Statistical Analysis Plan

A randomized, blinded study of fluorescence detection of pediatric primary central nervous system tumors in subjects receiving tozuleristide and imaged with the Canvas System

Protocol BB-006

Sponsored by:

Blaze Bioscience, Inc.

Version: 5.1

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NCT03579602

CONFIDENTIALITY STATEMENT

Approval Signatures

Product:	Tozuleristide
Protocol Number:	BB-006
SAP Version:	5.1
Version Date:	[redacted]

The individuals signing below have reviewed and approve this statistical analysis plan.

[redacted]

[redacted]

Date

[redacted]

Date

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[redacted]

[redacted]

Glossary of Abbreviations

Abbreviation	Description
AE	adverse event
ASP	all-subject population
BSA	body surface area
CI	confidence interval
CNS	central nervous system
CRF	case report form
CTCAE	Common Terminology Criteria for Adverse Events
CSF	cerebrospinal fluid
DAP	Data Access Plan
DNA	Deoxyribonucleic acid
ECG	Electrocardiogram
eCRF	electronic case report form
EOR	extent of resection
FAP	full analysis population
FLAIR	Fluid-Attenuated Inversion Recovery
GEE	general estimating equation
GTR	gross total resection
ITT	intent-to-treat
Max	Maximum
MedDRA	Medical Dictionary for Regulatory Activities
Min	Minimum
MRI	magnetic resonance imaging
NCI	National Cancer Institute
NIR	near infrared
NPV	negative predictive value
NTR	near total resection
PEAC	Protocol Endpoint Assessment Committee
PK	Pharmacokinetics
PKAP	pharmacokinetic analysis population

Abbreviation	Description
PPV	positive predictive value
PT	preferred term
QTc	corrected QT interval
SAE	serious adverse event
SAF	safety analysis population
SAP	Statistical Analysis Plan
SD	standard deviation
SI	standard international system of units
SMC	Safety Monitoring Committee
SOC	system organ class
STR	subtotal resection
T1	Longitudinal relaxation time
T2	Transverse relaxation time
TEAE	treatment emergent adverse event
WHO	World Health Organization

1 INTRODUCTION

This document outlines the statistical methods to be implemented within the scope of Protocol BB-006, entitled "A randomized, blinded study of fluorescence detection of pediatric primary central nervous system tumors in subjects receiving tozuleristide and imaged with the Canvas System". Results of the proposed analyses will become the basis of the clinical study report for this protocol.

The purpose of this plan is to provide general, and in some instances specific, guidelines from which the analysis will proceed. Nevertheless, an efficient data analysis should allow modifications to the original analysis plan to provide a more reliable and valid analysis of the data. Any such "post hoc" or "data driven" analyses will be identified in the final clinical study report. All planned analyses specified in this document will be performed. Any changes to this plan will either be reflected in amendments (to this plan) before the database lock or documented in the clinical study report.

2 STUDY OBJECTIVES

2.1 Primary Objectives

The primary objectives of this study in subjects undergoing neurosurgical resection of pediatric primary central nervous system (CNS) tumors are:

- To compare the sensitivity of tozuleristide and the Canvas near infrared (NIR) imaging system fluorescence to the sensitivity of surgical designation in equivocal tissue in situ;
- To compare the specificity of tozuleristide and the Canvas system fluorescence to the specificity of surgical designation in equivocal tissue in situ;
- To evaluate imaging efficacy as measured by sensitivity of tozuleristide and the Canvas system in facilitating intraoperative fluorescence detection and visualization of tumor in equivocal tissue in situ;
- To evaluate imaging efficacy as measured by specificity of tozuleristide and the Canvas system in facilitating intraoperative fluorescence detection and visualization of tumor in equivocal tissue in situ.

2.2 Secondary Objectives

The secondary objectives of this study are:

- [redacted]

- [redacted]
- [redacted]
- [redacted]

2.3 Exploratory Objectives

The exploratory objectives of this study will evaluate the:

- [redacted]
- [redacted]
- [redacted]

3 STUDY DESIGN

3.1 Overall Study Design

This is a randomized, blinded (central pathology, central fluorescence assessment and central radiology), multi-center Phase 2 study of a single dose of tozuleristide in subjects with pediatric primary CNS tumors; intraoperative imaging will be performed with the Canvas system.

