CANCER

IIT2020-09-Yu-BBIST001

A Phase 2 study of fluorescence detection of adult primary central nervous system tumors in subjects receiving tozuleristide and imaged with the Canvas System

Principal Investigator: **John Yu, MD**
Cedars-Sinai Medical Center
127 S San Vicente Blvd, A6600
Los Angeles, Ca 90048
Email: yuj@cshs.org
Telephone: 310-423-7900

Sub-Investigator(s)	Department/Division
Ray Chu, MD	Neurosurgery
Chirag Patil, MD	Neurosurgery
Jeremy Rudnick, MD	Neuro-Oncology
Jethro Hu, MD	Neuro-Oncology
Xuemo Fan, MD	Pathology

Biostatistician: Mourad Tighiouart, PhD

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Signature Page

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

Principal Investigator (PI) Signature	**Date**

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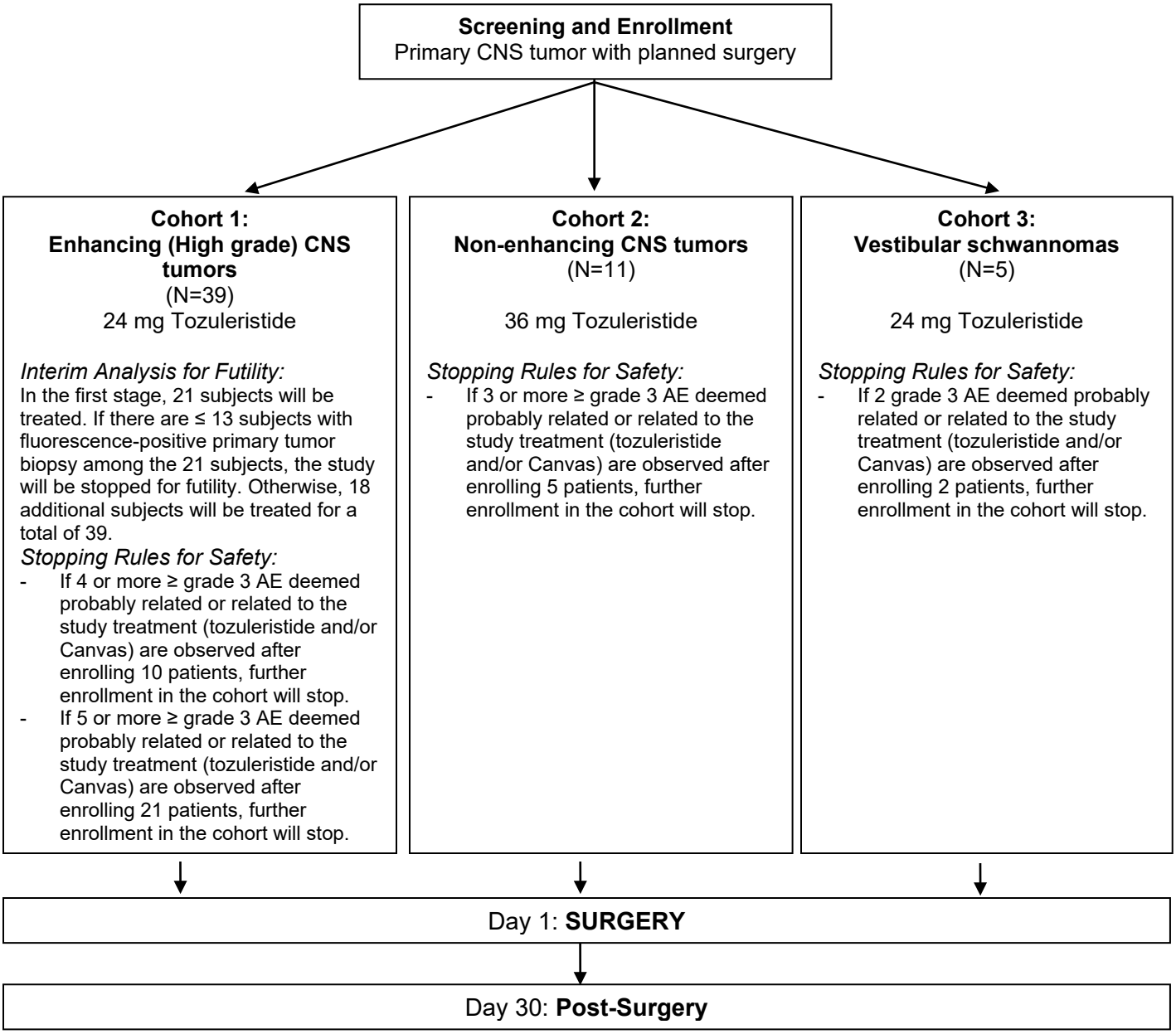
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LIST OF ABBREVIATIONS

^{o}C	degrees Celsius
AE	adverse event
ALA	5-aminolevulinic acid
ALT	alanine aminotransferase
AUC	area under the curve
AUC_{0-t}	AUC from zero to the last measurable time point
BSA	body surface area
CI	confidence interval(s)
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration equation
C_{max}	maximum observed concentration
CNS	central nervous system
CRF	case report form
CS	Clinically significant
CTCAE	Common Terminology Criteria for Adverse Events
CTX	chlorotoxin
CTEP	Cancer Therapy Evaluation Program
CTMS	Clinical Trial Management System
EOR	Extent of resection
FDA	Food and Drug Administration
FI	fluorescence intensity
FIH	first in human
FN	false negative
FP	false positive
FPFV	first patient first visit
GCP	Good Clinical Practice
GTR	gross total resection
H&E	hematoxylin and eosin
HDD	hard-disk drive
hr	hour
IB	investigator’s brochure
ICG	indocyanine green
ICH	International Conference on Harmonization
IND	investigational new drug application
IRB	Institutional Review Board
IV	intravenous
MedDRA	Medical Dictionary for Regulatory Activities

MRI	magnetic resonance imaging
NCI	National Cancer Institute
NIR	near infrared
NPV	negative predictive value
NTR	near total resection
OTC	over-the-counter
PK	pharmacokinetic(s)
PPV	positive predictive value
PRMC	Protocol Review and Monitoring Committee
ROI	region of interest
SAE	serious adverse event
SGPT	serum glutamic pyruvic transaminase
SIRIS	Synchronized Infrared Imaging System
STR	subtotal resection
t	time
TEAE	treatment emergent adverse event
TN	true negative
TNR	true negative rate
TP	true positive
TPR	true positive rate
USP	United States Pharmacopeia
V	volume
WHO	World Health Organization

STUDY SCHEMA



Co-Primary Endpoints: Percentage of patients with fluorescence-positive primary tumor biopsy. True positive rate of fluorescence, defined as the percentage of tumor-positive and fluorescence-positive tissue biopsies among all tumor-positive tissue biopsies. True negative rate of fluorescence, defined as the percentage of tumor-negative and fluorescence-negative tissue biopsies among all tumor-negative tissue biopsies.

