.7.6 Retreatment Criteria

All eligible consented participants enrolled into the study will be treated on Day 1. No retreatment of Gleolan is permitted per protocol even if the patient experiences emesis in the acute dosing interval or if surgery does not take place for any reason.

7 Discontinuation of Study Participation

Consented and eligible study participants will be rescreened for eligibility on Day 1 pre-dose to ensure specific criteria are still met. If the Day 1 pre-dose eligibility is not met, the patient will not receive Gleolan and will not be enrolled in the study.

7.1 Participant Discontinuation/Withdrawal from the Study

A participant may withdraw from the study at any time at his/her own request or may be withdrawn at any time at the discretion of the Investigator for safety, behavioral, compliance, or administrative reasons. This is expected to be uncommon.

At the time of discontinuing from the study, if possible, an early discontinuation visit should be conducted. See [Section 1.2](#) Schedule of Events for data to be collected at the time of study discontinuation and follow-up and for any further evaluations that need to be completed. The participant will be permanently discontinued both from the study drug and from the study at that time. If the participant withdraws consent for disclosure of future information, the Sponsor may retain and continue to use any data collected before such a withdrawal of consent. If a participant withdraws from the study, he/she may request destruction of any tissues or bodily fluids taken and not evaluated, and the Investigator must document this in the site study records.

7.2 Lost to Follow Up

A participant will be considered lost to follow-up if he or she repeatedly fails to return for scheduled visits and is unable to be contacted by the study site. The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site must attempt to contact the participant and reschedule the missed visit as soon as possible and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain whether or not the participant wishes to and/or should continue in the study.
- Before a participant is deemed LTFU, the Investigator or designee must make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a registered letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's source documentation and eCRF.
- Should the participant be deemed LTFU, he/she will be considered to have withdrawn from the study.

8 Study Assessments and Procedures

8.1 Efficacy Assessments

Study objectives and endpoints are detailed in [Table 7](#).

Table 7. Efficacy Objectives and Endpoints

Efficacy Objective	Endpoint/Estimand
Primary (Clinical Usefulness)	
To determine the percentage of participants for which Gleolan-induced PpIX fluorescence allows the surgeon to visually obtain correct information as to the presence or absence tumor in tissue where there is uncertainty regarding that tissue's tumor status based on white light (WL) visualization alone.	<p><i>Per Protocol Population (Primary)</i> <i>Intent-to-Treat (ITT) Population (Supportive)</i></p> <p>The percentage of participants who have at least one indeterminate tissue or unexpected fluorescent End of Surgery (EOS) tissue where Gleolan-induced PpIX fluorescence status is consistent with histology.</p> <p><i>Null Hypothesis: Percentage $\leq 30\%$</i> <i>Alternative Hypothesis: Percentage $> 30\%$</i></p> <p><i>Expected Response: Percentage $\geq 50\%$</i></p>

Table 8. Key Secondary Efficacy Endpoints

Efficacy Objective	Endpoint/Estimand
Key Secondary Efficacy Objective	
To determine the biopsy-level PPV of Gleolan for the real-time visualization of tissue locations on the tumor margin in newly diagnosed or recurrent meningioma during resection surgery.	<p><i>Biopsy Efficacy Analysis Population (Primary)</i> <i>All Biopsy Population (Supportive)</i></p> <p>Positive Predictive Value (PPV) of Gleolan-induced PpIX fluorescence status of biopsied tissue locations at the margin of the tumor.</p> <p>$PPV = TP_{BL} / (TP_{BL} + FP_{BL})$</p> <p><i>Null Hypothesis: PPV $\leq 60\%$</i> <i>Alternative Hypothesis: PPV $> 60\%$</i></p> <p><i>Expected Response: PPV = 80%</i></p>
To determine the biopsy-level NPV of Gleolan for the real-time visualization of tissue locations on the tumor margin in newly diagnosed or recurrent meningioma during resection surgery.	<p><i>Biopsy Efficacy Analysis Population (Primary)</i> <i>All Biopsy Population (Supportive)</i></p> <p>Negative Predictive Value (NPV) of Gleolan-induced PpIX fluorescence status of biopsied tissue location at the margin of the tumor.</p> <p>$NPV = TN_{BL} / (TN_{BL} + FN_{BL})$</p> <p><i>Null Hypothesis: NPV $\leq 40\%$</i> <i>Alternative Hypothesis: NPV $> 40\%$</i></p> <p><i>Expected Response: NPV = 60%</i></p>

Efficacy Objective	Endpoint/Estimand
Key Secondary Efficacy Objective	
<p>To determine the participant-level PPV of Gleolan for the real-time visualization of bulk tumor in newly diagnosed or recurrent meningioma during resection surgery.</p>	<p><i>Per Protocol Population</i></p> <p>Positive Predictive Value (PPV) of Gleolan-induced PpIX fluorescence status of the single bulk tumor biopsied tissue location obtained from each study participant.</p> $PPV = TP_{BL} / (TP_{BL} + FP_{BL})$ <p><i>Null Hypothesis: $PPV \leq 60\%$</i> <i>Alternative Hypothesis: $PPV > 60\%$</i></p> <p><i>Expected Response: $PPV = 80\%$</i></p>
<p>To determine the biopsy-level diagnostic accuracy of meningioma identification with (i) Gleolan-induced PpIX fluorescence status under BL vs. (ii) visualization under WL, among indeterminate tissue and unexpected fluorescent EOS tissue locations, as assessed by the operating surgeon.</p>	<p><i>Biopsy Efficacy Analysis Population (Primary)</i> <i>All Biopsy Population (Supportive)</i></p> <p>Diagnostic accuracy of Gleolan-induced PpIX fluorescence status among indeterminate tissue and unexpected fluorescent EOS tissue locations is at least 20% greater than the diagnostic accuracy of the surgeons' assessment of indeterminate tissue and unexpected fluorescent EOS tissue locations under WL:</p> $\text{Diagnostic Accuracy}_{BL} - \text{Diagnostic Accuracy}_{WL} \geq 20\%$ $[(TP_{BL} + TN_{BL}) / (TP_{BL} + TN_{BL} + FP_{BL} + FN_{BL})] * 100 - [(TP_{WL} + TN_{WL}) / (TP_{WL} + TN_{WL} + FP_{WL} + FN_{WL})] * 100 \geq 20\%$ <p><i>Null Hypothesis: $DA_{BL} - DA_{WL} \leq 20\%$</i> <i>Alternative Hypothesis: $DA_{BL} - DA_{WL} > 20\%$</i></p> <p><i>Expected Response $\geq 30\%$</i></p>
PpIX= protoporphyrin IX , PPV=positive predictive value, NPV=negative predictive value, EOS=end of surgery	

Table 9. Other Secondary Efficacy Endpoints

Efficacy Objective	Endpoint/Estimand
Other Secondary Efficacy Objective	
To further determine the biopsy-level diagnostic performance of Gleolan-induced PpIX fluorescence status for the real-time visualization of meningioma during resection surgery.	<p><i>Biopsy Efficacy Analysis Population</i></p> <p>Diagnostic performance of Gleolan-induced PpIX fluorescence status will be computed for indeterminate tissue biopsies and unexpected fluorescent EOS tissue biopsies (combined).</p> <ul style="list-style-type: none"> • <i>Biopsy-level sensitivity</i> • <i>Biopsy-level specificity</i> <p><i>Expected Responses > 70%</i></p>
To demonstrate that the biopsy-level concordance between the Biopsy Review Panel with the operating surgeon is better with BL visualization of Gleolan-induced PpIX fluorescence status than with WL visualization.	<p><i>Biopsy Efficacy Analysis Population</i></p> <p>The concordance between the Surgeon and Biopsy Review Panelists' assessment of white light (WL) visualization to identify tissue as likely or unlikely to be meningioma among indeterminate tissues.</p> <p>Concordance WL of (Majority Biopsy Review Panelists) and (Surgeon) - Fleiss Kappa < 0.4</p> <p><i>Expected Response < 0.4 (Fleiss Kappa)</i></p>
	<p><i>Biopsy Efficacy Analysis Population</i></p> <p>The concordance between the Surgeon and Biopsy Review Panelists' assessment of blue light (BL) visualization to identify fluorescence status of indeterminate tissues</p> <p>Concordance BL of (Majority Biopsy Review Panelists) and (Surgeon) - Fleiss Kappa > 0.6</p> <p><i>Expected Response > 0.6 (Fleiss Kappa)</i></p>