Eligible patients will be randomized into one of the two treatment arms in a 1:10 (no drug treatment tozuleristide) allocation:

> Arm 1: Approximately 9% of subjects will be randomized to receive standard of care neurosurgery without tozuleristide.
>
> Arm 2: Approximately 91% of subjects will be randomized to receive standard of care neurosurgery and tozuleristide.

Within this Statistical Analysis Plan (SAP), study-related tissue collection may be referred to as specimens or biopsies.

During the surgical procedure for each subject, and once the tumor has been accessed, the Canvas system, which is attached to the surgical microscope, will be used to capture and display intraoperative *in situ* images (white light and fluorescence) of the region from which surgical specimens for biopsy or excision will be collected. All clinical decision-

making regarding tissue resection will be made based on the routine neurosurgical practices, and tissue resection will take place under normal light conditions in line with best clinical practices.

In addition to primary tumor biopsy, regions of equivocal nature (uncertain if tumor or not tumor) will be identified before fluorescence in the operating room is determined.

[redacted]

Tozuleristide and the Canvas system are intended as an aid to surgical decision-making. As such, when a region of interest in the brain/spine is noted to be fluorescent, it is always the surgeon's decision whether to biopsy/resect or not, if safe to do so.

[redacted] luorescence assessment by the imaging operators (local assessment) will be used in the primary efficacy analyses.

Subjects who are randomized to Arm 1 or Arm 2 and need to undergo an additional surgery for their brain tumor will be eligible to receive tozuleristide without being subject to randomization. Subjects must meet all study eligibility requirements and re-consent for study prior to re-enrollment for open-label treatment of tozuleristide (re-treatment).

[redacted]

3.2 Central Pathology

The pathology assessment of a tissue specimen (primary, equivocal or other) as tumor or not tumor, for both arms, will be performed by a centralized and independent panel of pathologists without knowledge of fluorescence status or treatment arm (blinded pathology).

3.3 Central Radiology

Post-operative centralized and independent MRI assessment [redacted]

3.4 [redacted]

[redacted]

3.5 Independent Safety Monitoring Committee

An Independent Safety Monitoring Committee (SMC) will have responsibility for oversight of safety on the study. The SMC members and its responsibilities and processes will be described in the SMC charter.

3.6 Protocol Endpoint Assessment Committee

A Protocol Endpoint Assessment Committee (PEAC) will monitor the progress of the study [redacted]

[redacted]

4 STUDY ENDPOINTS

4.1 Primary Efficacy Endpoints

The primary efficacy endpoints are:

1. Ratio of sensitivity of tozuleristide fluorescence in equivocal tissue biopsies from subjects with fluorescence-positive tumors compared to the sensitivity of surgical designation from the same biopsies under white light conditions

2. Ratio of specificity of tozuleristide fluorescence in equivocal tissue biopsies from subjects with fluorescence-positive tumors compared to the specificity of surgical designation from the same biopsies under white light conditions

3. Sensitivity of tozuleristide fluorescence to detect tumor in equivocal tissue biopsies from subjects with fluorescence-positive tumor

4. Specificity of tozuleristide fluorescence to detect tumor in equivocal tissue biopsies from subjects with fluorescence-positive tumor

Fluorescence positivity for the primary endpoints is defined as weak or strong fluorescence as determined by the imaging operator fluorescence assessment based on displayed fluorescence signal and exposure setting.

[redacted]

[redacted]

4.2 Secondary Endpoints

Study endpoints related to the secondary efficacy objective:

1. [redacted]
2. [redacted]
3. [redacted]
4. [redacted]

[redacted]

Study endpoints related to the safety objective:

1. All adverse events (AE) will be graded according to the National Cancer Institute's Common Terminology for Adverse Events (NCI CTCAE, Version 5.0)

2. Serious adverse events (SAEs)

3. AE attribution of relatedness to tozuleristide and/or Canvas system (related vs. unrelated)

4. Laboratory parameters

5. Electrocardiogram (ECG) parameters

6. Vital signs

Study endpoints related [redacted]:

1. [redacted]
2. [redacted]
3. [redacted]
4. [redacted]

Study endpoints related [redacted]

- [redacted]

Study endpoints related [redacted]:

- [redacted]
    1. [redacted]

[redacted]

4.3 Exploratory Endpoints

[redacted]

5 STATISTICAL HYPOTHESES AND CRITERIA OF SIGNIFICANCE

[redacted]

6 DETERMINATION OF SAMPLE SIZE

Approximately 114 subjects are planned for treatment in this study.

