STATISTICAL ANALYSIS PLAN

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

Protocol Number: NXDC-MEN-301

Phase: Phase 3

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2 LIST OF ABBREVIATIONS

Only abbreviations and terms relevant to the SAP are repeated herein. The reader is referred to the protocol for the complete and comprehensive list of abbreviations and definitions of terms forthe study.

ABP	All Biopsy Population
ADaM	Analysis Data Model
AE	Adverse Event
ALA	Aminolevulinic Acid
ALA HCl	Aminolevulinic Acid Hydrochloride
ALT	alanine transaminase
AST	aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical
BEAP	Biopsy Efficacy Analysis Population
BIL	Bilirubin
BL	Blue Light
BRP	Biopsy Review Panel
BUN	Blood urea nitrogen
CBC	Complete Blood Count
CDISC	Clinical Data Interchange Standards Consortium
CFR	Code of Federal Regulations
CMP	Complete Metabolic Panel
CRF	Case report form
CSR	Clinical Study Report
CTCAE	Common Terminology for Adverse Events
CV	Coefficient of variation
DD	Drug Dictionary
DSMB	Data Safety Monitoring Board
EOS	End of Surgery
FDA	Food and Drug Administration
FL	Fluorescence
FN_{BL}	False Negative Blue Light
FN_{WL}	False Negative White Light
FP_{BL}	False Positive Blue Light
FP_{WL}	False Positive White Light
GEE	Generalized Estimating Equation
ICH	International Council for Harmonisation
ISO	International Organization for Standardization
ITI	Intent-to-Image
ITT	Intent-to-Treat
MedDRA	Medical Dictionary for Regulatory Activities
MRI	Magnetic Resonance Imaging
NDA	New Drug Application
NXDC	NX Development Corporation
NPV	Negative Predictive Value
PP	Per Protocol
PpIX	Protoporphyrin IX

PPV	Positive Predictive Value
PT	Preferred Term
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Standard Deviation
SDTM	Study Data Tabulation Model
SOC	System Organ Class
TEAE	Treatment-emergent Adverse Event
TESAE	Treatment Emergent Serious Adverse Event
TN_{BL}	True Negative Blue Light
TN_{WL}	True Negative White Light
TP_{BL}	True Positive Blue Light
TP_{WL}	True Positive White Light
US	United States
WHO	World Health Organization
WL	White Light
WOCBP	Women of Childbearing Potential

3 INTRODUCTION

3.1 Preface

This document presents a statistical analysis plan (SAP) for NX Development's Protocol (NXDC-MEN-301), *A Phase 3 Multicenter Study of Gleolan (Aminolevulinic AcidHydrochloride) to Enhance Visualization of Tumor in Patients with Newly Diagnosed or Recurrent Meningiomas*.

Reference materials for this statistical plan includes the protocol NXDC-MEN-301 (v 3.0 Dated: 04Apr2022).

The statistical analysis plan (SAP) described hereafter is an *a priori* plan. The SAP will befinalized and approved prior to database lock. Whereas the protocol presents a general discussion of statistical procedures, this SAP provides greater detail and further clarifies the analyses. Nevertheless, in the event of any discrepant directives between the protocol and this SAP, the SAP will supersede the protocol.

3.2 Rationale of Study

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

The study objectives are:

Primary Endpoint

> 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 meningioma tumor in tissue where there is uncertainty regarding that tissue's tumor status based on white light (WL) visualization alone.

Key Secondary Endpoints

1. To determine the biopsy-level PPV of Gleolan-induced PpIX fluorescence for the real-time visualization of tissue locations on the tumor margin in newly diagnosed or recurrent meningioma during resection surgery.

2. To determine the biopsy-level NPV of Gleolan-induced PpIX fluorescence for the real-time visualization of tissue locations on the tumor margin in newly diagnosed or recurrent meningioma during resection surgery.

3.3 Purpose of Analyses

The purposes of the planned analyses described in this SAP are to assess 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. Results from the analyses completed will be included in the final clinical study report for NXDC-MEN-301, and may also be utilized for regulatory submissions, manuscripts, or other clinical development activities.

Post-hoc exploratory analyses not identified in this SAP may be performed to further examine the study data. These analyses will be clearly identified, where appropriate, in the final clinical study report. Additional analyses not prospectively identified in this SAP may also be completed for publications, or regulatory or funding inquiries. These analyses, if performed, may not be reported in the clinical study report, but will be fully documented in the document containing the additional analyses.

4 STUDY POPULATIONS, OBJECTIVES AND ENDPOINTS

4.1 Analysis Populations

There will be five analysis populations defined for this study.

Table 1. Analysis Population

Population	Definition
Participant-Level Populations	
Safety Analysis Population	Participants who meet the eligibility criteria for the study and receive any amount of Gleolan.
Intent-to-Image (ITI) Population	Participants who meet the eligibility criteria for the study and receive any amount of Gleolan. This population will be used for determining the primary study endpoint at the participant level.
Per Protocol (PP) Population	Participants who are dosed with Gleolan who undergo tumor resection and have a histologically-confirmed meningioma (WHO Grade I, II, or III) from a bulk tissue sample.
Biopsy-Level Populations	
All Biopsy Population (ABP)	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 (BEAP)	The Biopsy Efficacy Analysis Population will be made up of indeterminate tissues that meet the criteria defined in Section 4.8.1 of the protocol and of unexpected fluorescent EOS tissues that meet the criteria in Section 4.8.2 of the protocol.
Blinded Biopsy Population	This includes all biopsies that come from participants in the ITI 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 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.

4.2 Primary Objective and Endpoint

Table 2. Primary Objective and Endpoint

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 of tumor in tissue where there is uncertainty regarding that tissue's tumor status based on white light (WL) visualization alone.	*Intent-to-Image (ITI) Population (Primary)* *Per Protocol Population (Supportive)* 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 alone is consistent with histology, among all participants who meet the eligibility criteria for the study and receive any amount of Gleolan. This endpoint will be derived by counting in the numerator the number of participants who have at least one true positive (TP) or true negative (TN) result (i.e., a success) with respect to the central laboratory neuropathologist's histological assessment. All participants in the ITI population will be included in the denominator. For a participant to be considered a success, only biopsies considered 'non-obvious' for tumor status by the Biopsy Review Panel (BRP) will be eligible to be assessed in the numerator. *Null Hypothesis: Percentage ≤ 30%* *Alternative Hypothesis: Percentage > 30%* *Expected Response: Percentage ≥ 50%*

4.3 Secondary Objectives and Endpoints

Table 3. Key Secondary Efficacy Endpoints

Sequence	Key Secondary Efficacy Objective	Endpoint/Estimand
1	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.	*Biopsy Efficacy Analysis Population (Primary)* *All Biopsy Population (Supportive)* Positive Predictive Value (PPV) of Gleolan-induced PpIX fluorescence status of biopsied tissue locations at the margin of the tumor (indeterminate tissue and unexpected fluorescent EOS tissue (combined)). $PPV = TP_{BL}/(TP_{BL}+FP_{BL})$ *Null Hypothesis: PPV ≤ 60%* *Alternative Hypothesis: PPV > 60%* *Expected Response: PPV ≥ 80%*
2	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.	*Biopsy Efficacy Analysis Population (Primary)* *All Biopsy Population (Supportive)* Negative Predictive Value (NPV) of Gleolan-induced PpIX fluorescence status of biopsied tissue location at the margin of the tumor (indeterminate tissue only). $NPV = TN_{BL}/(TN_{BL}+FN_{BL})$ *Null Hypothesis: NPV ≤ 40%* *Alternative Hypothesis: NPV > 40%* *Expected Response: NPV ≥ 60%*