STUDY SUMMARY

Title	A phase 2 study of fluorescence detection of primary central nervous system tumors (CNS) in subjects receiving tozuleristide and imaged with the Canvas System
Protocol Number	IIT2020-09-Yu-BBIST001
Phase	Phase 2
Methodology	Open label, single arm
Study Duration	Approximately 38 months (36 months accrual + 2 months for last patient)
Study Center(s)	Cedars-Sinai Medical Center
Objectives	The primary objectives of this study are: - To evaluate preliminary efficacy of 24 mg tozuleristide, administered intravenously and imaged with the Canvas imaging system during surgical resection to demonstrate fluorescence among a homogenous population of primary enhancing CNS tumors. - Among tumors that demonstrate tozuleristide fluorescence, to preliminarily estimate the true positive rate and true negative rate of fluorescence in tissue biopsies from peripheral regions of primary CNS tumors, as well as sensitivity and specificity of tozuleristide fluorescence for distinguishing tumor from non-tumoral tissue. The secondary objectives of this study are: - To evaluate the safety of tozuleristide and Canvas imaging system to provide fluorescence guidance for removal of primary brain tumor. - To preliminarily determine if the presence of residual fluorescence at the time of surgery corresponds to residual tumor evident on post-operative MRI images, or if the absence of fluorescence corresponds to evidence of no gross residual tumor on post-operative MRI. The exploratory objectives of this study are: - To explore the presence of fluorescence with administration of 36 mg of tozuleristide among a cohort of 11 patients with non-gadolinium enhancing primary brain tumors. - To explore the presence of fluorescence with administration of 24 mg of tozuleristide among a cohort of 5 patients with vestibular schwannomas and to determine whether fluorescence aids in distinguishing tumor from the cranial nerve to which it is adherent. - To explore the ability of the Modus IR device to detect tozuleristide fluorescence in situ and ex vivo in subjects undergoing resection of primary gadolinium-enhancing CNS tumors or in subjects undergoing resection of vestibular schwannoma. - To explore the extent of resection in subjects receiving tozuleristide and undergoing maximal safe resection as compared to historical controls. - To explore the size and intensity of the fluorescence in the images captured by the microscope and exoscope in subjects receiving tozuleristide.

Number of Subjects	55 evaluable subjects: - 39 subjects with primary gadolinium-enhancing (high-grade) CNS tumor - 11 subjects with primary non-gadolinium enhancing CNS tumor - 5 subjects with schwannoma tumor Note: Up to 60 subjects may be enrolled to account for subject replacement to obtain 55 evaluable subjects.
Diagnosis and Main Inclusion Criteria	- Primary CNS tumor for which maximal safe resection is indicated - At least 18 years of age
Study Product(s), Dose, Route, Regimen	- Single IV bolus dose of 24 or 36 mg tozuleristide at least 1 hour and no more than 24 hours before start time of surgery - Canvas imaging system attached to a Zeiss Pentero 900 surgical microscope prior to surgical removal of the tissue - Modus IR fluorescence imaging as an accessory of the Modus X surgical exoscope
Duration of administration	Single IV bolus dose of tozuleristide
Statistical Methodology	Descriptive

1.0 BACKGROUND AND RATIONALE

1.1 Disease Background

For patients with primary malignant brain tumors, surgery is a primary treatment modality with maximal safe tumor resection providing the greatest chance of long-term and progression free survival. Extent of resection for primary CNS neoplasms, including both low and high grade glial or glioneuronal tumors, is one of the most important factors impacting extent of survival, time to progression, and quality of life. (Smith 2008, Yang 2013, Pollack 1995, Rogers 2005, Zeltzer 1999, Jakacki 2015, Chi 2009) Aggressive tumor resection must be balanced with preservation of normal brain structures to avoid neurological impairment. However, it is often difficult for surgeons to distinguish tumor from normal brain tissue, particularly at tumor margins, due to the infiltrative nature of the tumors and their similar appearance. Development of intraoperative imaging technologies that can aid surgeons in identifying tumor margins in real time should lead to more complete and precise resection, but this remains a significant unmet clinical need.

Even the most experienced neurosurgeons are challenged by the difficulty of distinguishing cancer tissue from healthy brain. 5-aminolevulinic acid (ALA) is an intraoperative imaging agent that was developed and approved for clinical use in Europe for adults with glioblastoma. In a randomized controlled trial of adults with glioblastoma, ALA was significantly more likely to result in gross total resection, with 65% in the ALA group achieving gross total resection compared to 36% in the control group ($p<0.05$). In addition, the ALA group achieved a significantly ($p<0.05$) greater 6-month survival of 41% compared to 21% in the control arm (Stummer 2006). The study of ALA in adults with glioblastoma demonstrates that intraoperative imaging may have a greater impact on patient survival (20% difference) than any medical therapy developed to date, including temozolomide which resulted in 15% improvement in survival in the initial landmark study leading to United States Food and Drug Administration (FDA) approval (Stupp 2005). A recent systematic review and meta-analysis concluded that the intraoperative imaging agent ALA was superior to neuronavigation for adults with high grade glioma, resulting in improved extent of resection, survival, and quality of life (Zhao 2013). However, ALA is not likely to be applicable to large scale use for CNS tumors. Several reports of off-label use of ALA indicate that 5-ALA does not show fluorescence in low grade glioma and most metastatic tumors as well. Even some high-grade tumors fail to show fluorescence. Furthermore 5-ALA is an oral agent with many known side effects such as photosensitivity and gastrointestinal distress.