[redacted]

[redacted]

7 ANALYSIS POPULATIONS

The full analysis population (FAP) for efficacy analyses will consist of all subjects who

1. meet all eligibility criteria,

2. undergo neurosurgery, and

3. have central pathology tumor assessment and imaging operator fluorescence assessment of equivocal tissue specimens obtained from the first neurosurgery on study.

Subjects in the FAP will be classified into 4 groups:

- Group 1 (Arm 1): subjects who do not receive tozuleristide, regardless of fluorescence outcomes and tumor diagnosis

- Group 2 (Arm 2 Fluorescent): subjects who receive tozuleristide, have at least one fluorescence-positive study tissue specimens of any type (primary, equivocal, and other) based on imaging operator fluorescence assessment, and the subject's central pathology consensus diagnosis is tumor, ambiguous, or not definitive

- [redacted]

[redacted]

A subject is evaluable for efficacy if the subject meets the three criteria of the FAP [redacted]

The FAP will be the primary analysis population for all efficacy analyses, summaries, and listings.

The safety analysis population (SAF) will consist of all subjects who receive any amount of tozuleristide or receive neurosurgery prior to any re-treatment. The SAF will be used for all safety data summaries and listings.

[redacted]

The all-subject population (ASP) is defined as all subjects who sign informed consent. The ASP will be used for screen failure summaries and listings.

The intent-to-treat (ITT) population is defined as all subjects who are randomized. The ITT population will be used for disposition summaries, protocol deviations, and listings.

No subjects will be excluded from any analysis populations due to protocol deviations.

8 PLANNED METHODS OF ANALYSIS

8.1 Overall Plan

In general, descriptive statistics will be provided for all the study endpoints and will be presented in tables and/or graphs. Continuous variables will be summarized using the number of observations (n), mean, median, standard deviation, minimum and maximum. Categorical variables will be summarized with frequency counts and percentages. Percentages will be rounded to one decimal place, with the denominator being the number of subjects in the relevant population (N), unless otherwise stated.

Statistical tests will be performed at either a one-sided significance level of 2.5% or a two-sided significance level of 5%. All confidence intervals (CIs) will be constructed at a two-sided 95% confidence level.

In addition, data collected after re-treatment will be listed and will not be included in the primary efficacy and safety analyses.

8.2 Key Definitions

Study Day is the day relative to the date of neurosurgery, where Day 1 is the day of the first neurosurgery performed in this study.

Study Day will be calculated using the first neurosurgery date as the reference date. If the date of interest occurs on or after the first neurosurgery date, study day will be calculated as (date of interest – first neurosurgery date) + 1. If the date of interest occurs prior to the first neurosurgery date, study day will be calculated as (date of interest – first neurosurgery date). There will be no study Day 0.

Unless otherwise specified, the baseline value for a measurement used in all statistical analyses will be the most recent non-missing measurement prior to the administration of the study drug for Arm 2 and prior to the first neurosurgery for Arm 1.

A change from baseline value is calculated as the post-baseline value minus the baseline value.

Treatment-emergent adverse event (TEAE) is defined as an adverse event that is newly occurring or worsens following neurosurgery (Arm 1) or treatment with tozuleristide (Arm 2).

8.3 Missing Data Handling

Missing data will not be imputed. Subjects with a missing variable at a particular time point (e.g., a laboratory variable at a visit week) will be excluded from the analysis of that variable at that time point unless otherwise specified.

Section 8.12.2 describes how to deal with partial AE start dates in determining treatment emergence.

Section 8.12.6 describes how to determine prior and concomitant medications with partial medication start dates. Every effort will be made to ensure that the attributes of an AE (seriousness, severity, relatedness to study drug/device/surgical procedure and outcome) are complete. Missing attributes will not be imputed and will be summarized as "unknown".

8.4 Visit Windows

All assessments will be included in the listings. Analytic visit windows will be applied to the following assessments that do not have a visit on the CRF:

Assessments	Analytic Visit
Study drug administration	Day 1
Central radiology	Protocol specified visit windows[1]
Central pathology	Day 1
[redacted]	[redacted]
[redacted]	[redacted]
[redacted]	[redacted]

Analytic visit will be set to the CRF visit for the assessments with a visit on the CRF.

8.5 Pooling of Centers

Data from all centers will be pooled prior to analysis.