Table 10. Exploratory Objectives and Endpoints

Efficacy Objective	Endpoint/Estimand
Exploratory	
To determine whether Gleolan can help surgeons accurately decide whether or not to resect indeterminate tissue and unexpected fluorescing end-of-surgery (EOS) tissue.	<p><i>Biopsy Efficacy Analysis Population</i></p> <p>The BL Diagnostic accuracy = $[(TP_{BL} + TN_{BL}) / (TP_{BL} + TN_{BL} + FP_{BL} + FN_{BL})] * 100$ of Gleolan-induced PpIX fluorescence status among <u>indeterminate</u> tissue and <u>unexpected fluorescent EOS</u> tissue.</p> <p>This is the percentage of <u>indeterminate tissue</u> and <u>unexpected fluorescent EOS</u> tissues correctly classified by Gleolan-induced PpIX fluorescence status among all indeterminate tissues and unexpected fluorescent EOS tissues.</p>
To further characterize the diagnostic performance of Gleolan for the real-time visualization of meningioma during resection surgery.	<p><i>Per Protocol Population</i></p> <p>Diagnostic performance of Gleolan at the patient level as assessed with the <u>single tissue</u> sample of bulk tumor.</p> <p> $Sensitivity_{BL} = TP_{BL} / (TP_{BL} + FN_{BL})$ $Specificity = TN_{BL} / (TN_{BL} + FP_{BL})$ $NPV_{BL} = TN_{BL} / (TN_{BL} + FN_{BL})$ $Diagnostic\ accuracy_{BL} = [(TP_{BL} + TN_{BL}) / (TP_{BL} + TN_{BL} + FP_{BL} + FN_{BL})] * 100$ </p> <p>(Proportion of biopsies that are meningiomas by the central histology neuropathologist that exhibit Gleolan-induced PpIX fluorescence status)</p>
To demonstrate that the consistency of the Biopsy Review Panel assessments with the operating surgeon assessments is better with Gleolan-induced PpIX fluorescence status than with WL visualization	<p><i>Biopsy Efficacy Analysis Population</i></p> <p>Among <u>indeterminate tissues</u>, the difference in diagnostic accuracy of Gleolan-induced PpIX fluorescence status as determined by the Biopsy Review Panel vs. the operating surgeon is smaller than the difference in diagnostic accuracy of WL visualization as determined by the Biopsy Review Panel vs. the operating surgeon.</p> <p> $\Delta [Diagnostic\ Accuracy_{WL} (Biopsy\ Review\ Panel) - Diagnostic\ Accuracy_{WL} (Surgeon)]$ $>$ $\Delta [Diagnostic\ Accuracy_{BL} (Biopsy\ Review\ Panel) - Diagnostic\ Accuracy_{BL} (Surgeon)]$ </p>

Efficacy Objective	Endpoint/Estimand
Exploratory	
To determine if tumor location influences diagnostic performance of Gleolan	<p><i>Per Protocol Population</i></p> <p>PPV of Gleolan-induced PpIX fluorescence status of the single <u>bulk tumor tissue sample</u> obtained from each study participant will be compared across subgroups of meningioma tumor location.</p>
To determine if time from dose administration of Gleolan to acquisition of bulk tumor biopsy affects diagnostic performance of Gleolan.	<p><i>Per Protocol Population</i></p> <p>PPV, NPV, Sensitivity, Specificity, and Diagnostic Accuracy of Gleolan-induced PpIX fluorescence status of the single <u>bulk tumor tissue sample</u> obtained from each study participant will be compared across subgroups of time from Gleolan dose to tissue sample acquisition (<2 hours and ≥2 hours).</p>
To determine if the likelihood of identifying indeterminate/unexpected fluorescent EOS tissue and the number of indeterminate/unexpected fluorescent EOS tissues collected is different for different tumor locations.	<p><i>Biopsy Efficacy Analysis Population</i></p> <p>Compared across subgroups of meningioma tumor location and tissue type:</p> <ul style="list-style-type: none"> • Number of indeterminate/unexpected fluorescent EOS tissues • Percentage of all indeterminate/unexpected fluorescent EOS tissues where Gleolan-induced PpIX fluorescence status resulted in a change in the surgeon's decision to remove or leave a piece of tissue
To determine the frequency with which Gleolan-induced PpIX fluorescence status allows the surgeon to make a change in a decision whether to remove or leave an indeterminate area of tissue.	<p><i>Biopsy Efficacy Analysis Population</i></p> <p>Percentage of all <u>indeterminate</u> tissues where Gleolan-induced PpIX fluorescence status resulted in a change in the operating surgeon's decision to remove or leave a piece of tissue and is in agreement with central histopathological results.</p>
To determine the frequency with which Gleolan-induced PpIX fluorescence status allows the surgeon to observe residual tumor and make a change in decision, after complete resection under WL at the EOS.	<p><i>Biopsy Efficacy Analysis Population</i></p> <p>Percentage of all <u>unexpected fluorescent EOS</u> tissues where Gleolan-induced PpIX fluorescence status resulted in a change in the operating surgeon's decision to remove or leave a piece of tissue, and is in agreement with central histopathological results.</p>

EOS = end of surgery; WL = white light

8.2 Safety Assessments

An objective of this Phase 3 study is to determine the safety and tolerability of Gleolan administered prior to surgery for meningioma resection. Safety assessments include:

- AEs reported from informed consent through 6 weeks post-surgery,
- Serious AEs from informed consent through 6 weeks post-surgery,
- Evaluation of Common Terminology Criteria for Adverse Events (CTCAE) Nervous System Disorder events,
- Changes in clinical laboratory measures, with emphasis on liver function,
- Incidence of surgically-related neurological deficits will be reported by relationship to Gleolan dosing.

8.2.1 Physical Examinations, Vital signs, and Medical History

- A physical examination will include, at a minimum, assessments of the cardiovascular, respiratory, GI, skin, and neurological systems. Height and weight will also be measured and recorded.
- Investigators should pay special attention to clinical signs related to previous serious illnesses.
- Medical History will be collected at Baseline for adequate screening.
- Vital signs will include temperature, pulse rate, respiratory rate, and blood pressure.

8.2.2 Neurological Exams

- Neurological exams will be completed on each participant per the Schedule of Events.
- These exams should include assessment of the participant's mental status, speech, cranial nerves, motor system, sensory, gait, and other as noted during exam.
- Neurological exams should be completed by an Investigator or a delegated and credentialed medical professional.
- Karnofsky performance status will be completed per protocol's Schedule of Events by the Investigator or delegated study staff.

8.2.3 Clinical Safety Laboratory Assessments

- See [Appendix 2](#) for the clinical laboratory tests to be performed and to the Schedule of Events ([Section 1.2](#)) for the timing and frequency. These labs should fall in line with routine SOC lab requirements while treating these participants.
- The Investigator must review the laboratory report, document this review, and record any clinically significant changes occurring during the study in the AE section of the eCRF. The laboratory reports must be filed with the source documents. Clinically significant abnormal laboratory findings are those which are not associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the participant's condition.
- All abnormal laboratory tests with values considered clinically significant during participation in the study-should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the Investigator or Medical Monitor.
 - If such values do not return to normal/baseline within a period of time judged reasonable by the Investigator, the etiology should be identified, and the Sponsor notified.

- All protocol-required laboratory assessments, as defined in [Appendix 2](#), must be conducted in accordance with the MOPS and the Schedule of Events.
- Any clinically significant observations in results of clinical laboratory are to be recorded as part of the patient's Medical History if observed as part of the screening laboratory results. All laboratory test values that are outside the normal range and the Investigator indicates that they are clinically significant should be recorded as AEs unless they are part of the patient's Medical History.
- If laboratory values from non-protocol specified laboratory assessments performed at the institution's local laboratory require a change in participant management or are considered clinically significant by the Investigator (e.g., SAE or AE or dose modification), then the results must be recorded in the eCRF.

8.3 Adverse Events (AE) and Serious Adverse Events (SAE)

8.3.1 Definitions

Adverse Events (AE): An AE is defined in the ICH Guideline for GCP as “any untoward medical occurrence in a patient or clinical investigation study participants administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment” (ICH E6:1.2). 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. AEs will be coded in accordance with the National Cancer Institute (NCI) CTCAE V5.0. [Link below.](https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf)

https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_5x7.pdf

Interventions for pretreatment conditions (e.g., elective cosmetic surgery) or medical procedures for treatment of reported baseline Medical History are not considered AEs, unless the treatment is acute and/or emergent.

Worsening of a pre-existing medical condition, (e.g., diabetes, migraine headaches, gout) is to be considered an AE if there is either an increase in severity, frequency, or duration of the condition or an association with significantly worse outcomes.

Suspected Adverse Reaction: A suspected adverse reaction means any adverse event for which there is a reasonable possibility that the study drug caused the adverse event. For the purposes of safety reporting, ‘reasonable possibility’ means there is evidence to suggest a causal relationship between the study drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than an adverse reaction.