Tozuleristide (also known as BLZ-100) is a targeted fluorescent molecule developed by Blaze Bioscience to illuminate cancer foci to facilitate surgical resection. The peptide component of tozuleristide (KNT-03) is a 36 amino acid peptide based on the natural chlorotoxin (CTX) peptide found in scorpion venom (Stroud 2011). The CTX peptide has been shown to specifically bind to many different types of tumors *in vitro* and *in vivo* via a molecular interaction with protein components of cholesterol-rich lipid rafts, including Annexin A2 (Kesavan 2010, Stroud 2011) and matrix metalloproteinase 2 (Deshane 2003). In transgenic and xenograft mouse models of glioma, medulloblastoma, prostate cancer, sarcoma, and colorectal cancer, CTX binds to both primary tumor and metastatic lesions (Veiseh 2007).

The fluorophore component of tozuleristide is a conjugatable form of indocyanine green (ICG). ICG is a cyanine dye that has been approved by the FDA and used as an *in vivo* fluorescent imaging agent since the 1950s. Following excitation, it has an emission wavelength higher than 800 nm which is minimally absorbed by water or hemoglobin, making it well-suited for intraoperative imaging when used with an appropriate detection device. ICG is frequently employed in vascular and ophthalmology surgery as well as for sentinel lymph node mapping. Visualization of the NIR fluorescence from ICG is accomplished using an imaging system, typically composed of a light source for excitation, a camera to detect NIR fluorescence and a monitor to display the images from the camera. Commercially available NIR imaging systems for ICG detection, such as the Fluobeam® 800 (Fluoptics) imaging system or the Artemis Handheld Imaging System/Spectrum (Quest Medical Imaging), are capable of detecting the ICG fluorophore on tozuleristide. Similarly, the investigational device, Synchronized Infrared Imaging System (SIRIS) (Teal Light Surgical) also can detect and display images from tozuleristide dependent fluorescence. The Canvas, which is the

imaging device that will be used for fluorescence assessment in support of the primary and secondary objectives of this study, is an evolution of the SIRIS system's design. The only major change is the modification of the imaging head for close integration with surgical microscopes. Whereas the SIRIS system employed a stand-alone wide field camera, the Canvas system packages the imaging optics and components into a design which is intended to unobtrusively integrate with existing surgical microscopes. In a subset of subjects, the Modus IR device, a commercially available NIR imaging system for visualizing ICG, will be evaluated for its ability to detect tozuleristide fluorescence in situ and ex vivo in subjects undergoing resection of primary gadolinium-enhancing CNS tumors or in subjects undergoing resection of vestibular schwannoma.

Preclinical data show that chlorotoxin-based dye conjugates bind specifically to a range of human and animal tumors and provide greatly improved image resolution compared with pre-operative magnetic resonance imaging (MRI) or direct intraoperative observation, and greater imaging time window flexibility (Mamelak 2006; Gribbin 2009). Thus, they may enable surgeons to visualize and remove tumor cells that would otherwise have remained undetected and more precisely view tumor margins, resulting in more complete surgical resection.

A completed Phase 1 study (Patil et al, 2019) demonstrated the safety and utility of BLZ-100 in 17 adult patients with a variety of primary CNS tumors. At doses of 9 mg and above, the terminal serum half-life for tozuleristide was approximately 30 minutes. Fluorescence signal was detected in both high- and low-grade glial tumors, with high grade tumors generally showing greater fluorescence intensity compared to lower grade tumors. In high-grade tumors, signal intensity increased with increased dose levels of tozuleristide, regardless of the time of dosing relative to surgery. While this study was limited due to the need for dose escalation, it clearly demonstrated the ability of tozuleristide to be detected in high- and low-grade primary brain tumors, both at initial diagnosis and recurrence. Further, it indicated that BLZ-100 was safe and well tolerated. As noted above, there was an apparent association between tumor grade and fluorescence. In the 10 subjects treated with 18, 24, or 30 mg tozuleristide, 3 of 4 high grade tumors but only 1 of 6 low grade tumors exhibited positive fluorescence *in situ*. Moreover, the 3 subjects treated with the highest dose (30 mg) were found to have low grade tumors, thus the effectiveness of tozuleristide doses >24 mg in adult glioma patients with high grade tumors or a broader range of tumor types remains unexplored. The study was limited by the absence of a dedicated high sensitivity imaging device attached to the operating microscope, which limited both practical utility and surgeon ergonomics. Blaze Bioscience has subsequently assessed a higher sensitivity fluorescent imaging system (Canvas, Teal Light Surgical) directly integrated into the operating microscope, that has been tested in pediatric patients and is currently approved for use in a Phase 2/3 trial of tozuleristide in pediatric patients with primary CNS tumors (BB-006). The FDA has approved its use in the ongoing (enrollment complete) BB-006 trial and considers the device to pose non-significant risk to patients. As of 31October2022, tozuleristide and the Canvas system have been employed in 107 patients with pediatric CNS tumors (96 pediatric patients and 11 adult patients) with no reported related serious adverse events from either investigational product. The combination of optimal dosing of tozuleristide and an integrated imaging system will likely lead to better tumor detection and surgeon utility.

Modus IR is an accessory of the Synaptive Medical surgical exoscope, Modus X. Modus IR is FDA cleared (K231986) as of March 25, 2024. When used with the appropriate imaging agent, light output at a specific wavelength excites the imaging agent, which emits light at a specific wavelength that is detected by the optical system, thereby allowing the user to differentiate the structure that the imaging agent has concentrated in.

1.2 Study Agent and Device

Investigational (study agent): *Tozuleristide* (also known as BLZ-100) supplied by Blaze Bioscience (Blaze Bioscience IND #122229).

For more information on tozuleristide, please refer to the current version of the tozuleristide Investigator's Brochure.

Investigational Device: Canvas, supplied by Blaze Bioscience. *Note: The Canvas does not have an IDE number. Information was submitted to FDA as part of the Tozuleristide IND #122229.*

For device information, please refer to the current version of the Canvas Investigator's Brochure.

Investigational Device: Modus IR, supplied by Synaptive Medical (Synaptive Medical, FDA cleared, K231986). Modus X is a commercially available device to be used with Modus IR accessory.

The Modus IR device will be labeled in accordance with all applicable federal guidelines. As the packaging of the device will not be provided to the participant, the research team will provide a document to the subject of the Modus IR device label, in accordance with all applicable federal guidelines.

Note: Synaptive has agreed to upgrade the robotic exoscope currently owned by Cedars-Sinai Medical Center to the next generation platform, Modus X, which includes NIR fluorescence capabilities and Modus IR. The Modus X is approved for clinical use, as long as the investigational feature (Modus IR) is manually locked by Synaptive Medical in between research use. Synaptive Medical will provide clear documentation of the investigational Modus IR feature being manually locked by Synaptive in between research cases.