8.6 Subject Accountability

Subject accountability will be summarized for the ITT population. Subjects who discontinue tozuleristide prematurely and the reason for tozuleristide discontinuation will be summarized for Arm 2. Subjects who withdraw from the study and the reason for

study discontinuation will be summarized by treatment arm. The number of subjects who do not undergo surgery will also be summarized by treatment arm.

The number of screen failures and the reason for screen failure will be summarized as a percentage of the all-subject population.

The number of subjects in each analysis population (Section 7) and the reason for excluding from the analysis population will be summarized as a percentage of the all-subject population by treatment arm.

Informed consent (including protocol version), screen failure, eligibility and randomization data will be listed.

8.7 Tissue Biopsy Accountability

Tissue biopsy/specimen accountability will be summarized for the ITT population by treatment arm. [redacted]

8.8 Protocol Deviations

Subject data will be examined for evidence of protocol deviations in order to assess how well the protocol was followed. This is described in the Protocol Deviations and Non-compliance Management Plan. All protocol deviations will be listed using the ITT population.

8.9 Demographic and Baseline Characteristics

Demographic and other baseline characteristics will be summarized with descriptive statistics by treatment arm for the full analysis population. Demographics include:

- Age at informed consent calculated as Age (years) = integer value of ((Date of Informed Consent – Date of Birth + 1) / 365.25),
- Gender,
- Ethnicity, and
- Race.

Baseline characteristics include:

- Baseline weight (kg),

- Baseline height (cm),
- Baseline body surface area (BSA m^2, using Mosteller formula),
- Karnofsky performance status,
- Lansky performance status,
- Received prior CNS cancer surgery (Yes, No),
- Time from the most recent prior CNS cancer surgery to current surgery,
- Outcome of the most recent prior CNS cancer surgery (GTR, NTR, STR, Biopsy only, Other),
- Received prior CNS cancer systemic therapy (Yes, No),
- Time from the most recent prior CNS cancer systemic therapy to current surgery,
- Received prior CNS cancer radiation therapy (Yes, No),
- Time from the most recent prior CNS cancer radiation therapy to current surgery,
- Location of current tumor (Brain – supratentorial, Brain – posterior fossa, Spinal cord, Other),
- Recurrent or progressive disease for current tumor (Yes, No),
- Evidence of metastatic disease (Yes, No)

All baseline data will be listed.

8.10 Medical History

Medical history will be coded by using the Medical Dictionary for Regulatory Activities (MedDRA). Medical history frequencies will be summarized by treatment arm for the full analysis population and will be ordered by descending frequency for total by system organ class (SOC) then, within a SOC, by overall descending frequency of preferred term (PT). If incidence for more than one term is identical, they will be sorted alphabetically.

8.11 Efficacy Analyses

8.11.1 Primary Efficacy Endpoints

8.11.1.1 Primary Analyses of Sensitivity and Specificity

The primary efficacy analyses will be based on Group 2 in the full analysis population (Section 7). Each of the 4 sets of statistical hypotheses will be tested at two-sided 5% level (Section 5) using the repeated measures log-linear model specified in this section. Each equivocal tissue specimen from Group 2 that is determined to be either tumor positive or tumor negative by central pathology, and has an image operator assessment and/or a surgeon assessment will be used in the analysis.

[redacted]

8.11.1.2 Secondary Analyses of Sensitivity and Specificity

1. [redacted]

[redacted]

2. [redacted]

[redacted]

13. [redacted]

8.11.2 Secondary Endpoints

8.11.2.1 [redacted]

[redacted]

[redacted]

[redacted]

[redacted]

8.11.2.2 [redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

[redacted]

8.11.2.3 [redacted]

[redacted]

8.11.2.4 [redacted]

[redacted]

8.11.3 [redacted]

[redacted]

8.12 Safety Analyses

All safety analyses will be based on the safety analysis population. Safety data collected after re-enrollment for open-label treatment of tozuleristide will be listed.

8.12.1 Exposure to Study Drug

The dose of tozuleristide administrated in volume (mL) and amount (mg) will be summarized as a continuous variable. Dose compliance as measured by the actual dose

received as the percentage of the intended dose [(Total Volume Infused/Scheduled Volume) x 100] will also be summarized.

8.12.2 Adverse Events

Adverse events will be summarized by the system organ class (SOC) and preferred term (PT) based on the MedDRA dictionary. The actual version will be documented in the CSR.