PpIX= protoporphyrin IX, PPV=positive predictive value, NPV=negative predictive value, EOS=end of surgery

Table 4. Other Secondary Efficacy Endpoints

Efficacy Objective	Endpoint/Estimand
Other Secondary Efficacy Objective	
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.	*ITI Population (Primary)* *Per Protocol Population (Supportive)* Positive Predictive Value (PPV) of Gleolan-induced PpIX fluorescence status of the single bulk tumor biopsied tissue location obtained from each study participant. $PPV = TP_{BL}/(TP_{BL}+FP_{BL})$ *Expected Response: PPV ≥ 80%*
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.	*Biopsy Efficacy Analysis Population (Primary)* *All Biopsy Population (Supportive)* Diagnostic accuracy of Gleolan-induced PpIX fluorescence status among indeterminate tissue is greater than the diagnostic accuracy of the surgeons' assessment of indeterminate tissue under WL: Diagnostic Accuracy $_{BL}$ – Diagnostic Accuracy $_{WL}$ $[(TP_{BL}+TN_{BL})/(TP_{BL}+TN_{BL}+FP_{BL}+FN_{BL})]*100 – [(TP_{WL}+TN_{WL})/(TP_{WL}+TN_{WL}+FP_{WL}+FN_{WL})] *100$ *Expected Response ≥ 30%* Diagnostic accuracy will also be calculated for BL for unexpected fluorescent EOS tissues only and for both indeterminate tissue and unexpected fluorescent EOS tissue locations combined.
To further determine the biopsy-level diagnostic performance of Gleolan-induced PpIX fluorescence status for the real-time visualization of meningioma during resection surgery.	*Biopsy Efficacy Analysis Population* Diagnostic performance of Gleolan-induced PpIX fluorescence status will be computed for indeterminate tissue and unexpected fluorescent EOS tissue (combined). • *Biopsy-level sensitivity* • *Biopsy-level specificity* *Expected Responses > 70%*

4.4 Exploratory Objectives and Endpoints

Table 5. Exploratory Endpoints

Efficacy Objective	Endpoint/Estimand
Exploratory	
To further characterize the diagnostic performance of Gleolan for the real-time visualization of meningioma during resection surgery.	*Per Protocol Population* Diagnostic performance of Gleolan at the patient level as assessed with the single bulk tumor biopsied tissue sample. $Sensitivity_{BL} = TP_{BL}/(TP_{BL}+FN_{BL})$ $Specificity_{BL} = TN_{BL}/(TN_{BL}+FP_{BL})$ $NPV_{BL} = TN_{BL}/(TN_{BL}+FN_{BL})$ $Diagnostic\ Accuracy_{BL} = [(TP_{BL}+TN_{BL})/(TP_{BL}+TN_{BL}+FP_{BL}+FN_{BL})]*100$ (Proportion of biopsies that are meningiomas by the central histology neuropathologist that exhibit Gleolan-induced PpIX fluorescence status)
To determine if tumor location influences diagnostic performance of Gleolan	*Per Protocol Population* PPV of Gleolan-induced PpIX fluorescence status of the single bulk tumor biopsied tissue sample obtained from each study participant will be compared across subgroups of meningioma tumor location.
To determine if time from dose administration of Gleolan to acquisition of bulk tumor biopsy affects diagnostic performance of Gleolan.	*Per Protocol Population* PPV, NPV, Sensitivity, Specificity, and Diagnostic Accuracy of Gleolan-induced PpIX fluorescence status of the single bulk tumor biopsied tissue sample obtained from each study participant will be compared across subgroups of time from Gleolan dose to tissue sample acquisition (<2 hours and ≥2 hours).
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.	*Biopsy Efficacy Analysis Population* Compared across subgroups of meningioma tumor location and tissue type: • Number of indeterminate/unexpected fluorescent EOS tissues. • Percentage of all indeterminate tissues where Gleolan-induced PpIX fluorescence status resulted in a change in the surgeon's decision to remove or leave a piece of tissue.

Efficacy Objective	Endpoint/Estimand
Exploratory	
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.	*Biopsy Efficacy Analysis Population* The percentage of all indeterminate 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 the fluorescence status agrees with central histopathological results.
To determine the frequency with which Gleolan-induced PpIX fluorescence status allows the surgeon to observe residual tumor and then resect the tissue, after complete resection under WL at the EOS.	*Per Protocol Population* The percentage of participants who have at least one unexpected fluorescent EOS tissue where Gleolan-induced PpIX fluorescence status resulted in the operating surgeon's decision to resect the tissue and the central histopathological result confirms tumor.

5 STUDY METHODS

5.1 General Study Design and Plan

As background for the statistical methods presented below, this section provides an overview of the study design and plan of study execution. The NXDC-MEN-301 protocol is the definitive reference for all matters discussed in what follows.

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 15 months with individual study participation lasting for approximately 2 months (from informed consent through 6 weeks post-surgery).

Participants about to undergo resection for suspected meningioma [World Health Organization (WHO) Grade I, II, III] will be screened and informed consent will be obtained prior to surgery and prior to study participation. 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 fluorescencestatus for the selection of protocol-driven tissue locations and to assess fluorescence status (for further detail see Definitions and Terms in Section 5.2).

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

The overall study schema is presented in Figure 1. The schedule for assessments is presented in Table 6.