Adverse Reaction: An adverse reaction means any AE caused by a study drug. Adverse reactions are a subset of all suspected adverse reactions where there is reason to conclude that the drug caused the event.

Unexpected Adverse Event: Expectedness will be determined by the Sponsor according to the designated Reference Safety Information within the IB. Any updates or substantial amendments

will be considered accordingly. The Reference Safety Information for the study drug will be the IB. An adverse event or suspected adverse reaction is considered “unexpected” if it is not listed in the Reference Safety Information or is not listed with the specificity or severity that has been observed. Unexpected, as used in this definition, also refers to AEs or suspected adverse reactions that are mentioned in the Reference Safety Information as occurring with a class of drugs or as anticipated from the pharmacological properties of the study drug, but are not specifically mentioned as occurring with the particular study drug under investigation.

Intensity of Event (Severity)

All AEs will be assessed by the Investigator using the CTCAE V5.0 criteria. For events not included in the grading system, the following guidelines will be used to quantify intensity.

Mild: Events requiring minimal or no treatment and that do not interfere with the study participant’s daily activities.

Moderate: Events that result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.

Severe: Events that interrupt a study participant’s usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.

Life threatening: Any AE that places the study participant, in the view of the Sponsor-Investigator, at immediate risk of death from the reaction as it occurred, e.g., it does not include a reaction that had it occurred in a more severe form, might have caused death.

Death related to AE: The initial and greatest severity of an AE should be captured and documented within the eCRF.

Serious Adverse Event (SAE)

An SAE is defined as an AE meeting one of the following conditions:

Any untoward medical occurrence that:

- Results in death,
- Is life-threatening,
- Requires inpatient hospitalization or prolongation of existing hospitalization,
- Results in persistent or significant disability/incapacity, or
- Is a congenital anomaly/birth defect.
- Any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the Investigator.

An elective hospital admission will not be considered as a SAE.

Clarification of the difference in meaning between “severe” and “serious”

The term “severe” is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache).

This is not the same as “serious”, which is based on the outcome or action criteria usually associated with events that pose a threat to life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

8.3.2 Time Period and Frequency for Collecting AE and SAE Information

All AEs and SAEs will be recorded from the time the Informed Consent is obtained through the End of Study visit (6 weeks after surgery). All AEs must be graded for intensity and relationship to study drug. The Investigator is responsible for recording all AEs that are observed during this study according to the definition and followed until resolved or LTFU even if beyond the 6 week window of the study calendar.

The Investigator is ultimately responsible for recording all AEs and SAEs during the study conduct. The descriptions and grading scales found in the revised NCI CTCAE version 5.0, which will be utilized for adverse event reporting are available online at:

<http://ctep.cancer.gov/reporting/ctc.html>.

8.3.3 AEs Reportable per protocol

- Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (e.g., electrocardiogram, radiological scans, vital signs measurements), that are considered clinically significant in the medical and scientific judgment of the Investigator.
- Liver function tests that meet or exceed Hy’s law and considered attributable to the study drug.
- Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction.
- Neuro change from baseline that is considered clinically significant in the medical and scientific judgment of the Investigator.
- Worsening of a pre-existing medical condition, (e.g., diabetes, migraine headaches, gout) is to be considered an AE if there is either an increase in severity, frequency, or duration of the condition or an association with significantly worse outcomes.
- In the case of death, only record “Fatal” for the event causing death. AEs that are ongoing at the end of the study or time of death are to be noted as “continuing.”
- The Investigator is responsible for reviewing laboratory test results and determining whether an abnormal value in an individual patient represents a significant change from baseline. In general, abnormal laboratory findings without clinical significance (based on the Investigator’s judgment) should not be recorded as AEs; however, laboratory value changes requiring therapy or adjustment in prior therapy are considered AEs. Any clinically significant observations in results of clinical laboratory are to be recorded as part of the patient’s Medical History if observed as part of the screening laboratory results.

8.3.4 Relationship to Study Drug

The Investigator’s assessment of an AE/SAE’s relationship to study drug is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. All AEs must have their relationship to the study drug assessed using the following terms: unrelated,

unlikely, possible, probable or definite. In a clinical study, the study drug must always be suspect. To help assessment, the following guidelines are used:

Attribution: An assessment of the relationship between the AE and the study drug. CTCAE does not define an AE as necessarily “caused by a therapeutic intervention.” After naming and grading the event, the clinical Investigator must assign an attribution to the AE using the following attribution categories (Table 11).

Table 11. Adverse Event Attribution Categories

RELATIONSHIP	ATTRIBUTION	DESCRIPTION
Unrelated to study drug (No Reasonable Possibility)	Unrelated	The AE is clearly not related to the study drug
	Unlikely	The AE is doubtfully related to the study drug
Related to study drug (Reasonable Possibility)	Possible	The AE may be related to the study drug
	Probable	The AE is likely related to the study drug
	Definite	The AE is clearly related to the study drug

The Investigator should use medical judgment to determine whether he/she assumes a reasonable causal relationship, including in his/her evaluation all relevant factors and factual evidence such as:

- temporal course and latency;
- known pharmacological properties of the product;
- and alternative explanations (e.g. other drugs, Medical History, concomitant diseases).

The expression “reasonable causal relationship” means to convey in general that there is evidence or argument to suggest a causal relationship. Assessments must be done by the Investigator and/or other medically qualified study personnel to whom the tasks have been delegated and will be documented on the AE/SAE form.

8.3.5 Method of Detecting AEs and SAEs

AEs may be reported by a healthcare professional or the study participant (or, when appropriate, by a caregiver, or the participant’s LAR). The Investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study intervention or study procedures, or that caused the participant to discontinue the study.

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and non-leading verbal questioning of the participant is the preferred method to inquire about AE occurrences. AEs can be found in review of individual study participant’s medical record or be reported to the Principal Investigator or other site designee by the participant (or, when appropriate, by a caregiver, surrogate, or the participant’s LAR).

8.3.6 Recording and Follow-Up of AE and/or SAE

8.3.6.1 AE and SAE Recording

- When an AE/SAE occurs, it is the responsibility of the Investigator to review all documentation (e.g., hospital progress notes, laboratory reports, and diagnostics reports) related to the event.
- If an SAE occurs the Investigator has 24 hours from knowledge of event to report to the Sponsor.
- If a protocol reportable AE occurs the Investigator or site delegated personnel will record AE data into the eCRF system. All AEs will be described using the sign, symptom, or medical diagnosis on the AE Electronic Data Capture (EDC) page in standard medical terminology in order to avoid the use of vague, ambiguous, or colloquial expressions. Each AE will be defined as serious or non-serious according to the definitions in [Section 8.3.1](#). The Investigator will evaluate the severity of each AE and causal relationship of the event to the administration of study medication/study procedure and to the underlying disease.
- If the AE is **serious**, the Investigator must complete, in addition to the “adverse event” page in the eCRF, a “SAE Report Form” at the time the SAE is detected. This form must be marked as “**initial**” report and sent **immediately (e.g., within 24 hours upon becoming aware of the SAE)** to the safety contact of the Sponsor’s Pharmacovigilance (PV) Vendor:



- Every attempt should be made to describe all AEs in terms of a diagnosis. If a clear diagnosis has been made, individual signs and symptoms should not be recorded unless they represent atypical or extreme manifestations of the diagnosis, in which case they should be reported as separate events. If a clear diagnosis cannot be established, each sign and symptom must be recorded individually.
- There may be instances when copies of medical records for certain cases are requested by NXDC. In this case, all study participant identifiers, with the exception of the study participant’s number, will be redacted on the copies of the medical records before submission.
- The Investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.

8.3.6.2 Follow-up of AEs and SAEs

After the initial AE/SAE report, the Investigator is required to proactively follow each participant at subsequent visits/contacts. All SAEs will be followed until resolution, stabilization, the event is otherwise explained, or the participant lost to follow-up (as defined in [Section 4.6](#)). All reportable AEs will be followed until resolution or stabilization or through end of the study participant’s participation in the study. If a study participant has any AEs that have not resolved at the end of their participation in the study, the AE will be listed as ongoing. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.

- The Investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by the Sponsor to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals.
- If a participant dies during participation in the study or during a recognized follow-up period, the Investigator will provide the Sponsor with a copy of any postmortem findings including death certificate, autopsy results (if available) and histopathology.
- New or updated information will be recorded in the originally completed eCRF.
- The Investigator will submit any updated SAE data to NXDC within 24 hours of receipt of the information.