Adverse events will be defined as treatment-emergent if they are newly occurring or worsen following neurosurgery (Arm 1) or treatment with tozuleristide (Arm 2). Adverse events with incomplete start dates will be considered treatment emergent if:

- Day and month are missing and the year is equal to or after the year of the first neurosurgery for Arm 1 or the first dose date for Arm 2;
- Day is missing and the year is after the year of the first neurosurgery for Arm 1 or the first dose date for Arm 2;
- Day is missing and the year is equal to the year of the first neurosurgery for Arm 1 or the first dose date for Arm 2 and the month is equal to or after the month of the first neurosurgery for Arm 1 or the first dose date for Arm 2;
- Year is missing; or
- Start date is completely missing.

However, if the end date of the AE is before the first neurosurgery for Arm 1 or the first dose date for Arm 2, this AE will not be treatment emergent regardless of the completeness of its start date.

Adverse events will be graded using the NCI CTCAE, version 5.0 if applicable, or the protocol specified severity stated in Section 7.1 of the protocol.

Treatment-emergent AEs, SAEs, severity of AEs, tozuleristide-related AEs, Canvas-related AEs, neurosurgical-related AEs and AEs leading to withdrawal from the study will be summarized by MedDRA preferred term, system organ class and treatment arm for the safety analysis population. In the event of multiple occurrences of the same AE with the same preferred term in the same subject, the AE will be counted once at the greatest severity/relatedness.

The summaries presenting frequency of AEs by SOC and PT will be ordered by overall descending frequency of SOC and then, within a SOC, by overall descending frequency of PT for Arm 2.

Only the TEAEs from Arm 1 and Arm 2 will be included in the summary tables. All AEs will be included in the listings. Additional listings will be provided for SAEs, AEs leading to death and AEs leading to study withdrawal.

In addition, treatment-emergent AEs for the re-treatment period are defined as newly occurring or worsen following the open-label treatment of tozuleristide. All AEs reported during the re-treatment period will be listed.

8.12.3 Laboratory Parameters

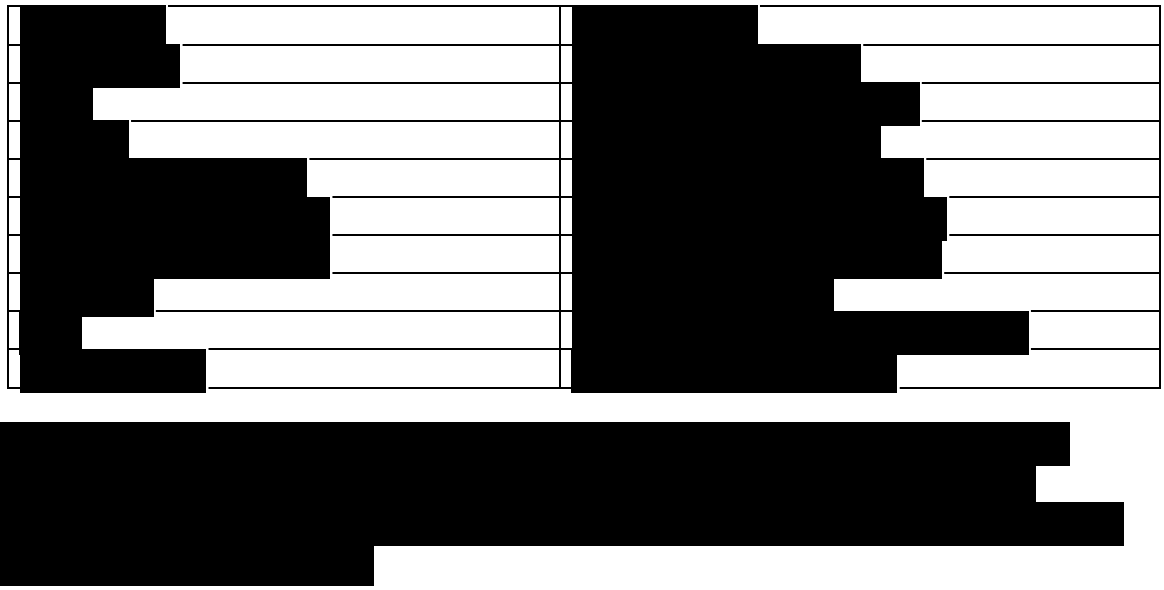
All summaries will be based on results in standard international (SI) system units. Reference ranges for all laboratory parameters collected throughout the study are provided by the respective laboratory. A laboratory value that is outside the reference range is considered either high abnormal (value above the upper limit of the reference range) or low abnormal (value below the lower limit of the reference range).