Figure 1. Overall Study Schema

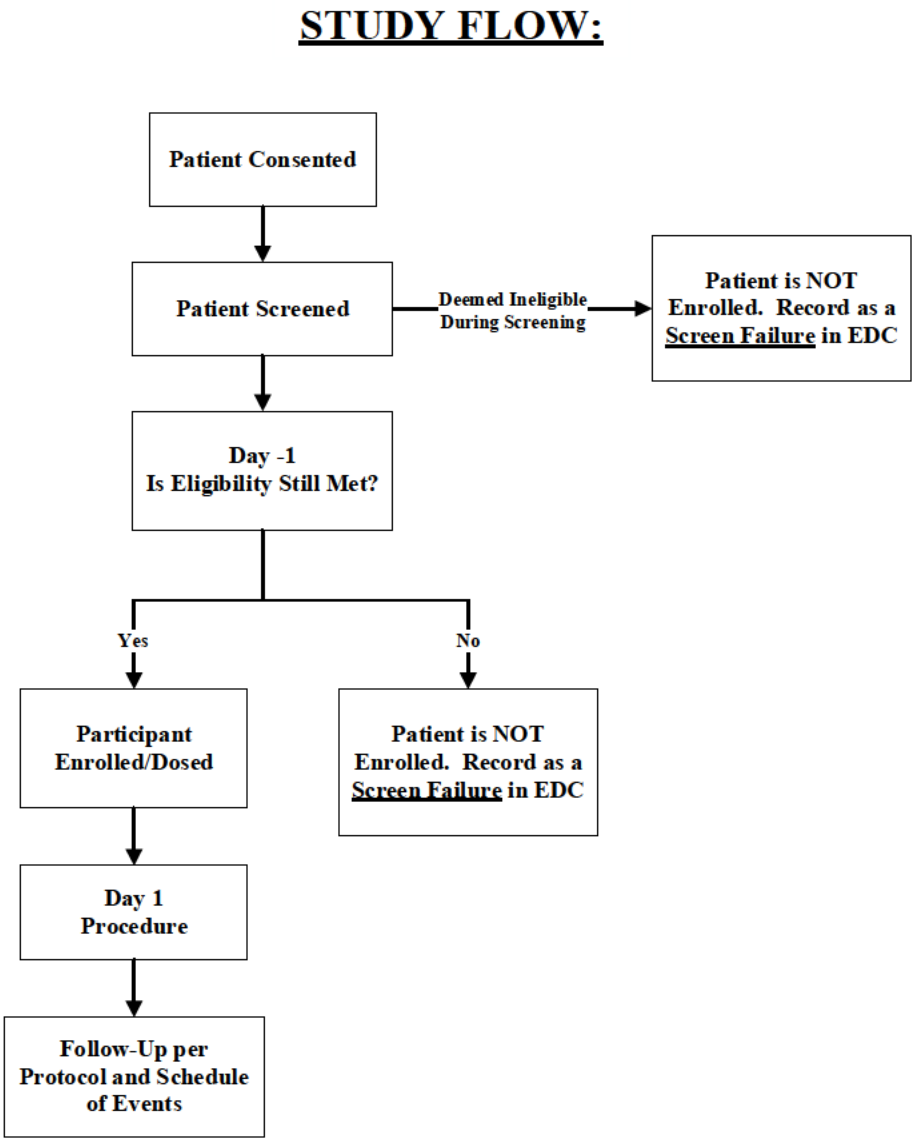


Table 6. Schedule of Events

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, CO_2 (Optional), total protein, albumin, AST=aspartate aminotransferase, ALT=alanine transaminase, BIL= Bilirubin, alkaline phosphatase)
f. Baseline MRI is standard of care 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 standard of care, not protocol mandated
g. Urine or blood pregnancy test for Women of Childbearing Potential (WOCBP). Type of test dependent on site's standard of care
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 standard of care.

5.2 Definitions and Terminology

Table 7. Definitions and Terminology

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 if it 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 a 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.
True Positive $_{\text{blue light}}$ (TP_{BL})	Tissue with Gleolan-induced PpIX fluorescence that is confirmed to be meningioma by the central histology neuropathologist.

Term	Definition
False Positive blue light (FP_{BL})	Tissue with Gleolan-induced PpIX fluorescence that is determined by the central histology neuropathologist to contain no meningioma cells (non-neoplastic tissue).
True Negative blue light (TN_{BL})	Tissue with *no* 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 *no* 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 neuro-pathologist.
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 of PPV performed at the biopsy level.
Diagnostic $Accuracy_{BL}$ of Gleolan-induced PpIX Fluorescence Status to Identify Meningioma Tumor Tissue	$(TP_{BL}+TN_{BL})/(TP_{BL}+TN_{BL}+FP_{BL}+FN_{BL})$ Proportion of biopsies correctly classified by Gleolan-induced PpIX fluorescence among all biopsies evaluated.

Term	Definition
Diagnostic Accuracy$_{WL}$ of Meningioma Tumor Tissue Identification Under WL Visualization	$(TP_{WL}+TN_{WL})/(TP_{WL}+TN_{WL}+FP_{WL}+FN_{WL})$ Proportion of biopsies correctly classified by visualization under WL among all biopsies evaluated.
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 *obviously* likely to be tumor or *obviously* 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-surgicalassessments 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-Image (ITI) Population	Participants who meet the eligibility criteria for the study and receive any amount of Gleolan. This population will be used for determining the primary study endpoint at the participant level.
Per Protocol Population	Participants who are dosed with Gleolan who undergo tumor resection, have a histologically-confirmed meningioma (WHO Grade I, II, or III) from a bulk tissue sample.
All Biopsy Population	All Biopsy Population includes all biopsies (bulk, indeterminate, or unexpected fluorescent 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 of the protocol and of unexpected fluorescent EOS tissues that meet the criteria in Section 4.8.2 of the protocol.
Blinded Biopsy Population	This includes all biopsies that come from participants in the ITI 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 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.

5.3 Randomization and Blinding

5.3.1 Randomization

This is an open-label single-arm study without randomization.

5.3.2 Blinding

A blinded histological assessment (presence or absence of meningioma cells) by the study's central neuropathology lab 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.

5.3.3 Bias Minimization for Efficacy Analyses

Biopsy Reviewers will independently perform a blinded post-surgical review of video and photos under WL and BL.

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 in the study.

The Biopsy Review Panel will be given independent online access to four sequential sessions where photos, videos and relevant data about the participant will be provided for evaluation. 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 and Blinded Biopsy Population.

Session 2 objective:

Evaluate a WL EOS Field of View image to identify location(s) of likely tumor tissue, ifany. 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 and Blinded Biopsy Population.

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).

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 or unexpected fluorescent EOS tissue.

Biopsy Review panelists reviewing photos and videos of the meningioma resection surgeries will record their assessments in the Biopsy Review Module of the electronic Case Report Form. The data will be part of the clinical trial database. All of the data recorded by the Biopsy Review panelists will be included with the datasets in the New Drug Application (NDA) submission. Whenever Biopsy Review data is provided in the NDA, it will be clearly identified so that the FDA reviewers can distinguish the results of Biopsy Review from data as originally collected.

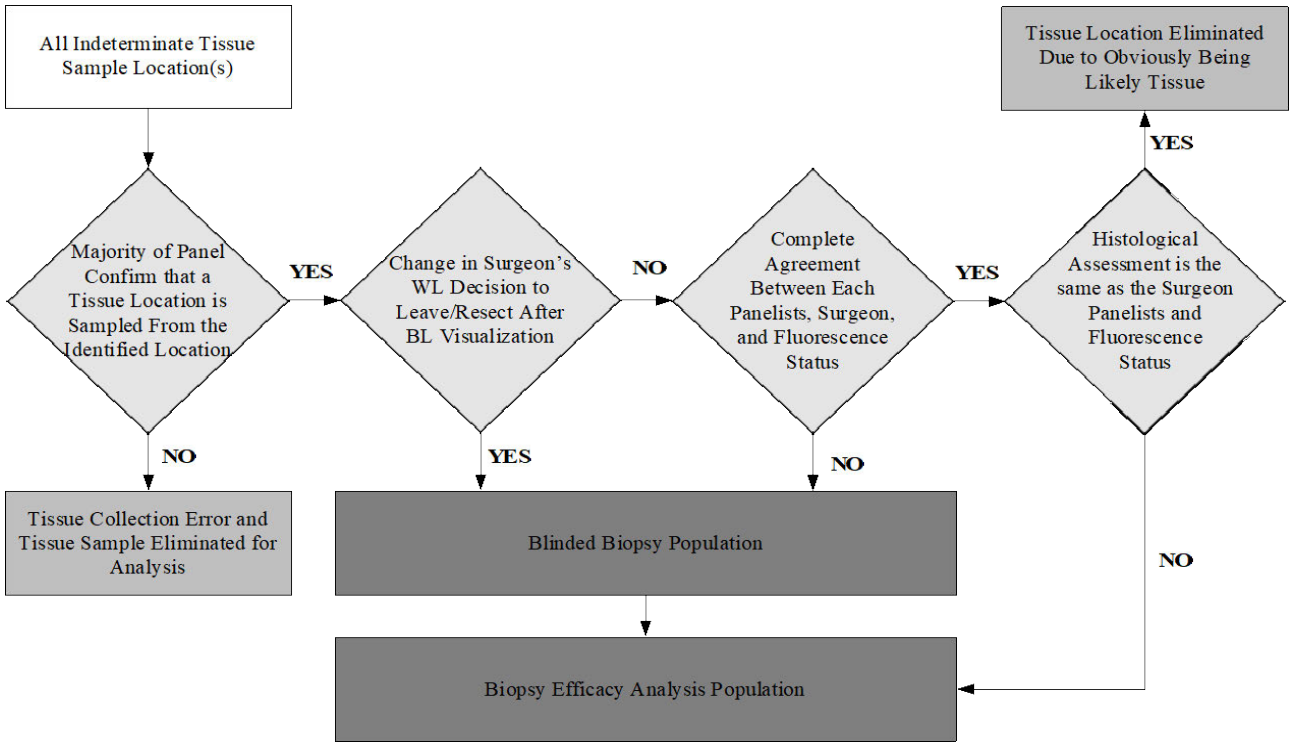
5.3.4 Criteria for Tissue Location Inclusion in Blinded Biopsy Population and Biopsy Efficacy Analysis Population