8.3.7 Regulatory Reporting Requirements for AEs and SAEs

AEs specific for Gleolan are found in the IB. Prompt notification by the Investigator to the Sponsor of a SAE is essential so that legal obligations and ethical responsibilities towards the safety of participants and the safety of a study drug under clinical investigation are met. The Sponsor has a legal responsibility to notify both the local Competent Authorities and other regulatory agencies about the safety of a study drug under clinical investigation. The Sponsor will comply with country-specific regulatory requirements relating to safety reporting to the Competent Authorities, Institutional Review Boards (IRB)/Independent Ethics Committees (IEC), and Investigators. For all studies, Investigator Safety Reports must be prepared for suspected unexpected serious adverse reactions (SUSAR) according to local regulatory requirements and Sponsor policy and forwarded to Investigators as necessary. An Investigator who receives an Investigator Safety Report describing a SAE or other specific safety information (e.g., summary or listing of SAEs) from the Sponsor will review and then file it in the site regulatory binder and will notify the IRB/IEC, if appropriate according to local requirements.

Events determined by the Investigator to involve injury or to be unanticipated problems involving risk will be reported to the IRB/IEC per site specific reporting guidelines. AEs that are determined by the Investigator not to involve injury or to be an anticipated risk, will be reported per IRB/IEC policy.

For reporting of safety events in U.S.:

An Investigational New Drug Application (IND) safety report will be reported to the FDA by the Sponsor when an event meets all three of the definitions for *suspected adverse reaction*, *serious* and *unexpected*. All FDA reporting will be consistent with 21 CFR 312.32 (c).

- Unexpected serious suspected adverse reactions will be reported to FDA as soon as possible but no later than within 15 calendar days following the Sponsor-Investigator initial receipt of the information.
- Unexpected fatal or life-threatening suspected adverse reactions will be reported to FDA as soon as possible but no later than 7 calendar days following the Sponsor-Investigator initial receipt of the information.

For reporting of safety events in Europe:

The Sponsor will report expedited all SUSARs to the Competent Authorities in accordance with the requirements of the countries. The expedited reporting will occur not later than 15 days after the Sponsor has first knowledge of the adverse reactions. For fatal or life-threatening cases, the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

Development Safety Update Report (DSUR)

The Sponsor will prepare and submit annual safety reports to Competent Authorities and concerned IRB/IECs.

8.3.8 Pregnancy Reporting

Pregnancies that occur while the participant is in the study (after Gleolan dosing) must be reported to the Investigator. In the event of a pregnancy, the participant should be referred to an obstetrician/gynecologist for further evaluation and counseling. Details of all pregnancies within 42 days after dosing in female participants and, if indicated, female partners of male participants will be collected after the start of study drug and until final study visit. If a pregnancy is reported, the Investigator should inform the Sponsor within 24 hours of learning of the pregnancy.

The Investigator will follow the participant (if Gleolan was administered) until completion of the pregnancy and must notify the Medical Monitor of the outcome within 5 days. The Investigator will provide this information as a follow-up to the initial report.

If the outcome of the pregnancy meets the criteria for immediate classification as an SAE (e.g., spontaneous abortion [any congenital anomaly detected in an aborted fetus is to be documented], stillbirth, ectopic pregnancy, neonatal death, or congenital anomaly), then the Investigator should report it as such. Furthermore, all neonatal deaths that occur within 30 days of birth are to be reported, without regard to causality, as SAEs. In addition, any infant death after 30 days that the Investigator suspects is related to the in-utero exposure to the study drug should also be reported.

8.3.9 Disease-Related Events and/or Disease-Related Outcomes

The following disease and procedure related events (DREs) are common in participants with undergoing standard surgical treatment for meningioma and can be serious/life threatening:

- deep vein thrombosis, thromboembolism;
- seizures;
- wound complications, infections
- major neurological deficits (hemiparesis, hemiplegia, aphasia)
- damage to nearby brain tissue
- cerebral edema

These DREs will be monitored by an independent DSMB on a routine basis as described in the DSMB Charter.

8.4 Study Drug abuse, misuse, overdose, and other special situations

Gleolan is administered as a single oral dose (20 mg/kg body weight) in this study; the same dosing regimen as the marketed product for the visualization of glioma. Administration of the study drug will be done by delegated study personnel who will also witness the study participant take their one-time oral dose. Study participants will not have access to the study drug at any other time, making the potential for an overdose, abuse or misuse unlikely.

This one-time study drug administration by a delegated study personnel also greatly reduces any chance for medication errors or occupational exposure.

In the event of an overdose, the Investigator or site designee should:

1. Contact the Medical Monitor and Sponsor immediately.
2. Closely monitor the participant for any AE/SAE potentially related to overdose and report per protocol.

8.5 Pharmacokinetic and Pharmacodynamic Measurements

No blood samples will be obtained for purposes of monitoring PK or PD measures.

9 Statistical Considerations

9.1 Sample Size Determination

Sample size estimation for study NXDC-MEN-301 is based on assumptions about the performance of Gleolan for the visualization of meningioma. No prospective clinical studies have yet been conducted that evaluate a visualization tool to aid in decision-making during meningioma surgery, either by the Sponsor or in the scientific literature. The primary efficacy endpoint is the percentage of participants who have at least one indeterminate or end of surgery (EOS) tissue location where Gleolan-induced PpIX fluorescence status is confirmed to be or not to be meningioma tumor by central histology (i.e., true positive or true negative). This endpoint will be derived by counting the number of participants who have at least one true positive or true negative result (a success) with respect to the central laboratory neuropathologist's histological assessment. The primary efficacy analysis will be based on the Per Protocol Population. The primary endpoint and overall study design have been developed in consultation with the U.S. Food and Drug Administration (FDA).

NXDC believes that if a minimum of 30% of study participants achieve the primary efficacy endpoint, that this is clinically meaningful. This means that at least 30% of the study participants will have at least one indeterminate or unexpected fluorescent EOS tissue where Gleolan-induced PpIX fluorescence status is consistent with histological assessment of meningioma.

The determination of sample size in this study based on modeling assumptions for three factors (Table 12):

Table 12. Sample Size Determination Modeling Assumptions

95% Confidence Interval Modeling Factor	Assumption
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Percent of Participants with Evaluable Tissue Location Biopsy in the BEAP	60%
Average Number of All Biopsies per Participant with a Tissue Location in BEAP	1.5
Minimum Diagnostic Accuracy of Gleolan-Induced Fluorescence	70%

BEAP = Biopsy Efficacy Analysis Population

The primary efficacy endpoint is the percentage of participants who have at least one additional indeterminate or EOS tissue location where Gleolan-induced PpIX fluorescence status is confirmed by central histology. The primary efficacy analysis will be based on the Per Protocol Population (participant level). With 66 patients included in the Per Protocol Population, there will be 90% power to test that the primary endpoint result is greater than 30% if the expected value is 50% with an alpha of 0.05. This sample size was calculated from a two-sided Z test for binomial proportion using SAS (v9.4) PROC Power.

In order to provide adequate data to assess the secondary efficacy and safety endpoints a total of 100 participants will be enrolled in the study.

9.2 Analysis Populations

Analysis populations are described in Table 13.

Table 13. Analysis Populations

Population	Definition
Safety Analysis Population	Participants who meet the eligibility criteria for the study and receive any amount of Gleolan.
Intent-to-Treat (ITT) Population	Participants who meet the eligibility criteria for the study and have at least one tissue included in the All Biopsy Population
Per Protocol Population	Participants who are dosed with Gleolan who undergo tumor resection, have a central histologically confirmed meningioma (WHO Grade I, II, or III), and have at least one tissue included in the Biopsy Efficacy Analysis Population. This population will be used for determining the primary study endpoint at the participant level.
Biopsy-Level Populations	
All Biopsy Population	All Biopsy Population includes all biopsies (bulk, indeterminate, or unexpected fluorescent End of Surgery (EOS) tissues), regardless of acceptance into the Biopsy Efficacy Analysis Population.
Biopsy Efficacy Analysis Population	The Biopsy Efficacy Analysis Population will be made up of indeterminate tissues that meet the criteria defined in Section 4.8.1 and for unexpected fluorescent EOS tissues that meet the criteria in Section 4.8.2 .

Population	Definition
Blinded Biopsy Population	This includes all biopsies which meet the requirements for the Biopsy Efficacy Analysis Population, without taking into account biopsy histology. This population will only be used for sample size readjustment and will remain blinded to histology status of biopsies and the diagnostic accuracy of Gleolan-induced PpIX fluorescence. The population will be used to estimate the percentage of participants with 1 or more biopsies in the Biopsy Efficacy Analysis Population, and the average number of biopsies in the Biopsy Efficacy Analysis Population per participant.

Participant level analyses will be based on the Safety, ITT, and Per Protocol populations.