The laboratory reference ranges will be provided on the listings of laboratory data with out of range values flagged (low or high).

Hematology and chemistry laboratory samples are scheduled to be collected at Screening (all subjects) and Day 8 (Arm 2 and re-treatment only). Coagulation laboratory samples will be collected at Screening (all subjects). For hematology and clinical chemistry summary tables, the actual values and change from baseline for each laboratory parameter will be provided by scheduled visit. Summaries will include data from scheduled assessments only, and all data will be reported according to the nominal visit date for which it was recorded (i.e., no visit windows will be applied).

Toxicity grading will follow the NCI CTCAE grades version 5.0. NCI CTCAE toxicity grading will be derived programmatically for laboratory variables where possible. Toxicity grading that requires investigator/clinical input will not be considered and only the numeric criteria will be used for the programmed grade assignments. If this results in the criteria for more than one grade being met, the highest (worst) CTCAE grade will be assigned. Where a result does not meet any of the CTCAE grading criteria, the record will be counted in the ‘No Grade’ category. For a parameter that can be low or high, e.g. ‘hemoglobin increased’ or ‘anemia’, if CTCAE criteria for a grade for ‘anemia’ is satisfied, then ‘hemoglobin increased’ will be set to ‘No Grade’.

[redacted]



[redacted]

All laboratory results in original and SI units will be included in data listings. Tests will be listed in CRF order.

All abnormal laboratory values will be included in the listings.

Pregnancy data will be listed.

8.12.4 Vital Signs

Vital signs will be collected at Day 1 pre-surgery (Arm 1)/pre-dose (Arm 2 and re-treatment), Day 1 between 1-30 minutes post-dose and Day 8 (Arm 2 and re-treatment). Summary tables of the actual values and change from baseline for each vital sign will be provided by scheduled visit. Summaries will include data from scheduled assessments only, and all data will be reported according to the nominal visit date for which it was recorded (i.e., no visit windows will be applied).

8.12.5 Electrocardiogram Parameters

Electrocardiogram (ECG) parameters (rhythm, ventricular rate, PR interval, QRS duration, QT interval and QTcF interval) will be collected pre-dose, between 1-30 minutes post-dose and Day 8 and will be summarized with descriptive statistics of the change from baseline value by time point for Arm 2 and re-treatment arm. Overall ECG result (normal or abnormal) will be summarized by time point. Summaries will include data from scheduled assessments only, and all data will be reported according to the nominal visit for which it was recorded (i.e., no visit windows will be applied).

[redacted]

8.12.6 Prior and Concomitant Medications

All medications will be coded using the latest version of the World Health Organization (WHO) Drug coding dictionary. The actual dictionary versions used will be presented in the CSR.

Concomitant medications will be summarized by standardized medication name and treatment arm for the safety analysis population. Subject incidence will be presented. Subjects who take the same medication (in terms of the standardized medication name) more than once will only be counted once for that medication.

Prior medications are those medications that were stopped before the first neurosurgery date for Arm 1 or the first dose date for Arm 2. Concomitant medications are medications taken at least once on or after the first neurosurgery date for Arm 1 or the first dose date for Arm 2.

If partial dates exist for concomitant medication, the start dates will be imputed to the first of the month (for missing day) or first of the year (for missing day and month) for purposes of assigning the medication to a prior or concomitant medication.

Prior medications will be listed only. Prior and concomitant medications will be listed separately, including derived study day.

8.12.7 Physical Examination

Physical examination will be listed for the safety analysis population.

9 [redacted]

10 INTERIM ANALYSIS

Interim analysis is not planned. [redacted]

[redacted]

[redacted]

12 REFERENCES

Pepe MS. *The Statistical Evaluation of Medical Tests for Classification and Prediction*. Oxford University Press; 2003

13 APPENDICES

13.1 Calculation of 95% Confidence Interval for Ratio of Paired Percentages

The 95% CI for the ratio of paired percentages (sensitivity and specificity) will be calculated using Pepe's formula (Pepe, 2003) as follows.