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 2 and Figure 3.

5.3.4.1 Indeterminate Tissues in Biopsy Efficacy Analysis Population

For inclusion in the Biopsy Efficacy Analysis Population, an indeterminate tissue location must meet Criteria described in Section 4.8.1 of the Protocol. The Blinded Biopsy Population which will be used for sample size re estimation only, does not rely on the histological assessment (Figure 2).

Figure 2. Criteria for Inclusion of an Indeterminate Tissue in the Blinded Biopsy Population and Biopsy Efficacy Analysis Population



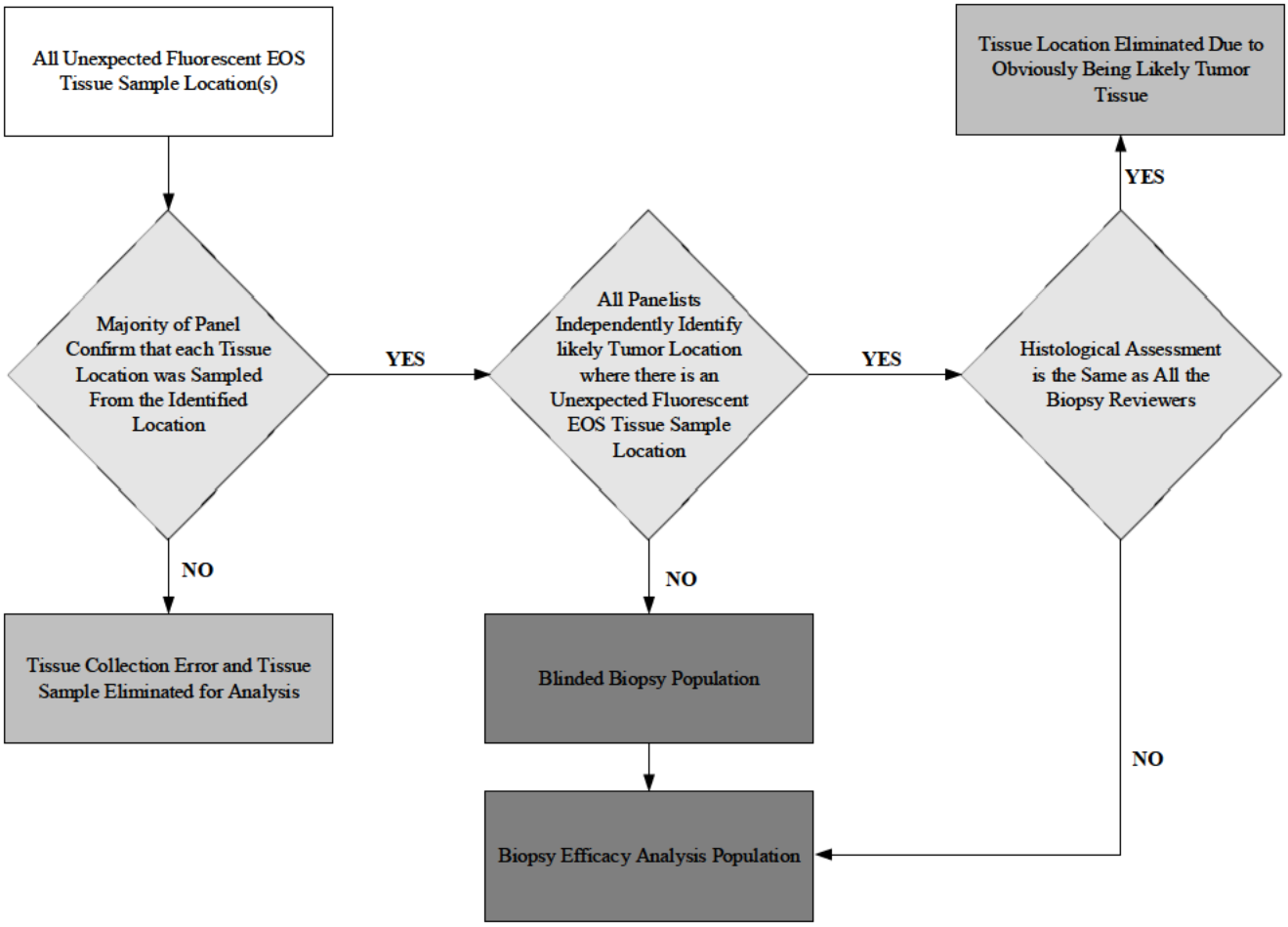
Indeterminate tissue locations will not be included in the Biopsy Efficacy Analysis Population when the following criteria are met (a) the surgeon selects an indeterminate tissue and declares it to be likely tumor and (b) all Biopsy Reviewers also assess the tissue to be likely tumor, and (c) the tissue exhibits Gleolan-induced fluorescence, and (d) histology confirms the tissue is tumor.

However, there is a possibility that indeterminate tissue locations might be included in the Biopsy Efficacy Analysis Population where the operating surgeon's assessment (likely tumor or unlikely tumor) is different from the assessment by (i) all three Biopsy Reviewers, and (ii) fluorescence status, and (iii) histology. These indeterminate tissue locations are included in the Biopsy Efficacy Analysis Population because the in-person visual and tactile information available to the operating surgeon may not be available to the reviewer and may provide greater details in assessing "likely tumor" or "unlikely tumor". Nevertheless, the Sponsor will also perform a supportive analysis for the primary and key secondary endpoints on a sub-population of the Biopsy Efficacy Analysis Population which excludes tissue locations where only the surgeon's assessment is different from the assessment by (i) all three Biopsy Reviewers, and (ii) fluorescence status, and (iii) histology. Furthermore, a supportive analysis will also be performed on the All Biopsy Population, which includes (a) all tissue locations which meet the criteria for the Biopsy Efficacy Analysis Population and (b) those tissue locations excluded from the Biopsy Efficacy Analysis Population where the operating surgeon's assessment (likely tumor or unlikely tumor) is consistent with the assessment by (i) all three Biopsy Reviewers, and (ii) fluorescence status, and (iii) histology.