Biopsy level analyses will be performed on the Biopsy Efficacy Analysis Population and All Biopsy Population.

9.3 Statistical Analyses

This section presents a summary of the definition and derivation of the primary and secondary efficacy and safety variables, and the planned statistical analyses. An SAP will be written that describes in detail the analyses to be conducted. Should any analyses described in this protocol differ from that described in the SAP, the procedures in the SAP will take precedence. Any deviations from the SAP will be documented.

9.3.1 General Considerations

Descriptive statistics (n, mean, median, standard deviation (SD), minimum, and maximum for continuous data; frequencies and percentages for categorical data) will be used to summarize study data. Confidence intervals will also be provided as appropriate.

9.3.2 Primary Efficacy Analysis

The primary endpoint is the percentage of participants who have at least one additional indeterminate or EOS tissue location where Gleolan-induced PpIX fluorescence status is confirmed by central histology. This endpoint will be derived by counting the number of participants who have at least one true positive or true negative result (a success) with respect to the central laboratory neuropathologist's histological assessment. The number and percentage of participants with a success will be provided along with a 95% confidence interval calculated using the Wilson (score) method. The primary efficacy analysis will be based on the Per Protocol Population.

The null hypothesis can be written as $p \leq 30\%$ and the alternative hypothesis can be written as $p > 30\%$. If the lower bound of the Wilson (score) confidence interval is above 30%, the study will be assumed to have met its primary efficacy endpoint.

9.3.3 Secondary Efficacy Analyses

The secondary efficacy endpoints are separated into two groups: key secondary efficacy endpoints and other secondary efficacy endpoints. These endpoints are summarized in [Table 3](#) and [Table 4](#). Information on the statistical hypotheses and estimates used for powering the key secondary efficacy endpoints are also provided in the SAP. The key secondary efficacy endpoints will be tested sequentially, each at a 0.05 level.

Key Secondary Efficacy Endpoints:

1. Biopsy-Level Positive Predictive Value (PPV) of Gleolan-induced PpIX fluorescence status of biopsied tissue locations at the margin of the tumor.

This analysis will be performed at the biopsy level. The primary analysis population is the Biopsy Efficacy Analysis Population. The All Biopsy Population will be used as a supportive analysis. A weighted estimator, proposed by Lee and Dubin (Lee and Dubin, 1994), which takes into account the correlation (clustering) of the biopsies within a participant, will be used to calculate the estimate and two-sided 95% confidence interval of the PPV.

2. Biopsy-Level Negative Predictive Value (NPV) of Gleolan-induced PpIX fluorescence status of biopsied tissue location at the margin of the tumor.

This analysis will be performed at the biopsy level. The primary analysis population is the Biopsy Efficacy Analysis Population. The All Biopsy Population will be used as a supportive analysis. A weighted estimator, proposed by Lee and Dubin, which takes into account the correlation (clustering) of the biopsies within a participant, will be used to calculate the estimate and two-sided 95% confidence interval of the NPV.

3. Participant-Level Positive Predictive Value (PPV) of Gleolan-induced PpIX fluorescence status of the single bulk tumor biopsied tissue location obtained from each study participant.

This analysis will be performed at the participant level. The primary analysis population is the Per Protocol Population. The ITT Population will be used as a supportive analysis. The PPV estimate along with a two-sided 95% confidence interval calculated using the Wilson (score) method will be provided.

4. Biopsy-Level Diagnostic accuracy of Gleolan-induced PpIX fluorescence status among indeterminate tissue and unexpected fluorescent EOS tissue locations is at least 20% greater than the diagnostic accuracy of the surgeons' assessment of indeterminate tissue and unexpected fluorescent EOS tissue locations under white light.

This analysis will be performed at the biopsy level. The primary analysis population is the Biopsy Efficacy Analysis Population. The All Biopsy Population will be used as a supportive analysis. A weighted estimator, proposed by Lee and Dubin, which takes into account the correlation (clustering) of the biopsies within a participant, will be used to calculate the estimates and 95% confidence intervals of the diagnostic accuracy of blue light and white light. The difference in diagnostic accuracy rates (blue light minus white light) will also be provided. The bootstrap method will be used to create a 95% confidence interval for the difference in diagnostic accuracy estimates between blue light and white light. Full details on the bootstrap confidence interval construction will be provided in an appendix to a future version of the SAP.

Other Secondary Efficacy Endpoints:

Biopsy-level sensitivity and specificity will be analyzed using the Biopsy Efficacy Analysis Population. The All Biopsy Population will be used as a supportive analysis. A weighted estimator, proposed by Lee and Dubin, which takes into account the correlation (clustering) of the biopsies within a participant, will be used to calculate the estimate and two-sided 95% confidence interval of both the sensitivity and specificity.

The concordance of Majority Biopsy Review Panelists and Surgeon under WL and the concordance of Majority Biopsy Review Panelists and Surgeon under BL will be summarized using a Fleiss Kappa statistic. A 95% confidence interval will also be provided.

9.3.4 Exploratory Efficacy

Exploratory efficacy endpoints are described in [Table 10](#). In general, point estimates and 95% confidence intervals using the Wilson (score) method will be provided for the exploratory endpoints. Analyses at the biopsy level will also be analyzed using the Lee and Dubin method to take into account the correlation (clustering) of the biopsies within a participant [17]. Participant-level analyses will be performed on the Per Protocol Population and ITT Population, while tissue-level analyses will be performed on the Biopsy Efficacy Analysis Population and where appropriate All Biopsy Population.

9.3.5 Safety Analyses

Safety variables include laboratory assessments, physical and neurological examinations, vital signs, and assessment of AEs and SAEs. All summaries will be performed on the Safety Population.

All AEs and SAEs will be classified as to whether they occurred before Gleolan treatment or after Gleolan treatment in the analysis.

Safety variables will be summarized with appropriate descriptive statistics (sample size, missing, mean, standard deviation, median, minimum and maximum for continuous variables; number and percentage for categorical variables). No formal statistical tests will be performed on safety evaluations.

AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA). All AEs that occur after dosing will be summarized. The number and percentage of participants with AEs will be displayed by System Organ Class and preferred term. Summaries in terms of severity and relationship to treatment will also be provided. SAEs will be summarized separately in a similar fashion. In the case of multiple occurrences of the same AE within the same participant, each participant will be counted only once for each SOC and preferred term. Participant listings of SAEs and of AEs causing discontinuation of the treatment will be produced. All AEs will be listed by participant.

All vital signs, laboratory data, Karnofsky Performance Scale, MRIs and any other appropriate quantitative safety data will be presented descriptively over all time points for the baseline and post-baseline evaluations, as well as change from baseline.

Neurological examination data will also be summarized descriptively. Medical History and physical examination results will be listed by participant.

9.4 Interim Analyses

A blinded re-estimation of the sample size will be undertaken by the Sponsor after approximately 35 participants have completed the study, to evaluate the adequacy of a 100 participant sample size. The Blinded Biopsy Population will be used for sample size re-estimation. The percent of participants in the Blinded Biopsy Population with an evaluable tissue location and the average number of all tissue locations in the Blinded Biopsy Population per participant will be assessed. If the actual results of these two variables differ substantially from the expected results the sample size for the study may be increased. Only blinded data will be used for this sample size re-estimation.

9.5 Handling of Missing Intra-Operative Tissue Location Data

The study is not expected to produce a significant amount of missing data; therefore, missing data will not be imputed. All the data will be reviewed, and any spurious data will be queried as appropriate. Missing information (e.g., intraoperative data) about a tissue sample that questions the validity of the sample may cause the tissue sample to be excluded from the Biopsy Efficacy Analysis Population.

9.6 Multiple Testing Procedures

The 4 key-secondary efficacy endpoints will be analyzed in a pre-specified sequential testing procedure to preserve the overall Type 1 error rate. All comparisons will utilize an alpha level of 0.05. No additional adjustment for multiplicity will be implemented for the other efficacy endpoints.

9.7 Data Safety Monitoring Board

A central independent DSMB will periodically assess the safety of Gleolan in the study analysis population.

NXDC or designee will be responsible for establishing the DSMB, assuring proper credentials of its members, and appointing a liaison to receive the minutes and other communications of the DSMB. The DSMB members will include a:

- Neurosurgeon with experience using Gleolan
- Neuro-oncologist
- Physician safety monitor
- Statistician

The DSMB chair will be responsible for convening meetings, distributing review materials, helping in the preparation of minutes and reports, and facilitating any protocol changes that may arise out of the deliberations of the committee. A charter for the DSMB will be prepared and agreed upon, at the first meeting.

Although the Sponsor has primary responsibility for the formal statistical analysis of Study data, the DSMB will perform an interim futility analysis (as described in Section 9.4).