For device information, please refer to the Modus IR 510(k) letter and user manual.

1.3 Nonclinical Studies

All non-clinical studies are described and summarized in the tozuleristide Investigator's Brochure.

1.4 Clinical Studies

1.4.1 Overview of Clinical Studies

Table 1 provides an overview of the clinical studies with tozuleristide. Nearly all subjects treated in the Phase 1 studies received the sterile 2 mg/mL liquid tris/mannitol formulation of the drug product, BLZ-100 Injection. The last 5 subjects enrolled in the dose expansion phase of study BB-004 and all subjects in the Phase 2/3 BB-006 study received the lyophilized formulation (histidine/mannitol), tozuleristide for injection.

Table 1. Clinical studies with tozuleristide

Study No. Phase / Status / Enrollment Period	Design Objectives	Population	Dosage and Frequency
BB-001 Phase 1 / Complete / FPFV: Dec 24, 2013 LPLV: Mar 3, 2015	Open-label, FIH, dose-escalation/expansion study Primary objectives: safety and tolerability Secondary objectives: PK, dose level for Phase 2 studies	Adult subjects with skin cancer undergoing surgical excision of their tumor(s)	21 subjects treated at doses of 1 (n=3), 3 (n=3), 6 (n=6), 12 (n=6), and 18 (n=3) mg IV, single 15 minute infusion 48 hours prior to surgery
BB-002 Phase 1 / Complete / FPFV: Nov 18, 2014 LPLV: Jan 27, 2016	Open-label, dose-escalation study evaluating doses up to 30 mg Primary objectives: safety and tolerability Secondary objectives: PK, fluorescent signal from excised brain tumor tissue, dose level for Phase 2 studies	Adult subjects with glioma undergoing surgical excision of their tumor(s)	17 subjects treated at doses of 3 (n=3), 9 (n=4), 18 (n=4), 24 (n=3), and 30 (n=3) mg IV, single bolus administration at least 2 hours prior to surgery
BB-004 Phase 1 / Complete / FPFV: Jun 17, 2015 LPLV: Aug 23, 2018	Open-label, dose-escalation and expansion study Primary objectives: safety and tolerability, PK, recommended Phase 2 dose	Pediatric[1] subjects with pediatric primary CNS tumors	29 subjects treated (32 doses administered) at doses of 1.7 (n=3), 3.5 (n=3), 6.9 (n=3), 13.9 (n=3), 15 (n=17), and 17.3 (n=3) mg/m^2 IV, single bolus administration at least 1 hour prior to surgery
BB-005 Phase 1 / Complete /	Open-label, exploratory study Primary objective: safety and	Adult subjects with solid tumors	30 subjects treated (23 breast cancer, 3 prostate cancer, 2

FPFV: Aug 4, 2015 LPLV: Dec 28, 2016	tolerability Secondary objectives: fluorescent signal intensity from excised tumor tissue, PK in subjects with breast cancer.	(breast, lung, prostate, colorectal, and "other") undergoing surgery	colorectal cancer, 1 lung cancer and 1 bladder cancer) at doses of 12 mg or 6 mg IV, single bolus administration at least 2 hours[2] prior to surgery
BB-006 Phase 2/3 / **Enrollment complete, study close out in progress** / FPFV: Nov 26, 2018 LPLV: June 13, 2022	Randomized, blinded pivotal study Primary objective: Efficacy of tozuleristide and Canvas system to identify equivocal tumor tissue Secondary objectives: Safety, extent of resection, PK, Canvas system performance	Subjects with pediatric primary CNS tumors (age >1 month and ≤30 years)	107 subjects treated Randomized 1:10: Arm 1 (11 subjects): no treatment, imaged with the Canvas system Arm 2 (105 subjects): treated with single dose of tozuleristide at 15 mg/m^2, imaged with the Canvas system Retreatment Arm includes 2 subjects initially on Arm 1 and 1 ` Ssubject initially on Arm 2 retreated 2 times

CNS = central nervous system; FIH = first in human; FPFV = first patient first visit; IV = intravenous; LPLV = last patient last visit; PK = pharmacokinetic

[1] One subject on the BB-004 study was 19 years old, but had a characteristically pediatric type tumor (myxopapillary ependymoma).

[2] One subject in the BB-005 study was granted an authorized deviation to receive tozuleristide approximately 1 hour before the scheduled start of surgery.

The above completed clinical studies are also presented in detail in the current version of the tozuleristide Investigator's Brochure. Study BB-006 enrollment is complete, and study close out is in progress.

In the clinical studies to date, visualization of tozuleristide has been performed using more than one imaging system, including the Fluobeam® 800 (Fluoptics) imaging system, the Artemis Handheld Imaging System/Spectrum (Quest Medical Imaging), the SIRIS (Teal Light Surgical) and most recently, the Canvas (in Study BB-004 and BB-006). The primary goal of NIR imaging in these dose-ranging studies has been detection of fluorescent signal in tumor tissue *ex vivo* following excision and in some cases, fluorescence imaging *in situ* prior to excision. The Fluobeam 800 and Spectrum are imaging systems cleared by the FDA for capturing and viewing fluorescent images for the visual assessment of blood flow for the evaluation of tissue perfusion and circulation using ICG. As the fluorophore moiety of tozuleristide is a conjugatable form of ICG the study procedures for intraoperative imaging of tozuleristide fluorescence were performed according to the manufacturers' instructions. The SIRIS is a clinical research instrument also capable of imaging ICG and is being developed to support clinical studies of tozuleristide in cancer patients.

In the adult and pediatric brain cancer clinical studies (BB-002, BB-004), intraoperative tumor imaging for tozuleristide was performed with the Fluobeam 800, SIRIS or Canvas prior to start of tumor excision and after completion of excision. Additional imaging was obtained at other time points at the discretion of the neurosurgeon. All clinical decision-making was made based on the routine neurosurgical practices with tissue resection taking place under normal light conditions in line with best clinical practices.

Overall, the results from the tozuleristide clinical studies have shown that tozuleristide is well tolerated with no serious safety findings. In adult patients with skin cancer (BB-001) or gliomas (BB-002), serum tozuleristide exposures based on mean C_{max} and AUC_{0-t} values increased modestly greater than dose-proportionally from 3 to 9 mg (~6- to 8-fold for the 3-fold increase in dose), but approximately dose-proportionally from 9 to 30 mg (~3- to 4-fold for the 3.3-fold increase in dose). Similar PK properties were observed in pediatric patients with CNS malignancies (BB-004).