5.3.4.2 Unexpected Fluorescent EOS Tissue in Analysis Populations

The criteria for an Unexpected Fluorescent EOS Tissue Location to be placed in the Biopsy Efficacy Analysis Population is described in Section 4.8.2 of the Protocol. Unexpected Fluorescent EOS Tissue Locations which meet the criteria located in Figure 3 are included in both the Blinded Biopsy Population and Biopsy Efficacy Analysis Population.

Figure 3. Criteria for Inclusion of an Unexpected Fluorescent EOS Tissue in the Blinded Biopsy Population and Biopsy Efficacy Analysis Population



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.

The Biopsy Efficacy Analysis Population will exclude tissue locations when histology confirms tumor, and all three Biopsy Reviewers identify likely tumor presence in the WL photo/video of the EOS Field of View at that EOS tissue location.

6 SAMPLE SIZE

6.1 Clinical Rationale for Study Sample Size

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 EOS tissue location where Gleolan-induced Protoporphyrin IX (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 ITI 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 end of surgery (EOS) tissue where Gleolan-induced PpIX fluorescence status is consistent with histological assessment of meningioma. This percentage is based on what is known about the performance of ALA HCl with meningioma resection from the scientific literature, plus the Principal Investigator's experience with ALA HCl in meningioma patients, as discussed below.

In a review of case reports, first patient series and clinical studies of intraoperative use of ALA-PpIX fluorescence status in intracranial meningiomas, Valdes et al. (2019) note that when using ALA-PpIX fluorescence status guidance, neurosurgeons can expect to observe visible intraoperative fluorescence in the majority of intracranial meningiomas (sensitivity: 77–96%). Additionally, Prof. Dr. Walter Stummer has utilized Gliolan (ALA HCl marketed in Europe) in a series of compassionate use meningioma patients in Germany undergoing fluorescence guided surgery (approximately 35 patients). Prof. Dr. Stummer and his team of surgeons observed that Gliolan aided in decision-making in approximately 60% of these patients where fluorescence was used for additional tissue information either to identify residual unexpected EOS tissue fluorescence or to identify indeterminate tissues that were assessed for visible fluorescence (personal experience Prof. Dr. Walter Stummer).

The study methods utilized in Phase 3 protocol NXDC-MEN-301 assume conditions which are not always possible during every surgery, e.g. when brain retraction can simply not be maintained for the sole purpose of the data collection and tissue location sampling process. Brain retraction can potentially injure the brain, and this can become more of an issue as soon as the margins of the tumor are reached in some cases. However, brain retraction is necessary for the sole purpose of resection and thus is clinically indicated. In contrast, brain retraction for the sole purpose of obtaining the information necessary for the protocol-dictated Biopsy Review process (i.e., clear documentation of a field of view by video and intraoperative photography, clear collection of biopsy while filming, and sufficient size of the biopsy for pathology) was considered to be feasible only in about 50% of cases where fluorescence status also provided useful information along the tumor and normal tissue interface.

Given these data, determination of sample size in this study based on modeling assumptions for three factors (Table 8).

Table 8. Sample Size Determination Modeling Assumptions

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

To achieve an average of 1.5 biopsies per participant, it was assumed that 40% of participants in the ITI Population had 0 indeterminate or EOS tissue samples, 31% had 1 indeterminate or EOS tissue samples, 27% had 2 indeterminate or EOS tissue samples, and 2% had 3 indeterminate or EOS tissue samples in our simulations. With 100 participants and 70% diagnostic accuracy it was calculated that 50% of all participants in the ITI Population would have at least 1 indeterminate or EOS tissue location where Gleolan-induced PpIX fluorescence status alone is confirmed by central histology. These estimates were considered to be conservative.

6.2 Sample Size Estimation

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 alone is confirmed by central histology.

The primary efficacy analysis will be based on the ITI Population (participant-level). With 66 patients included in the ITI 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, and to account for participants who were dosed with Gleolan but did not have the surgical procedure performed, a total of approximately 110 participants will be enrolled in the study.

7 GENERAL CONSIDERATIONS

7.1 Planned Study Analyses

7.1.1 Statistical Summaries: Descriptive and Inferential

Descriptive summaries of variables will be provided where appropriate. For continuous variables, the number of non-missing values (n) and the median, mean, standard deviation, minimum, and maximum will be tabulated. For categorical variables, the counts and percentages of each value will be tabulated. Expansion of descriptive table categories may occur if such elaborations are thought to be useful. Two-sided 95% confidence intervals will also be provided as appropriate.

For the summary statistics of all numerical variables unless otherwise specified, minimum and maximum will be displayed to the same level of precision as reported. Mean and median will be displayed to one level of precision greater than the data collected. The standard deviation / standard error will be displayed to two levels of precision greater than the data collected.

Change from baseline scores will be calculated as the post-baseline measurement minus the baseline value.

All collected data will be presented in listings. Data not subject to analysis according to this plan will not appear in any tables or figures but will be included in the data listings.

7.1.2 Interim Analyses and Data Monitoring

A blind re-estimation of the sample size will be undertaken one time by the Sponsor after approximately 65 participants have completed the study, to evaluate the adequacy of a 110 participant sample size.The Blinded Biopsy Population will be used for sample size re-estimation. The percentage 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. No unblinded information will be used for this sample size re-estimation.

7.2 Multiple Testing Procedures

The 2 key-secondary efficacy endpoints will be analyzed in a pre-specified sequential testing procedure to preserve the overall Type 1 error rate. The order is provided in Table 3. Both comparisons will utilize an alpha level of 0.05. No additional adjustment for multiplicity will be implemented for the other efficacy endpoints.

7.3 Covariates and Subgroups

7.3.1 Planned Covariates

No covariate analyses are planned.

7.3.2 Planned Subgroups

The following subgroups (Table 9) will be used for the primary efficacy endpoint, key secondary efficacy endpoints, overall summary of AEs, most common TEAE (in at least 5% of participants), and TESAE by system organ class (SOC), preferred term (PT), and relationship to the study drug. It is expected that some subgroup sample sizes will be very small so not all analyses will be able to be performed.

Table 9. Subgroups

Subgroup Label	Subgroup Values
Region	• US • Non-US
Race #1	• White • Black/African American • Asian • Other • Not reported
Race #2	• White • Other • Not reported
Ethnicity	• Hispanic • Not Hispanic • Unknown/Not reported
Gender	• Female • Male
Age #1	• ≤65 years • >65 years
Age #2	• ≤ study population median years • > study population median years
Tumor Status	• Recurrent • De novo
Tumor Grade	• Grade 1 • Grade 2+3 • Not specified/Other
Tumor Location	• Falx & parasagittal • Convexity • Sphenoid • Olfactory groove • Suprasellar • Posterior fossa • Intraorbital • Spinal • Planum sphenoidale • Other

7.4 Management of Analysis Data

7.4.1 Laboratory Data Handling

Unscheduled or repeated laboratory results will not be analyzed for the summary of continuous values but will be included in the laboratory shift tables as follows. Unscheduled tests will be included with the time of the nearest regularly scheduled test. If there is a scheduled test and one or more unscheduled tests assigned to the same time point, the most conservative test (i.e., a test with low or high results) will be used. Repeated tests will be included only if they reflect abnormal (low

or high) results, and the corresponding original results are normal. All laboratory values, for all visits, will be provided in by-participant listings.