10 Supporting Documentation and Operational Considerations

10.1 Regulatory and Ethical Considerations

This study will be conducted in accordance with the protocol and with the following:

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
- Applicable ICH GCP Guidelines
- Applicable laws and regulations

The protocol, protocol amendments, informed consent form (ICF), IB, and other relevant documents (e.g., advertisements, participant materials) must be submitted to an IRB/IEC by the Investigator or Sponsor on the Investigator's behalf and reviewed and approved by the IRB/IEC before the study is initiated.

Any amendments to the protocol will require IRB/IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study participants.

The Investigator will be responsible for the following:

- Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC
- Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures
- Providing oversight of the conduct of the study at the site and adherence to requirements of 21 CFR, ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations

10.2 Financial Disclosure

Investigators and sub-Investigators will provide the Sponsor with sufficient, accurate financial information as requested to allow the Sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate Competent Authorities. Investigators are responsible for providing information on financial interests for themselves and their legal dependents during the course of the study and for 1 year after completion of the study.

10.3 Study and Site Start and Closure

The site study start date is the date on which the clinical study will be open for recruitment of participants.

The first act of study enrollment will be the calendar date following the individual site initiation visit. No study activity, recruitment, or screening may occur before this date.

The site end of study date will be the last study visit for the last enrolled patient.

The Sponsor designee reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the Sponsor. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

The Investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Discontinuation of specific sites or of the study as a whole are handled as part of site performance evaluations as described in the MOPS.

If the Sponsor, Investigator, or Study Medical Monitor discover conditions during the study that indicate that the study or Clinical Trial Site should be terminated, this action may be taken after appropriate consultation between the Sponsor, Investigator, and Study Medical Monitor.

Reasons for the early closure of a study site by the Sponsor or Investigator may include but are not limited to:

- Failure of the Investigator to comply with any of the following:
 - the requirements of the IRB/IEC or local Competent Authorities, the Sponsor's procedures, and GCP guidelines
 - the protocol procedures for data collection and tissue sample collection required to fulfill the aims of the study within the first group of 4 participants at each institution,
 - the securing of adequate enrollment of participants by the Investigator,
 - the proper handling of the study drug.
 - the identification of any unexpected, serious, or unacceptable risk to participants enrolled in the study, and
 - Submission of knowingly false information from the research facility to the Sponsor, Clinical Monitor, or Competent Authorities.

In addition, a decision of the sponsor to suspend or discontinue testing, evaluation, or development of the study drug. If the study is prematurely terminated or suspended, the Sponsor shall promptly inform the Investigators, the IRBs/IECs, the Competent Authorities, and any Contract Research Organization(s) used in the study of the reason for termination or suspension, as specified by the applicable regulatory requirements. The Investigator shall promptly inform the study participant and should assure appropriate therapy and/or follow-up. If the Sponsor and/or the Investigator should discover conditions arising during the study that indicate it should be terminated, an appropriate schedule for termination will be instituted. The Sponsor also reserves the right to discontinue this study for administrative reasons at any time.

10.4 Document and Data Retention

The Investigator and site will maintain all essential study documents and source documentation which support the data collected on the study Subjects according to local regulations (e.g., FDA guidelines, 21 CFR 312.62) and no less than 15 years after the end of the Study. Documents must be retained for a period of 2 years after the latter of the following two dates: The date on which the investigation is 2 years after shipment and delivery of the drug for investigational use is discontinued and Competent Authorities has been so notified; or as required by local applicable

regulatory requirements or 2 years after the marketing application is approved for Gleolan. The Investigator will take measures to ensure these essential documents are not accidentally damaged or destroyed. If for any reason the Investigator withdraws responsibility for maintaining these essential documents, custody must be transferred to an individual who will assume responsibility and is approved by authorizing parties (e.g., IRB/IEC, Sponsor). Sponsor must receive written notification of this change and study-documented information recorded appropriately.

The Sponsor will be responsible for retaining the final data set and documents pertaining to this study for a period of at least 15 years, at least 2 years after the Gleolan marketing application is approved; or, if an application is not approved for Gleolan, until 2 years after shipment and delivery of the drug for investigational use is discontinued and Competent Authorities has been so notified; or as required by local applicable regulatory requirements.

10.5 Dissemination of Clinical Study Data

This protocol will be registered with clinical trial registries per country regulatory requirements. Results will also be disseminated via those registries as required.

10.6 Publication Policy

The results of this study may be published or presented at scientific meetings. If this is foreseen, the Investigator agrees to submit all manuscripts or abstracts to the Sponsor before submission. This allows the Sponsor to protect proprietary information and to provide comments.

Investigators may not publish segments of study data without written authorization from the Sponsor and the Steering Committee.

The Sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the Sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating Investigator will be designated by mutual agreement.

Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

10.7 Clinical Trial Insurance

The Sponsor will obtain Clinical Trial Insurance as needed and provided the necessary information to the investigative sites.

10.8 Contact Information for Trial Sites

The Sponsor or representative will maintain a list of all active clinical trial sites and maintain trial status on clinicaltrials.gov and clinicaltrialsregister.eu as applicable.

10.9 Investigator's Responsibilities

- The Investigator should be qualified by education, training (including all requested Gleolan training), and experience to assume responsibility for the proper conduct of the clinical study.

- The Investigator must have knowledge on the use, application, implementation or administration of the study drug and the requirements for clinical, efficacy and safety follow-up.
- The Investigator should be familiar with and trained on the appropriate use of study drug as described in the protocol and in the current IB.
- The Investigator should disclose any potential conflicts of interest, including financial, that interfere with the conduct of the clinical investigation or interpretation of the results.
- The Investigator should be trained on and comply with GCP regulations and the applicable regulatory requirements.
- The Investigator should demonstrate that the proposed Clinical Trial Site has the following:
 - One or more qualified Investigators;
 - Qualified site staff;
 - Adequate facilities for the foreseen duration of the clinical study;
 - Required number of eligible study participants needed within the agreed recruitment period.
- The Investigator must create and maintain source documentation throughout the clinical study and make it available as requested during monitoring visits and audits.
- The Investigator should permit monitoring and auditing by the Sponsor or Sponsor's designee and inspection by the appropriate Competent Authorities. Investigator should be accessible (when possible) to the monitor to respond to questions.
- The Investigator should have sufficient time to conduct and oversee the trial.
- The Investigator should ensure the IRB/IEC has the most up to date study related documentation (e.g., IB, Protocol).
- The Investigator should inform the study participant's primary physician about their patients' participation in the trial if permitted to do so by the study participant.
- The Investigator will provide the Sponsor with copies of any clinical-investigation-related communications between the Investigator and the IRB/IEC.
- The Investigator will permit trial-related monitoring, audits, IRB/IEC review, and regulatory inspection(s), providing direct access to source data/documents.
- The Investigator must be aware of the AE and adverse reaction reporting process, including reactions related to the use of study drug.
- The Investigator shall ensure accuracy, completeness legibility and timeliness of the data reported to the Sponsor in the eCRFs and in all required reports.
- The Investigator must have knowledge of the risk analysis of the study drug, knowledge of the requirements for storage, handling, administration, and return of the study drug including any hazard to those handling the product and close contacts and the risk to the environment. Investigator will also maintain study drug accountability records.
- The Investigator must ensure that the particular requirements for the use of the study drug are known.
- The Investigator shall ensure maintenance and calibration of the equipment relevant for the assessment of the clinical study is appropriately performed and documented, when applicable.
- The Investigator must be knowledgeable with the method of obtaining informed consent.
- The Investigator shall ensure and document appropriate training if any authorized designee is appointed to conduct the informed consent process.

- The Investigator must inform the study participant of the particular issues that arise for the study drug. In particular, both the ICF and any other written information to be provided to the participants should include an explanation of the following:
 - Provisions for study data protection and [REDACTED]
 - The arrangements for follow-up before and after the end of the trial, including after withdrawal from the study and including the information to be provided to the study participant for use in the event of problems arising after the end of the trial;
 - The length of follow-up;
 - The definition of the end of the trial and its relationship to the follow-up after the end of the trial;
- The Investigator shall provide adequate medical care to a study participant during and after their participation in a clinical study in the case of AEs.
- The Investigator shall ensure that clinical records are clearly marked to indicate that the participant is enrolled in a particular clinical study.
- The Investigator must provide the study participant with the following:
 - Information on any new significant findings occurring during the clinical study, including the need for additional medical care that may be required;
 - Well-defined procedures for possible emergency situations to the clinical study
 - Some means of showing their participation in the clinical study, together with identification and compliance information for the concomitant treatment measures
- The Investigator shall review the Clinical Study Report at the close-out of the clinical study.