The paired data of sensitivity between tozuleristide and surgical designation can be summarized in a 2 x 2 table of all tumor-positive biopsies by central pathology:

		Surgical Designation	
		Tumor*	Not Tumor*
Tozuleristide Fluorescence	Positive*	a	b
	Negative*	c	d

*See Section 4.1

[redacted]

The paired data of specificity between tozuleristide and surgical designation can be summarized in a 2 x 2 table of all tumor-negative biopsies by central pathology:

		Surgical Designation	
		Tumor*	Not Tumor*
Tozuleristide Fluorescence	Positive*	e	f
	Negative*	g	h

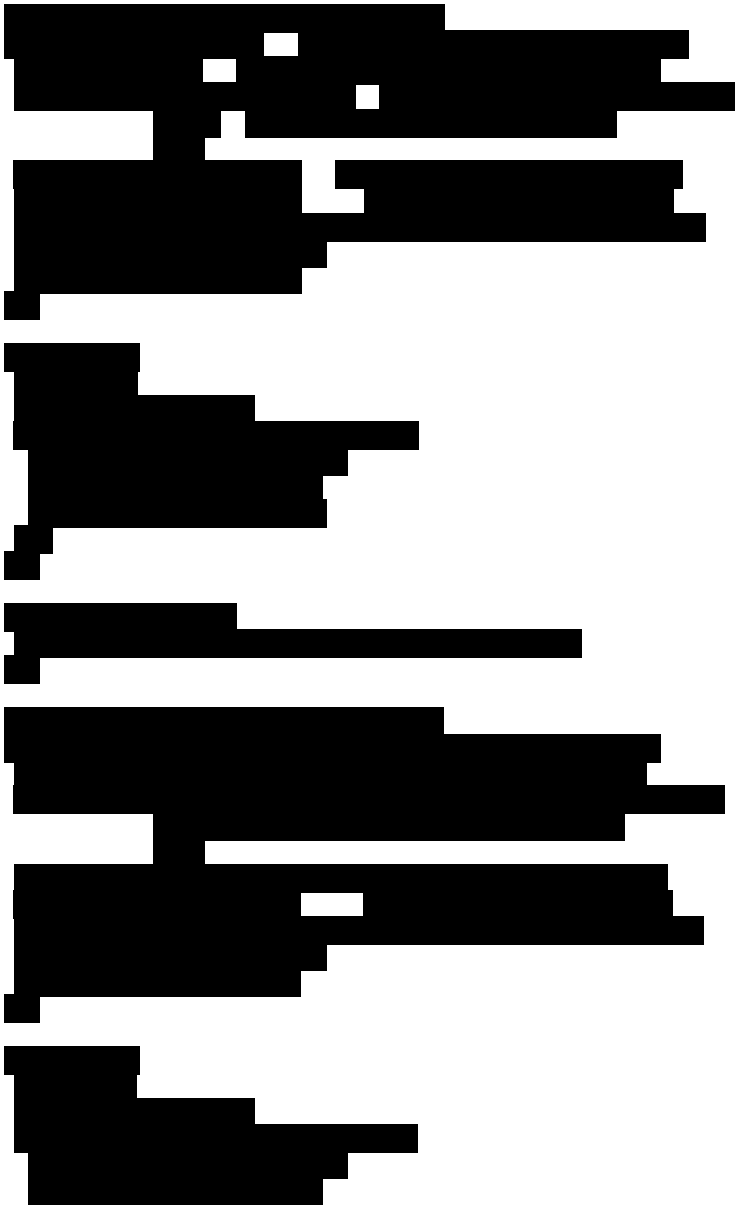
*See Section 4.1

[redacted]

13.2 Definition of Tumor Diagnosis

Subjects are eligible for enrollment based on MRI evidence consistent with a pediatric primary CNS tumor. Study specimens are subjected to pathological analysis and along with local pathology assessments, a diagnosis is assigned by Central Pathology. Tumor diagnosis is based on WHO Classification of Tumors of the Central Nervous System, 4th Edition, 2016. Subjects with ambiguous pathological diagnosis of tumor or no definitive pathological diagnosis are considered to have a pediatric primary CNS tumor. Subjects with a pathological diagnosis indicative of non-tumorous abnormality are considered to not have a pediatric primary CNS tumor.

13.3 Sample SAS Code for Repeated Measures Log-Linear Regression



13.4 [redacted]

[redacted]

- [redacted]
- [redacted]

[redacted]

[redacted]

[redacted]

[redacted]

- [redacted]
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- [redacted]
- [redacted]

[redacted]