7.4.2 Missing Data

All data recorded on the case report form (CRF) will be included in data listings that will accompany the clinical study report.

7.4.2.1 Handling of Missing Date Values

Partial or Missing Dates

The following conventions will be used to impute missing portions of dates for adverse events and concomitant medications, if warranted. Note that the imputed values outlined here may not always provide the most conservative date. In those circumstances, the imputed value may be replaced by a date that will lead to a more conservative analysis.

A. Start Dates
    1) If the year is unknown, then the date will not be imputed and will be assigned a missing value.
    2) If the month is unknown, then:
        i) If the year matches the first dose date year, then impute the month and day of the first dose date.
        ii) Otherwise, assign 'January.'
    3) If the day is unknown, then:
        i) If the month and year match the first dose date month and year, then impute the day of the first dose date.
        ii) Otherwise, assign the first day of the month.

B. Stop Dates
    1) If the year is unknown, then the date will not be imputed and will be assigned a missing value.
    2) If the month is unknown, then assign 'December.'
    3) If the day is unknown, then assign the last day of the month.

7.4.2.2 Imputation Methods

The imputation methods that will be utilized are summarized below.

- The primary efficacy endpoint analyzed with the ITI population will be imputed using (1) modified-worst case imputation and (2) worst case imputation. The modified-worst case imputation method (defined in Table 10) will be the primary imputation method with worst case imputation (defined in Table 11) being used as a sensitivity analysis.
- Participant-level endpoints analyzed with the PP population will not have missing data imputed.
- Biopsy-level endpoints analyzed with the BEAP or ABP will not have missing data imputed.

- Intercurrent events, such as only collecting bulk tumor samples for the primary efficacy endpoint, only collecting obvious tissue samples, and not having the surgery performed are discussed below.
- If FL status is 'Uncertain' or pathology results are 'Uncertain' then they will be considered missing for imputation purposes.

Primary Efficacy Endpoint

For the primary efficacy endpoint, for a participant to be considered a success, only biopsies considered 'non-obvious' for tumor status by the BRP will be eligible to be assessed in the numerator. Note that missing pathology results for a tissue will be imputed prior to determining if a tissue is 'non-obvious.' For the primary efficacy endpoint, all non-bulk and non-obvious tissues will first be assessed for a tissue success (TP or TN) or failure (FP or FN) using FL status and pathology results. After the eligible tissues are assessed, the participant will be assigned as meeting the primary efficacy endpoint (success) if one of the eligible tissues is a success (TP or TN). Otherwise, the participant is considered as failing the primary efficacy endpoint (failure). Participants may have bulk tissue samples only, only obvious tissues collected, no surgery, missing FL status, or missing pathology results. Details on handling these cases are provided in the two tables below.

Table 10. Primary Efficacy Endpoint - Modified-Worst Case Imputation – Primary Imputation Method

Scenario	Data Handling
Only bulk tumor tissue sample is available	Participant will be imputed as failing to meet the primary efficacy endpoint
Only "obvious"* tissues are available (non bulk)	Participant will be imputed as failing to meet the primary efficacy endpoint
No surgery	Probability of meeting the primary efficacy endpoint for the participant will be based on overall observed cases tissue success rate (the proportion of all tissues from all participants that are TP or TN)**
Missing FL status for a given tissue sample	If pathology is positive for meningioma, then FL status will be negative (FN) If pathology is negative for meningioma, then FL status will be positive (FP) If pathology is missing for meningioma, then the tissue will be assigned as a FP
Missing pathology results for a given tissue sample	If FL status is positive then the probability of TP (ie, success at the tissue level) will be based on the overall observed cases TP rate (the proportion of all tissues for all participants that are TP)** If FL status is negative then the probability of TN (ie, success at the tissue level) will be based on the overall observed cases TN rate (the proportion of all tissues for all participants that are TN)** If FL status is missing, then the tissue will be assigned as a FP

* See Section 5.3.4 for further details on identification of an 'obvious' tissue sample.
** A random number generator will randomly create a proportion between 0 and 1 (SAS® function RAND ("uniform")), if the randomly generated number is less than or equal to the overall observed cases tissue success rate (TP or TN, TP, or TN – depending on the scenario) then the participant will be assumed to be an overall success.

Table 11. Primary Efficacy Endpoint - Worst Case Imputation

Scenario	Data Handling
Only bulk tumor tissue sample is available	Participant will be imputed as failing to meet the primary efficacy endpoint
Only "obvious"* tissues are available (non bulk)	Participant will be imputed as failing to meet the primary efficacy endpoint
No surgery	Participant will be imputed as failing to meet the primary efficacy endpoint

Scenario	Data Handling
Missing FL status for a given tissue sample	If pathology is positive for meningioma, then FL status will be negative (FN) If pathology is negative for meningioma, then FL status will be positive (FP) If pathology is missing for meningioma, then the tissue will be assigned as a FP
Missing pathology results for a given tissue sample	If FL status is positive, then pathology will be negative (FP) If FL status is negative, then pathology will be positive (FN) If FL status is missing, then the tissue will be assigned as a FP

* See Section 5.3.4 for further details on identification of an 'obvious' tissue sample.

Imputation for Other Endpoints

If the relationship of an AE is missing, it will be considered treatment related. Missing AE severity will be coded as severe.

For other endpoints, unless otherwise specified, observed cases, without imputation, will be used for the analyses.

7.4.3 Handling of Early Termination Visit Information

If a participant is terminated early from this study the early termination visit data will be analyzed at the closest scheduled visit. If the closest visit has valid data, the early termination data will be assigned to the next available visit.

7.4.4 Pooling of Study Centers

Study centers will not be combined to create any pooled study centers (e.g., combining data fromlow enrolling sites). The primary and secondary efficacy analyses will include all study sites pooled together. Exploratory analyses may be performed by the study center.

7.4.5 Coding Conventions for Events and Medications

All adverse events, and medical history will be mapped to the Medical Dictionary for Regulatory Activities (MedDRA® Version 24.0) system for reporting (preferred term and body system).

Prior and concomitant medications will be coded using WHO-DD (Drug Dictionary) (Version March 2021).

7.4.6 Analysis Software

Data manipulation, tabulation of descriptive statistics, calculation of inferential statistics, and graphical representations will be performed primarily using SAS® (release 9.4 or higher) for Windows. If the use of other software is warranted, the final clinical study report will detail what software was used and for what purposes.

7.4.7 Study Data

All study data will be formulated into regulatory compliant data sets to provide transparency, traceability, and integrity of trial analysis results from the collection source. Observed study data will be mapped to the CDISC Study Data Tabulation Model (SDTM) and serve as the source data from the trial. All study analyses will be completed using analysis data sets that are derived from the SDTM and follow the CDISC Analysis Data Model (ADaM) architecture. All planned analyses will be performed using the ADaM data sets developed for this study.

8 SUMMARY OF STUDY DATA

8.1 Participant Disposition

A summary of the analysis sets includes the number and percentage of participants for the following categories: participants screened, participants in the Safety Population, participants in the ITI Population, participants in the Per Protocol Population, and participants in the Biopsy Efficacy Analysis Population and All Biopsy Population. All percentages will be based on the number of participants in the Safety Population.

End of trial information will also be summarized in this table, including the number of participants completing the study and the number of participants who prematurely discontinuedthe study with reasons for withdrawal. All percentages will be based on the number of participants in the Safety Population.