1.4.2 Safety Summary: Tozuleristide

Summary: As of 31 October 2022, a total of 43 serious adverse events (SAE) have been reported in 28 subjects across all Blaze-sponsored clinical trials to date with tozuleristide. All events were considered unrelated to tozuleristide or the Canvas device. See section 1.4.2.2 for details.

1.4.2.1 Summary of Treatment Emergent Adverse Events

As of 31 October 2022, a total of 19 related or possibly related treatment emergent AEs (TEAE) have been reported by 15 subjects in clinical studies BB-001 (n=5), BB-004 (n=1), and BB-005 (n=8). There were no related TEAEs reported in study BB-002.

In the BB-006 trial (enrollment complete, study close-out in progress), five tozuleristide treatment related AEs have been reported: Grade 1 injection site joint pain, proteinuria, and rash maculopapular, in one subject each; and Grade 2 vomiting and hypoalbuminemia, in one subject each.

The overall incidence and number of subjects experiencing adverse events considered related to drug at any tozuleristide dose level was low in these studies (see Table 2 for a summery based on data from 95 Phase 1 subjects). The most commonly reported events considered related to drug were in the Gastrointestinal Disorder system organ class. Across the studies, there was no trend by dose level or patient population in the incidence or severity of adverse events considered related to tozuleristide.

Overall, the clinical studies in adults have shown that tozuleristide is safe and well-tolerated.

Table 2 Tozuleristide treatment related TEAEs by grade from monitored Phase 1 trials

System Organ Class (SOC) Preferred Term	Grade 1 n	Grade 2 n	Grade 3 n	Grade 4 n	Grade 5 n	Total Number of Subjects[1] (N=95) n (%)
Subjects with at least one related TEAE[2]	8	1	1			10 (10.5)
Total number of TEAE reported	13	4	1			18
Gastrointestinal disorders	5	1				6 (6.3)
Nausea	2					2 (2.1)
Abdominal distension		1				1 (1.1)
Abdominal pain	1					1 (1.1)
Abdominal pain upper	1					1 (1.1)
Diarrhea	1					1 (1.1)
Frequent bowel movements	1					1 (1.1)
Nervous system disorders	3					3 (3.2)
Headache	2					2 (2.1)
Dysgeusia	1					1 (1.1)
Investigations	1	1	1			2 (2.1)
ALT increased			1			1 (1.1)
AST increased		1				1 (1.1)
Elevated Alkaline Phosphatase	1					1 (1.1)

System Organ Class (SOC) Preferred Term	Grade 1 n	Grade 2 n	Grade 3 n	Grade 4 n	Grade 5 n	Total Number of Subjects[1] (N=95) n (%)
Vascular disorders		2				2 (2.1)
Hypertension		2				2 (2.1)
Metabolism and nutrition disorders	1					1 (1.1)
Hypoalbuminemia	1					1 (1.1)
Renal and urinary disorders	1					1 (1.1)
Chromaturia	1					1 (1.1)
Skin and subcutaneous tissue disorders	1					1 (1.1)
Pruritus	1					1 (1.1)

TEAE were coded with Medical Dictionary for Regulatory Activities (MedDRA) Version 17.1 (BB-001) or Version 20.0 (BB-004 and BB-005) preferred terms and are presented by decreasing incidence in SOC for "All Subjects".

[1]For total number of subjects by SOC, subjects are counted once at the highest severity of TEAE reported by that subject within the SOC

[2]For total TEAE by severity, subjects are counted once at the highest severity reported for that subject

1.4.2.2 Summary of Serious Adverse Events

As of 31October2022, a total of 43 serious adverse events (SAE) have been reported in 28 subjects across all Blaze-sponsored clinical trials to date with tozuleristide. All events were considered unrelated to tozuleristide or the Canvas device. These are listed below in Table 3.

Table 3 Listing of Serious adverse Events in All Blaze-Sponsored tozuleristide Clinical Trials

Study/ Tozuler-istide Dose	Subject #/ Date of Dose	SYSTEM ORGAN CLASS Preferred Term	Event Onset Date	Event Stop Date	Event Severity	Relationship to tozuleristide	Outcome of Event at End of Study
BB-002 3 mg	BB-002-T101 20 Nov 2014	INFECTIONS AND INFESTATIONS Post procedural infection	29 Nov 2014	23 Dec 2014	Gr. 3	Unrelated	Resolved
BB-002 18 mg	BB-002-T302 14 May 2015	INFECTIONS AND INFESTATIONS Post procedural infection	19 May 2015	Ongoing	Gr. 3	Unrelated	Ongoing
BB-002 18 mg	BB-002-T304 9 Jul 2015	RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS Pulmonary embolism	28 Jul 2015	18 Aug 2015	Gr. 3	Unrelated	Resolved
BB-004 1.7 mg/m^2	BB-004-T102 24 June 2015	INJURY, POISONING AND PROCEDURAL COMPLICATIONS Wound complication	2 Jul 2015	7 Jul 2015	Gr. 3	Unrelated	Resolved
BB-004 17.3 mg/m^2	BB-004-T503 22 Aug 2016	INFECTIONS AND INFESTATIONS Meningitis	4 Sep 2016	27 Sep 2016	Gr. 3	Unrelated	Resolved
BB-004 15 mg/m^2	BB-004-T606 3 Apr 2017	NERVOUS SYSTEM DISORDERS	5 Apr 2017	10 Apr 2017	Gr. 4	Unrelated	Resolved