11 References

1. *Gleolan Prescribing Information* 2019.
2. *Gliolan Summary of Product Characteristics*. 2018.
3. Knipps, J., et al., *Fluorescence Behavior and Dural Infiltration of Meningioma Analyzed by 5-Aminolevulinic Acid–Based Fluorescence: Operating Microscope Versus Mini-Spectrometer*. *World neurosurgery*, 2017. **108**: p. 118-127.
4. Coluccia, D., et al., *Intraoperative 5-aminolevulinic-acid-induced fluorescence in meningiomas*. *Acta neurochirurgica*, 2010. **152**(10): p. 1711-1719.
5. Millesi, M., et al., *Analysis of the surgical benefits of 5-ALA–induced fluorescence in intracranial meningiomas: experience in 204 meningiomas*. *Journal of neurosurgery*, 2016. **125**(6): p. 1408-1419.
6. Della Puppa, A., et al., *Predictive value of intraoperative 5-aminolevulinic acid–induced fluorescence for detecting bone invasion in meningioma surgery*. *Journal of neurosurgery*, 2014. **120**(4): p. 840-845.
7. Valdes, P., et al., *5-Aminolevulinic acid-induced protoporphyrin IX fluorescence in meningioma: Qualitative and quantitative measurements in vivo*. *Neurosurgery*, 2014;. **10** p. 74-82.
8. Apra, C., M. Peyre, and M. Kalamarides, *Current treatment options for meningioma*. *Expert review of neurotherapeutics*, 2018. **18**(3): p. 241-249.
9. Sotoudeh, H. and H.R. Yazdi, *A review on dural tail sign*. *World journal of radiology*, 2010. **2**(5): p. 188.
10. *Investigator's Brochure Gleolan Aminolevulinic Acid Hydrochloride*. 2020.
11. Potapov, A., et al., *Intraoperative fluorescence diagnostics in surgery of intracranial meningiomas: analysis of 101 cases*. *Zhurnal voprosy neirokhirurgii imeni NN Burdenko*, 2018. **82**(2): p. 17-29.
12. Marbacher, S., et al., *Use of fluorescence to guide resection or biopsy of primary brain tumors and brain metastases*. *Neurosurgical focus*, 2014. **36**(2): p. E10.
13. Simpson, D., *The recurrence of intracranial meningiomas after surgical treatment*. *Journal of neurology, neurosurgery, and psychiatry*, 1957. **20**(1): p. 22.
14. Kaneko, S., et al., *Fluorescence-Based Measurement of Real-Time Kinetics of Protoporphyrin IX After 5-Aminolevulinic Acid Administration in Human In Situ Malignant Gliomas*. *Neurosurgery*, 2019. **85**(4): p. E739-E746.
15. Stummer, W., et al., *Randomized, Prospective Double-Blinded Study Comparing 3 Different Doses of 5-Aminolevulinic Acid for Fluorescence-Guided Resections of Malignant Gliomas*. *Neurosurgery*, 2017. **81**(2): p. 230-239.
16. Peng, Q., et al., *5-Aminolevulinic acid-based photodynamic therapy: principles and experimental research*. *Photochemistry and photobiology*, 1997. **65**(2): p. 235-251.
17. Lee, E.W. and N. Dubin, *"Estimation and sample size considerations for clustered binary responses*. *Statistics in Medicine*. Vol. 13. 1994. 1241-1252.

12 Appendices

Appendix 1 Administrative Structure

Name/Affiliation	Address/Phone Number	Responsibility
NXDC	[REDACTED]	Sponsor Project Director
[REDACTED]	[REDACTED]	Medical Monitor
[REDACTED]	[REDACTED]	Site Management and Monitoring, Regulatory Submission in Europe, EDC and Data Management
[REDACTED]	[REDACTED]	Safety Reporting

Appendix 2 Clinical Laboratory Evaluations

Investigators must document review of each laboratory safety report.

Pregnancy

- A serum pregnancy test may diagnose pregnancy ~6 to 10 days after fertilization; a urine pregnancy test, because it is less sensitive, will diagnose pregnancy a few days after a serum pregnancy test.
- As a minimum, a pregnancy test should be performed at screening to confirm absence of pregnancy and at the end of relevant systemic exposure. Additional testing may be required between the screening visit and the first dose of study intervention on Day 1. Consider additional pregnancy testing if the interval is:
 - ≤4 days, a repeat highly sensitive serum pregnancy test usually is not required
 - >4 days, a repeat serum pregnancy test should be obtained
- The tests detailed in the schedule of events will be performed at the local lab.
- Additional tests may be performed at any time during the study as determined necessary by the Investigator or required by local regulations.

Liver Function

- Study participants will be monitored by the Investigator and study team for any changes in hepatic clinical laboratory measures.
- Investigators should follow Hy's law when assessing and reporting AEs/SAEs involving liver function.
- Additional tests may be performed during study participation to monitor any elevated values.

Routine/Standard of Care

- Labs collected per site SOC during screening/baseline, study procedure and routine follow up visits will be collected and assessed for AEs/SAEs reportable per protocol.
- Comprehensive metabolic panel (CMP) and complete blood count (CBC) should be done following the Schedule of Events.
- Some of these labs may need to be repeated during the period of study participation if deemed necessary by the Investigator, the Sponsor or IRB/IEC.

Appendix 3 Further Information on Contraception

There is very limited data about the use of Gleolan during pregnancy. There is some limited evidence from animal studies of an embryotoxic effect of Gleolan following light exposure.

A WOCBP is defined as any female who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, or a bilateral oophorectomy) or is not postmenopausal (defined as amenorrhea > 12 consecutive months, or women on hormone replacement therapy (HRT) with documented plasma follicle-stimulating hormone (FSH) level > 35 mIU/mL). Even women who are using oral, implanted, or injectable contraceptive hormones or mechanical products (diaphragm, condoms, spermicides) to prevent pregnancy or practicing abstinence or where partner is sterile (e.g., vasectomy), should be considered to be WOCBP.

Prior to study enrollment, WOCBP must be advised of the importance of avoiding pregnancy during trial participation and the potential risk factors for an unintentional pregnancy. In addition, men enrolled on this study should understand the risks to any sexual partner of childbearing potential.

WOCBP, defined as all women physiologically capable of becoming pregnant, or fertile men, must agree to use highly effective methods of contraception throughout the study. Male participants should not donate sperm for 42 days after the dose of study drug. Women are considered postmenopausal and not of childbearing potential if they have had 12 months of natural (spontaneous) amenorrhea with an appropriate clinical profile (e.g., age appropriate, history of vasomotor symptoms) or have had surgical bilateral oophorectomy (with or without hysterectomy) or tubal ligation at least six weeks ago. In the case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment if she is considered not of childbearing potential.

Highly effective contraception methods include:

- Total abstinence when this is in line with the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.
- Female sterilization (have had surgical bilateral oophorectomy with or without hysterectomy), total hysterectomy, or tubal ligation at least six weeks before taking study treatment. In case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment
- Male sterilization (e.g., vasectomy)
- Use of oral, injected or implanted hormonal methods of contraception or placement of an intrauterine device (IUD) or intrauterine system (IUS), or other forms of hormonal contraception that have comparable efficacy (failure rate <1%), for example hormone vaginal ring or transdermal hormone contraception.
- In case of use of oral contraception, women should have been stable on the same pill for a minimum of 3 months before taking study drug. Note: While oral contraceptives are allowed, they should be used with caution as there is no data available indicating if these drugs are contraindicated when taken by participants that are dosed with Gleolan.

WOCBP who are pregnant during the screening period will not be eligible for the trial. Participants will be evaluated prior to dosing for pregnancy status and if a positive pregnancy test is determined, the participant will not receive study drug.

Appendix 4 COVID-19 Pandemic Protocol Amendment

Study Modifications During the Novel Coronavirus Disease (COVID-19) Pandemic

This Amendment to Protocol MEN-301 is intended to detail the modifications to study conduct and site monitoring that will be implemented during this study due to potential impacts of the global COVID-19 pandemic. In light of potential disruptions during the COVID-19 pandemic, certain participant and site-specific modifications may be made to study assessments and procedures to ensure the safety of all participants and staff. The allowances outlined in this COVID-19 Pandemic Protocol Amendment aim to ensure continued periodic safety assessments, retention of study data integrity, and to avoid study interruption.