A summary of the tissue disposition will also be provided. This table will summarize the number of tissues included and excluded from each analysis population (All Biopsy Population and Biopsy Efficacy Analysis Population) along with the reasons for exclusion.

A by-participant data listing of study completion information including the reason for premature study withdrawal, if applicable, will be presented.

8.2 Protocol Deviations

All protocol violations will be presented in a data listing.

8.3 Demographics and Baseline Characteristics

Participant demographic data and baseline characteristics (including time from Gleolan dose to tissue sample acquisition and time from Gleolan dose to induction of anesthesia) will be tabulated and summarized descriptively. The demographic data and baseline characteristics will be summarized by participants in the Safety, ITI, Per Protocol, Biopsy Efficacy Analysis Population

and the All Biopsy Population. Individual participant demographics and baseline characteristics will be provided in the listings.

8.4 Medical and Surgical History

Medical and surgical history will be summarized descriptively by System Organ Class (SOC) and Preferred Term (PT) using the Safety population.

Participant medical and surgical history data including specific details will be presented in a listing.

8.5 Prior and Concurrent Medications

The number and percentages of all concomitant medications will be summarized by Anatomical Therapeutic Chemical (ATC) level 4 and PT. The total number of concomitantmedications and the number and percentages of participants with at least one concomitant medication will be summarized. All summaries will be performed using the Safety Population.

The number and percentages of all prior medications will be summarized similarly to concomitant medications in a separate table.

A concomitant medication is defined as any medication taken on or after the day of the first dose of study drug. A prior medication is defined as any medication that starts and ends prior to the first dose of study drug.

8.6 Treatment Compliance

Dosing information, including date and time of administration, time from administration to initiation of anesthesia, time from administration to completion of surgery, defined as when the Surgeon declares resection complete, and total dose will be listed by participant.

9 EFFICACY ANALYSES

9.1 Primary Efficacy

The primary endpoint is the percentage of participants who have at least one indeterminate tissue or unexpected fluorescent EOS tissue where Gleolan-induced PpIX fluorescence status alone is consistent with 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. Note that for a participant to be considered a success, only biopsies considered 'non-obvious' for tumor status by the BRP will be eligible to be assessed in the numerator.

The number and percentage of participants with a success will be provided along with a two-sided 95% confidence interval calculated using the Wilson (score) method. The primary efficacyanalysis will be based on the ITI Population (participant level).

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.

See Section 7.4.2.1 for the handling of missing data.

The analysis will be repeated for the PP population as a sensitivity analysis. Additional methods to determine if a tissue is 'non-obvious' may also be utilized (e.g., removing the requirement for location confirmation if the video/photograph is not available).

9.2 Secondary Efficacy

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 Table 3. As discussed in Section 7.2 the key secondary efficacy endpoints will be tested sequentially, each at a 0.05 level.

Key Secondary Efficacy Endpoints:

1. Biopsy-level 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 Generalized Estimating Equation (GEE) model that takes into account the correlation (clustering) of the biopsies within a participant will be used to calculate the estimate and 95% confidence interval of the PPV. The model used a binomial distribution function with an exchangeable working correlation matrix and utilized a robust variance estimator. If the GEE model has convergence issues then 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.

2. Biopsy-level 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 GEE model that takes into account the correlation (clustering) of the biopsies within a participant will be used to calculate the estimate and 95% confidence interval of the NPV. The model used a binomial distribution function with an exchangeable working correlation matrix and utilized a robust variance estimator. If the GEE model has convergence issues then 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.

Other Secondary Efficacy Endpoints:

The analysis of participant-level PPV of Gleolan-induced PpIX fluorescence status of the single bulk tumor biopsied tissue location obtained from each study participant will be performed at the participant level. The primary analysis population is the ITI Population. The PP 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.

The analysis of biopsy-level diagnostic accuracy of Gleolan-induced PpIX fluorescence status among indeterminate tissue locations is greater than the diagnostic accuracy of the surgeons' assessment of indeterminate tissue locations under WL 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 GEE model that takes into account the correlation (clustering) of the biopsies within a participant will be used to calculate the estimates and 95% confidence intervals of diagnostic accuracy of BL and WL. The model used a binomial distribution function with an exchangeable working correlation matrix and utilized a robust variance estimator. The difference in diagnostic accuracy rates (BL minus WL) will also be provided for indeterminate tissues. Diagnostic accuracy will also be calculated for BL for unexpected fluorescent EOS tissues only and for both indeterminate tissue and unexpected fluorescent EOS tissue locations combined. If the GEE model has convergence issues then 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 estimates and two-sided 95% confidence intervals.

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 GEE model that takes into account the correlation (clustering) of the biopsies within a participant will be used to calculate the estimates and 95% confidence intervals of sensitivity and specificity. The model used a binomial distribution function with an exchangeable working correlation matrix and utilized a robust variance estimator. If the GEE model has convergence issues then 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 estimates and two-sided 95% confidence intervals.

9.3 Exploratory Efficacy

Exploratory efficacy endpoints are described in Section 4.4. In general, point estimates and 95% confidence intervals using the Wilson (score) method will be provided for the exploratory endpoints. Participant-level analyses will be performed on the Per Protocol Population, while tissue-level analyses will be performed on the Biopsy Efficacy Analysis Population and All Biopsy Analysis Population. No data imputation for missing data will be performed for the exploratory efficacy endpoints.

A listing of all false positive and all false negative results for bulk, indeterminate, and unexpected fluorescent EOS tissues will be provided.

10 SAFETY ANALYSES

All Safety analyses will be conducted using the Safety Population.

10.1 Adverse Events

The number and percent of participants with any treatment-emergent adverse events (TEAEs) will be displayed by system organ class and preferred term. Within each preferred term, participants will be counted only once if they had more than one event reported during the study.

TEAEs will also be summarized by greatest reported severity for each event preferred term. Counts indicate participants reporting one or more TEAEs that map to the severity grade classification for each preferred term. At each level of summarization (SOC or event PT) participants are only counted once and the worst severity case of repeated instances of the same TEAE will be used in tabulations. TEAEs will also be summarized by strongest investigator assessment of relationship to study drug in a similar manner.

Analyses of AEs will be performed for those events that are considered treatment emergent, where treatment emergent is defined as any AE with onset or worsening on or after the first dose of study drug.

Treatment emergent summarization will be characterized by serious or not, the severity and the relationship with the study drug. The most common TEAEs (incidence ≥5%) will also be summarized by PT. A conservative approach will be taken to assess the relationship of an AE to study drug; if the relationship of an event is missing, it will be considered treatment related. Missing severity will be coded as severe.

All TEAEs will be listed individually by participant. In addition, a separate listing will be produced for AEs that are not treatment emergent.

10.2 Deaths, Serious Adverse Events and Other Significant Adverse Events

10.2.1 Deaths

All deaths, regardless of causality, will be provided in listings and written clinical narratives.

10.2.2 Serious Adverse Events

The number and percent of participants with Treatment Emergent Serious Adverse Event (TESAE) will be displayed by SOC and PT, and relationship to study drug. Within each preferred term, participants will be counted only once if they had more than one TESAE event reported during the study.

A listing will be produced for all participants who reported serious TEAEs.