Study/ Tozuler-istide Dose	Subject #/ Date of Dose	SYSTEM ORGAN CLASS Preferred Term	Event Onset Date	Event Stop Date	Event Severity	Relationship to tozuleristide	Outcome of Event at End of Study
		Spinal cord compression					
BB-004 15 mg/m^2	BB-004-T613 25 Jan 2018	NERVOUS SYSTEM DISORDERS Subdural hygroma	8 Feb 2018	8 Feb 2018	Gr. 4	Unrelated	Resolved
BB-004 15 mg/m^2	BB-004-T617 21 May 2018	NERVOUS SYSTEM DISORDERS Hydrocephalus	28 May 2018	28 May 2018	Gr. 3	Unrelated	Resolved
BB-004 15 mg/m^2	BB-004-T617 21 May 2018	GASTROINTESTINAL DISORDERS Colitis	28 May 2018	6 Jun 2018	Gr. 3	Unrelated	Resolved
BB-005 6 mg	BB-005-B2-031 5 Oct 2016	INFECTIONS AND INFESTATIONS Cellulitis	15 Oct 2016	20 Oct 2016	Gr. 2	Unrelated	Resolved
BB-006 15mg/m^2	BB-006-010-001 17-Jul-2019	METABOLISM AND NUTRITION DISORDERS Dehydration	7-Aug-2019	10-Aug-2019	Gr. 3	Unrelated	Resolved
BB-006 No treatment	BB-006-010-002 N/A	NERVOUS SYSTEM DISORDERS Basal ganglia stroke	29 Aug 2019	21 Oct 2019	Gr. 3	Unrelated	Resolved with sequelae
BB-006 No treatment	BB-006-010-002 N/A	INJURY, POISONING AND PROCEDURAL COMPLICATIONS Tension pneumocephalus	29 Aug 2019	2 Sep 2019	Gr. 3	Unrelated	Resolved
BB-006 No treatment	BB-006-010-002 N/A	RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS Pulmonary embolism	22-Sep-2019	2-Mar-2020	Gr. 3	Unrelated	Resolved
BB-006 No treatment	BB-006-010-003 N/A	NERVOUS SYSTEM DISORDERS Cerebrospinal fluid leakage	20-Sep-2019	24-Sep-2019	Gr. 3	Unrelated	Resolved
BB-006 15mg/m^2	BB-006-007-002 24-Oct-2019	NERVOUS SYSTEM DISORDERS Headache	1-Dec-2019	5-Dec-2019	Gr. 3	Unrelated	Resolved
BB-006 15mg/m^2	BB-006-007-002 24-Oct-2019	EYE DISORDERS Blurred vision	1-Dec-2019	1-Dec-2019	Gr. 1	Unrelated	Resolved
BB-006 15mg/m^2	BB-006-007-002 24-Oct-2019	GASTROINTESTINAL DISORDERS Vomiting	1-Dec-2019	1-Dec-2019	Gr. 1	Unrelated	Resolved
BB-006 15mg/m^2	BB-006-007-002 24-Oct-2019	NERVOUS SYSTEM DISORDERS Dizziness	1-Dec-2019	1-Dec-2019	Gr. 1	Unrelated	Resolved
BB-006 15mg/m^2	BB-006-005-001 2-Feb-2020	NERVOUS SYSTEM DISORDERS Headache	2-Mar-2020	Ongoing	Gr. 2	Unrelated	Ongoing
BB-006 15mg/m^2	BB-006-005-001 2-Feb-2020	NERVOUS SYSTEM DISORDERS Facial muscle weakness	2-Mar-2020	Ongoing	Gr. 1	Unrelated	Ongoing
BB-006 15mg/m^2	BB-006-005-001 2-Feb-2020	NERVOUS SYSTEM DISORDERS Hydrocephalus	2-Mar-2020	Ongoing	Gr. 3	Unrelated	Ongoing
BB-006 15mg/m^2	BB-006-005-001 2-Feb-2020	NERVOUS SYSTEM DISORDERS Tremor limb	13-Mar-2020	Ongoing	Gr. 1	Unrelated	Ongoing
BB-006 15mg/m^2	BB-006-005-001	GASTROINTESTINAL DISORDERS	13-Mar-2020	14-Mar-2020	Gr. 1	Unrelated	Resolved

Study/ Tozuler-istide Dose	Subject #/ Date of Dose	SYSTEM ORGAN CLASS Preferred Term	Event Onset Date	Event Stop Date	Event Severity	Relationship to tozuleristide	Outcome of Event at End of Study
	2-Feb-2020	Bowel incontinence					
BB-006 15mg/m^2	BB-006-001-009 21-Apr-2020	IMMUNE SYSTEM DISORDERS Anaphylactis	22-Apr-2020	23-Apr-2020	Gr. 3	Unrelated	Resolved
BB-006 15mg/m^2	BB-006-010-017 27-Jul-2020	NERVOUS SYSTEM DISORDERS Cerebrospinal fluid leakage	30-Jul-2020	5-Aug-2020	Gr. 2	Unrelated	Resolved
BB-006 15mg/m^2	BB-006-010-018 4-Aug-2020	INJURY, POISONING AND PROCEDURAL COMPLICATIONS Posterior fossa syndrome	9-Aug-2020	Ongoing	Gr. 3	Unrelated	Ongoing
BB-006 15mg/m^2	BB-006-010-019 27-Aug-2020	INJURY, POISONING AND PROCEDURAL COMPLICATIONS Posterior fossa syndrome	2-Sep-2020	Ongoing	Gr. 3	Unrelated	Ongoing
BB-006 15mg/m^2	BB-006-010-021 15-Sep-2020	GASTROINTESTINAL DISORDERS Vomiting	14-Sep-2020	21-Sep-2020	Gr. 3	Unrelated	Resolved
BB-006 15mg/m^2	BB-006-002-003 8-Sep-2020	NERVOUS SYSTEM DISORDERS Hydrocephalus	26-Sep-2020	30-Sep-2020	Gr. 3	Unrelated	Resolved
BB-006 15mg/m^2	BB-006-002-003 8-Sep-2020	GASTROINTESTINAL DISORDERS Vomiting	19-Sep-2020	30-Sep-2020	Gr. 2	Unrelated	Resolved
BB-006 15mg/m^2	BB-006-002-003 8-Sep-2020	NERVOUS SYSTEM DISORDERS Lethargy	26-Sep-2020	30-Sep-2020	Gr. 2	Unrelated	Resolved
BB-006 15mg/m^2	BB-006-007-009 2-Mar-2021	RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS Respiratory failure	4-Mar-2021	4-Mar-2021	Gr. 4	Unrelated	Resolved
BB-006 15mg/m^2	BB-006-007-009 2-Mar-2021	NERVOUS SYSTEM DISORDERS Depressed level of consciousness	4-Mar-2021	4-Mar-2021	Gr. 3	Unrelated	Resolved with sequelae
BB-006 15mg/m^2	BB-006-001-028 15-Jun-2021	NERVOUS SYSTEM DISORDERS Hydrocephalus	29-Jun-2021	30-Jun-2021	Gr. 3	Unrelated	Resolved
BB-006 15mg/m^2	BB-006-006-004 14-Sep-2021	METABOLISM AND NUTRITION DISORDERS Hyponatremia	23-Sep-2021	24-Sep-2021	Gr. 3	Unrelated	Resolved
BB-006 15mg/m^2	BB-006-008-005 23-Sep-2021	INFECTIONS AND INFESTATIONS Meningitis	8-Oct-2021	25-Oct-2021	Gr. 3	Unrelated	Resolved
BB-006 15mg/m^2	BB-006-008-005 23-Sep-2021	NERVOUS SYSTEM DISORDERS Obstructive hydrocephalus	22-Oct-2021	25-Oct-2021	Gr. 3	Unrelated	Resolved
BB-006 15mg/m^2	BB-006-006-005 4-Oct-2021	NERVOUS SYSTEM DISORDERS Hydrocephalus	7-Oct-2021	10-Oct-2021	Gr. 3	Unrelated	Resolved
BB-006 15mg/m^2	BB-006-006-006 9-Feb-2022	INJURY, POISONING AND PROCEDURAL COMPLICATIONS Posterior fossa syndrome	14-Feb-2022	25-Feb-2022	Gr. 2	Unrelated	Resolved with sequelae
BB-006 15mg/m^2	BB-006-016-014	INJURY, POISONING AND PROCEDURAL	27-Feb-2022	3-Mar-2022	Gr. 3	Unrelated	Resolved