This study will continue to be implemented per protocol at all sites and for all participants, except in cases where the impact of COVID-19 prevents the performance of scheduled assessments or procedures as described in the current study protocol. In these cases, scheduled visits should still be conducted, and scheduled assessments should still be performed per protocol, to the extent possible for each participant at each scheduled visit. Certain assessments may be performed remotely (e.g. via phone/video meetings, in-home medical assessment, or at other local laboratories or local satellite clinics affiliated with hospital clinic). Upon alleviation of COVID-19 risks, it is expected that the study will be conducted according to the current protocol and best practices determined by the site's Principal Investigator on a per participant and per site basis. In all cases, deviations to the protocol will be recorded within the Electronic Data Capture (EDC) system utilized for the trial and described in the Clinical Study Report.

1 Timeframe for this COVID-19 Pandemic Protocol Amendment

The start and end dates of the implementation of this COVID-19 Pandemic Protocol Amendment will be determined for each site individually, through consultation between the Sponsor and the site's Principal Investigator.

2 Participation of COVID-19 Positive Patients

Any patient who has symptoms known to be associated with COVID-19 and has been determined by the Institution's standard of care to be positive for the disease, prior to receipt of the study medication, will be excluded from the trial and will be ineligible to receive study medication at time of surgery. The criteria for the patient to undergo surgery will be at the discretion of the site's Investigator and his/her Institution. Any participant who becomes COVID-19 positive after surgery, will be retained in the study. All participants who have a Serious Adverse Events (SAEs) suspected to be related to COVID-19 by the investigator will be assessed for COVID-19 per site standard of care and data must be recorded in the participant's medical history.

3 Notification of Study Participants and Staff about Modifications to Study Procedures

Study participants are to contact the site Investigator or study contact by email, mail, and/or phone/video meeting if they present with symptoms of COVID-19. To enable the expeditious implementation of the modifications outlined in this Amendment, site study staff are also

encouraged to contact study participants by phone/video meeting to present any future modifications as detailed in this Amendment, and to address any questions. If required by the site, the conversational script for the phone/video meeting used by the site study staff will be submitted to the Institutional Review Board (IRB)/Independent Ethics Committee (IEC).

4 Visit Schedule and On-site Study Visits

Participants should attempt to complete as many of the scheduled study visits as possible at the study site. This COVID-19 Pandemic Protocol Amendment allows for wider visit windows for some study visits and allows for study visits to take place via phone/video meeting, or at other local laboratories or local satellite clinic affiliated with or approved by the hospital clinic. These modifications and allowances can be made on a visit, participant or site-specific basis, as needed.

Every attempt should be made to conduct the assessments at the specified assessment time point. All data collected remotely (e.g., via phone/video meeting, or at local laboratories or satellite clinics affiliated with the hospital clinic) will be recorded in the eCRF. Specific information should be captured in the eCRF for missed or assessments conducted in a manner (including data collected remotely) that is not in accordance with the schedule of events specified in the Protocol, including the relationship to COVID-19.

SAEs resulting from suspected COVID-19 exposure or testing (regardless of test results) must be reported to the Sponsor and clearly described as COVID-19 related. If a study participant tests positive for COVID-19, this should be documented as an AE and the Sponsor must be notified within 24 hours in order to plan for any possible interruptions in study visits. This information, summarized in the Clinical Study Report, will be helpful to the Sponsor and the Regulatory Authorities (e.g. FDA).

5 Follow-up Examination

In the event participants are unable to access the site for on-site study visits, assessments may be recorded by a local satellite clinic affiliated with hospital clinic or a phone/video meeting, optimally no longer than 5 days after the protocol dictated window. Data will be recorded in the eCRF with notation that the assessments were recorded at a remote visit. Missing data will be identified as protocol deviations related to COVID-19 within the eCRF.

6 Collection of Adverse Events and Concomitant Medications

In the event participants are unable to access the site for on-site study visits, adverse events and concomitant medications will be reviewed with the participant by phone/video meeting follow up with the Investigator or designee. Data will be recorded in the eCRF.

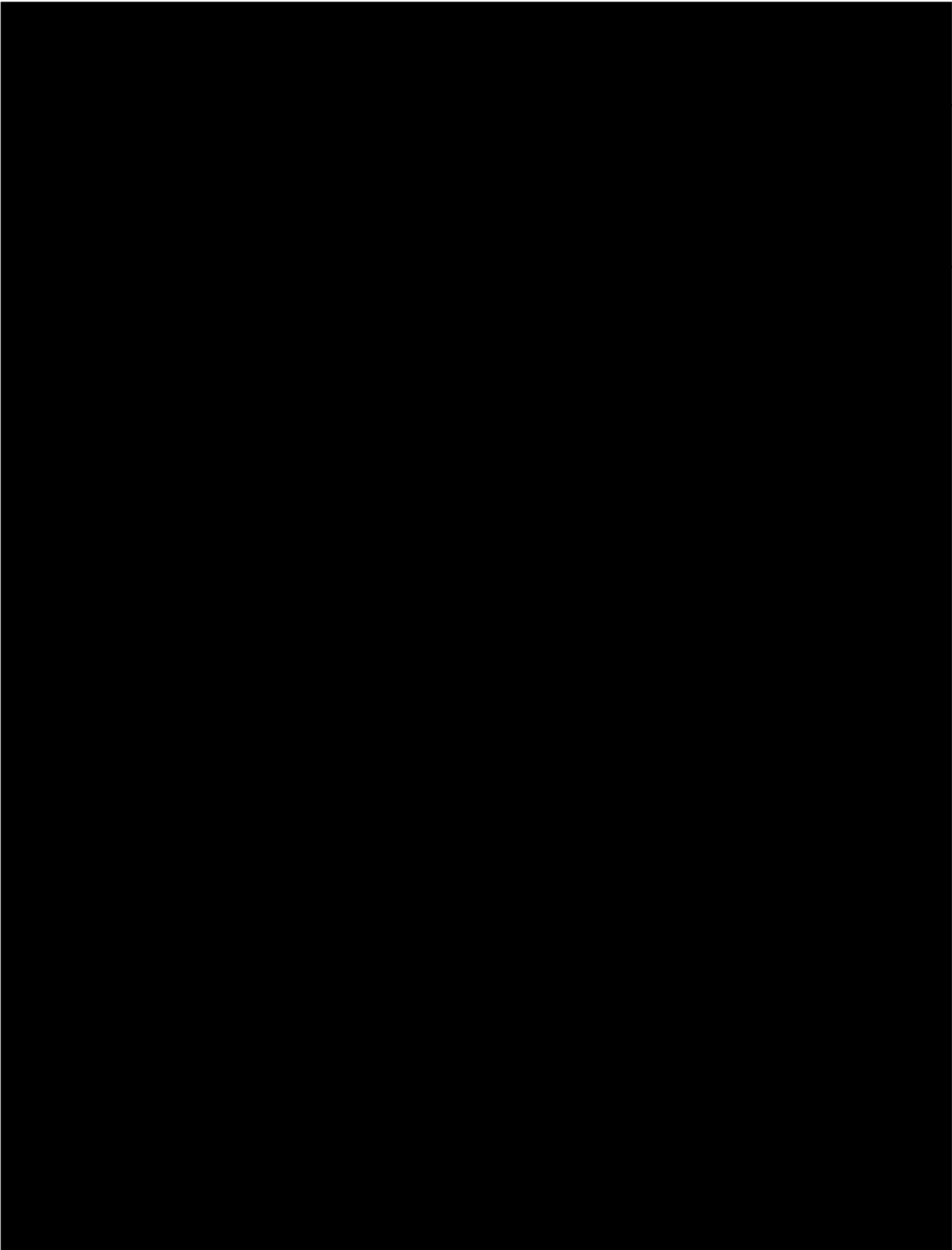
7 Protocol Deviations

Protocol deviations will continue to be recorded in the EDC system as specified per study protocol, with protocol deviations reported by the Investigator to the IRB/IEC and Sponsor or designee within

24 hours of discovery, or according to local site requirements. In addition, all deviations resulting from the COVID-19 pandemic will be recorded with documented reasons pertaining to COVID 19.

8 Site Monitoring

In accordance with applicable local regulations, Good Clinical Practice (GCP), and the procedures of the Sponsor or its designees, the Study Monitor will periodically contact the site to conduct on-site monitoring reviews. During the COVID-19 pandemic, the extent, nature, and frequency of monitoring reviews will be largely based on the site's ability to accommodate on-site monitoring visits, site policies pertaining to hospital and medical record access and local and federal regulations regarding COVID-19 related travel restrictions. The Study Monitor(s) designated by the Sponsor will follow all required hygienic regulations required by local authorities and carry an Access Permission issued by the hospital where the site is located. Through frequent communications (e.g., letter, e-mail, and phone/video meeting) as applicable, the Study Monitor will ensure that the site continues to conduct all study procedures and evaluations according to protocol and regulatory requirements. All source data verification will be performed by Monitor(s) at the study site only.



✔ Agreement completed.

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