10.2.3 Adverse Events Leading to Discontinuation of Study Drug

The number and percent of participants with TEAE's leading to discontinuation, or interruption, of study drug will be displayed by SOC and PT. Within each preferred term, participants will be counted only once if they had more than one TEAE leading to discontinuation, or interruption, of study drug reported during the treatment period.

A listing will be produced for all participants who discontinued study drug due to TEAEs.

10.3 Clinical Laboratory Evaluations

Clinical Laboratory results will be summarized descriptively by visit for the observed value as well as for the change from baseline value. In addition, laboratory shift tables will be provided for all laboratory parameters where abnormal/normal status can be ascertained. Listings of individual laboratory parameters by visit with normal ranges and abnormality assessments will also be completed by participant.

10.4 Vital Signs

Vital sign results will be summarized descriptively by visit for the observed value as well as for the change from baseline value. All vital sign data by participant will be presented in a listing. Unscheduled visit results will not be summarized but will be included in data listings.

10.5 Physical Examinations

Physical examinations results will be presented in data listings.

10.6 Neurological Examinations

Neurological examination results will be summarized using the number and percentage of participants who exhibit each item of the exam categories (mental status, speech, cranial nerves, motor system, sensory, and other). Results will also be summarized by CTCAE grade.

Neurological examination results will be presented in data listings.

10.7 Other Safety Measures

MRI results will be presented in data listings.

Karnofsky Performance Scale will be summarized descriptively by visit for the observed value as well as for the change from baseline value. All Karnofsky Performance Scale data will be presented in listings.

Pregnancy test results will be presented in data listings.

11 CHANGES IN ANALYSES FROM PROTOCOL

- ITI Population added
- ITT Population removed
- Per Protocol Population definition updated
- Blinded Biopsy Population definition updated to use ITI population
- Primary efficacy endpoint updated
- Key secondary efficacy endpoints PPV of the single bulk tumor biopsied tissue and diagnostic accuracy of indeterminate tissue and EOS tissue moved to other secondary endpoints
- Removed hypotheses for other secondary endpoints
- Primary analysis based on ITI population (primary) and PP (supportive)

- Participant-level PPV based on ITI population (primary) and PP (supportive)
- GEE models replaced the Lee and Dubin analyses. Lee and Dubin analyses will be utilized if the GEE model has convergence issues.
- Clarified that diagnostic accuracy of BL will be compared with WL for indeterminate tissues only; diagnostic accuracy will also be calculated for BL for unexpected fluorescent EOS tissues only and for both indeterminate tissue and unexpected fluorescent EOS tissue locations combined.
- Removed concordance between Biopsy Review Panel and surgeon, second mention of diagnostic accuracy of BL, diagnostic accuracy comparisons between Biopsy Review Panel and surgeon, and change in the operating surgeon's decision to remove or leave a piece of EOS tissue analyses
- Removed change in operating surgeon's decision for EOS tissues endpoint
- Added exploratory endpoint for percentage of participants who have at least one unexpected fluorescent EOS tissue where Gleolan-induced PpIX fluorescence status resulted in the operating surgeon's decision to resect the tissue and the central histopathological result confirms tumor
- Sample size justification updated
- Interim analysis updated
- Subgroup analyses added
- Missing data imputation methods updated – modified-worst case and worst case imputation added
- Most common TEAEs (incidence ≥5%) summary added

12 REPORTING CONVENTIONS

The following reporting conventions will be adopted for the presentation of study data. These conventions will enhance the review process and help to standardize presentation with common notations.

12.1 General Reporting Conventions

- All tables and data listings will be developed in Landscape Orientation, unless presented as part of the text in a CSR.
- Figures will be presented in Landscape Orientation, unless presented as part of the text in a CSR.
- Legends will be used for all figures with more than one variable or item displayed.
- Figures will be presented in color with groups distinguished by different symbols and colors. Lines in figures should be wide enough to view the line after being photocopied.
- Specialized text styles, such as bolding, italics, borders, shading, superscripted and subscripted text will not be used in tables, figures, and data listings unless they add significant value to the table, figure, or data listing.
- Only standard keyboard characters should be used in tables and data listings. Special characters, such as non-printable control characters, printer specific, orfont specific

characters, will not be used on a table, figure, or data listing. Hexadecimal character representations are allowed (e.g., μ, α, β).

- All titles will be centered on a page. The ICH numbering convention is to be used for all tables, figures, and data listings.
- All footnotes will be left justified and the bottom of a page. Footnotes must be present on the page where they are first referenced. Footnotes should be used sparingly and must add value to the table, figure, or data listing. If more than fourfootnote lines are planned, then a cover page may be used to display footnotes.
- Missing values for both numeric and character variables will be presented as blanks in a table or data listing. A zero (0) may be used if appropriate to identifywhen the frequency of a variable is not observed.
- All date values will be presented as YYYY-MM-DD (e.g., 2013-05-17) ISO 8601 format.
- All observed time values will be presented using a 24-hour clock HH:MM:SS format (e.g., 01:35:45 or 11:26). Seconds should only be reported if they were measured as part of the study, also in ISO 8601 format.
- Time durations will be reported in mixed HHh MMm SSs notation (e.g., 5h 32m, or 27h 52m 31s). The use of decimal notation to present (display) time durations should be avoided (e.g. 0.083h = 5m) unless it is necessary to show the computation of time differences in a table, figure, or data listing, in which caseboth notations may be used to display the time duration.
- All tables, figures, and data listings will have the Table, Listing, or Graph status (DRAFT, FINAL), and a date/time stamp on the bottom of each output.
- All analysis programs developed for a table, figure, or data listing display will be self-contained to facilitate transfer of programs to multiple computing environments and transfer to a regulatory agency (if requested).

12.2 Population Summary Conventions

- Population(s) represented on the tables or data listings will be clearly identified in the last title of the Table as “Population: <name of population>” and will be identical in name to that identified in the protocol or SAP.
- Consistent terminology will be used to define and identify a population.
- Sub-population(s) or special population(s) descriptions will provide sufficient detail to ensure comprehension of the population (e.g., Per Protocol Males >60 years of age) used for analysis in a table or figure.
- Population sizes may be presented for each treatment or dosing category as totals in the column header as (N=xxxx), where appropriate.
- Population sizes shown with summary statistics are the samples sizes (n) of participants with non-missing values.
- All population summaries for categorical variables will include all categories that were planned and for which the participants may have had a response. Percentages corresponding to null categories (cells) will be suppressed.

- All population summaries for continuous variables will include: N, mean, SD, minimum, and maximum. Other summaries (e.g. number missing, median, quartiles, 5%, 95% intervals, CV or %CV) may be used as appropriate.
- All percentages are rounded and reported to xx.x%. A percentage of 100% will be reported as 100%. No value of 0% will be reported. Any computation of a percent that results in 0% is to be presented as a blank.
- Population summaries that include p-values will report the p-value to four decimal places with a leading zero (0.0001). All p-values reported on default output from statistical software (i.e., SAS® Software version) may be reported at the default level of precision. P-values <0.0001 should be reported as <0.0001 not 0.0000.

13 REFERENCES

Lee, E.W. and N. Dubin, *"Estimation and sample size considerations for clustered binary responses.* Statistics in Medicine. Vol. 13. 1994. 1241-1252.

Valdes PA, Millesi M, Widhalm G, Roberts DW. 5-Aminolevulinic acid induced protoporphyrinIX (ALA-PpIX) fluorescence guidance in meningioma surgery. J Neuro-oncol. 2019.