Study/ Tozuler-istide Dose	Subject #/ Date of Dose	SYSTEM ORGAN CLASS Preferred Term	Event Onset Date	Event Stop Date	Event Severity	Relationship to tozuleristide	Outcome of Event at End of Study
	10-Jan-2022	COMPLICATIONS Pseudomeningocele					
BB-006 15mg/m^2	BB-006-016-014 10-Jan-2022	NERVOUS SYSTEM DISORDERS Headache	6-Mar-2022	8-Mar-2022	Gr. 3	Unrelated	Resolved
BB-006 15mg/m^2	BB-006-016-017 1-Apr-2022	PSYCHIATRIC DISORDERS Mental status changes	11-Apr-2022	13-Apr-2022	Gr. 3	Unrelated	Resolved

Note: Adverse events were coded with Medical Dictionary for Regulatory Activities (MedDRA) version 17.1 (BB-002), or version 20.0 (BB-004, BB-005) or version 21.0 and version 25.0 (BB-006) system organ class (SOC) and preferred terms. Adverse event severity was determined using the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 for studies BB-002, BB-004, and BB-005 and version 5.0 for study BB-006. This table includes final data from study BB-001 (n=21), study BB-002 (n=17), study BB-005 (n=30), study BB-004 (n=32), and study BB-006 (n=118).

1.4.3 Efficacy/Imaging summary: BB-002 and BB-004 studies

1.4.3.1 BB-002

Fluorescent signal intensity from excised tumor samples was a secondary endpoint for this study and was assessed using Odyssey analysis of cryosections and by *ex vivo* imaging of tissues immediately after excision using Fluobeam or SIRIS. This approach allowed for comparisons of regions of tissue fluorescence with areas of tumor determined by histopathology. Fluorescence was also compared with expression of other biomarkers related to BLZ-100 such as Annexin A2 and those related to brain cancer, an exploratory endpoint. The exploratory study objective to evaluate the fluorescent signal from areas of brain tumor in situ was accomplished using intraoperative imaging with Fluobeam or SIRIS. Imaging results are summarized in Table 4 and discussed in detail in the following sections.

The goal of the sub-study, added to the protocol with amendment 2, was to demonstrate the feasibility of using the SIRIS device to collect imaging data.

Table 4 Summary of Imaging Results

Subj. No.	Post-Surgery Cancer Diagnosis	WHO Grade	BLZ-100 Dose	*In situ Score*[a]	*In situ* Device	*Ex vivo Score*[a]	*Ex vivo* Device(s)	Highest Mean[b] Odyssey Score from Tissue Sections
T101	Astrocytoma	III	3 mg	Negative	FB	Negative	FB	409
T102	Glioblastoma	IV	3 mg	Negative	FB	Weak	FB/ SIRIS	1890
T103	Glioblastoma	IV	3 mg	Negative	FB	Weak	FB/ SIRIS	556
T201	Oligodendroglioma	II	9 mg	Weak	FB	Weak	FB/ SIRIS	1336
T202	Anaplastic astrocytoma	III	9 mg	Weak	FB	Weak	FB/ SIRIS	6204
T203	Glioblastoma multiforme	IV	9 mg	Negative	FB	Weak	FB/ SIRIS	4560
T204	Oligodendroglioma	II	9 mg	Negative	FB	Negative	FB	389

T301	Oligodendroglioma	II	18 mg	Negative	FB/ SIRIS	Negative	SIRIS	191
T302	Glioblastoma	IV	18 mg	Weak	FB/ SIRIS	Strong	SIRIS	32,141
T303	Glioblastoma	IV	18 mg	Strong	SIRIS	Strong	SIRIS	5729
T304	Oligodendroglioma	II	18 mg	Weak	FB	Strong	FB/ SIRIS	17,033
T401	Anaplastic oligodendroglioma	III	24 mg	Negative	FB	Weak	FB	1821
T402	Gliomatosis cerebri	IV	24 mg	Strong	SIRIS	Strong	SIRIS	36,109
T403	Pilocytic astrocytoma	I	24 mg	Negative	SIRIS	Strong	SIRIS	4543
T501	Oligodendroglioma	II	30 mg	Negative	SIRIS	Weak	SIRIS	644
T502	Oligodendroglioma	II	30 mg	Negative	SIRIS	Negative	SIRIS	480
T503	Oligoastrocytoma	II	30 mg	Negative	SIRIS	Negative	SIRIS	971

a. Three reviewers independently scored the available images for each case, and reviewed them together at the end of the study. The final score assigned was based on consensus after review (at least 2 out of 3). In cases where multiple images were available (e.g. more than one stopping point *in situ*, or more than one tissue sample *ex vivo*) the score is reported for the image showing the strongest fluorescence or contrast.
b. A representative section corresponding to the brightest section of the tumor was analyzed
FB = Fluobeam® 800; SIRIS = Synchronized Infrared Imaging System

1.4.3.1.1 In Situ and Ex Vivo Imaging by BLZ-100 Dose: BB-002

In the 3 mg cohort, contrast between tumor and surrounding normal brain was not observed *in situ* in any of the 3 subjects (Table 4). Fluorescence signal was detected in *ex vivo* samples in 2 of the 3 cases. Long exposure times (up to 1 second) were needed to obtain these images.

In the 9 mg cohort, contrast with surrounding normal brain was observed *in situ* in 2 of 4 subjects and a fluorescence signal was detected *ex vivo* in 3 of 4 subjects. Exposure times of 300 msec to 1 sec were required to obtain images in this cohort.

In the 18 mg cohort, contrast with surrounding normal brain was observed *in situ* in 3 of 4 subjects and a fluorescence signal was detected *ex vivo* in 3 of 4 subjects. Intraoperative images for Subject T303 are shown in Figure 1. Exposure times required for imaging in this cohort were generally shorter (< 33 msec) than those needed in cohort 2.

In the 24 mg cohort, contrast with surrounding normal brain was observed *in situ* in 1 of 3 subjects and a fluorescence signal was detected *ex vivo* in 3 of 3 subjects.

In the 30 mg cohort, contrast with surrounding normal brain was observed *in situ* in 0 of 3 subjects and a fluorescence signal was detected *ex vivo* in 1 of 3 subjects.

In the 10 subjects treated with 18, 24, or 30 mg tozuleristide, 3 of 4 high grade tumors but only 1 of 6 low grade tumors exhibited positive fluorescence *in situ*, and the 3 subjects in the 30 mg cohort had low grade tumors. These findings indicate an apparent association between tumor grade and fluorescence, and it is not known if tozuleristide doses >24 mg in adult glioma patients with high grade tumors or a broader range of tumor types will lead to greater incidence and/or intensity of tumor fluorescence.

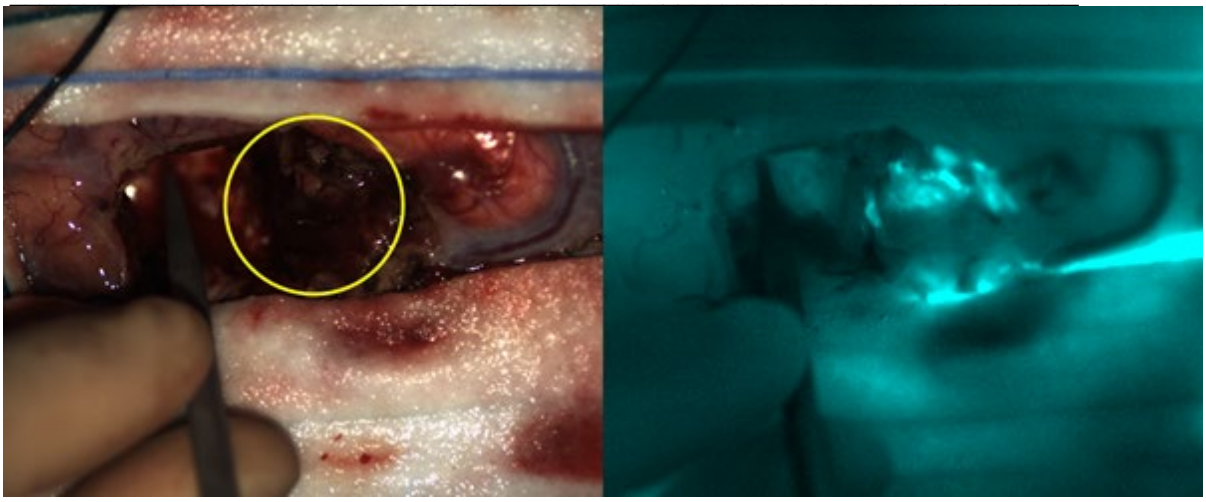


Figure 1 Intraoperative images obtained for Subject T303 using the SIRIS.
The area of exposed tumor is circled in the white light image (left). Fluorescence is visible within the area of tumor in the near-infrared image at right (For visual clarity 135 msec exposure is shown).

1.4.3.1.2 In Situ and Ex Vivo Imaging by Tumor Type: BB-002

Post-surgery diagnosis was high grade glioma for 9 subjects (glioblastoma: n=4; anaplastic astrocytoma; n=1; astrocytoma: n=1; glioblastoma multiforme: n=1; gliomatosis cerebri: n=1; anaplastic oligodendroglioma: n=1) and low grade glioma for 8 subjects (pilocytic astrocytoma: n=1; oligodendroglioma: n=6; oligoastrocytoma; n=1; Table 4).

Overall 12/17 tumors demonstrated positive fluorescence on *ex vivo* imaging. At doses of 9 mg or higher, 6/6 high grade tumors showed positive fluorescence *ex vivo*. At these doses 4/8 (50%) of low grade tumors also showed positive fluorescence *ex vivo*.

Overall, a contrast with surrounding normal brain was observed *in situ* for 6 of the 17 subjects. When considered by tumor type, fluorescence signal was observed *in situ* for 4 of 9 high grade gliomas and 2 of 8 low grade gliomas. Deep tumors accessed through narrow openings were difficult to image *in situ* and in some tumors, necrotic tissue may have blocked the signal from underlying viable tumor. The *in situ* imaging may also have been affected by ambient light in the operating room.

1.4.3.1.3 Imaging of Sections Using Odyssey and Comparison to In Situ and Ex Vivo Results: BB-002

Odyssey fluorescence data generally agreed with *ex vivo* imaging results obtained with freshly excised tissues and with tumor histopathology. Amongst the 7 mean Odyssey scores >4500/mm^2, 5 corresponded to *ex vivo* scores of strong (high grade: n=3; low grade: n=2) and 2 were weak (high grade: n=2). Odyssey scores showed a significant relationship with *in situ* results (Figure 2A). Overall, no relationship between Odyssey scores and dose of BLZ-100 was observed (Figure 2B); however, a significant relationship was observed for the high grade gliomas ($p<0.01$; Figure 2C).

Odyssey scan data were available for sections scored as normal brain parenchyma from 4 cases (T304, T401, T402, and T403), and mean fluorescence intensities of 491, 331, 892, and 401 MFI/mm^2 were recorded (mean 529 ± 251 MFI/mm^2). Tumor sections considered positive for fluorescence at these dose levels ranged from 1821 to 36,109 MFI/mm^2 (3.44 to 68.3 times the mean normal brain MFI/mm^2).

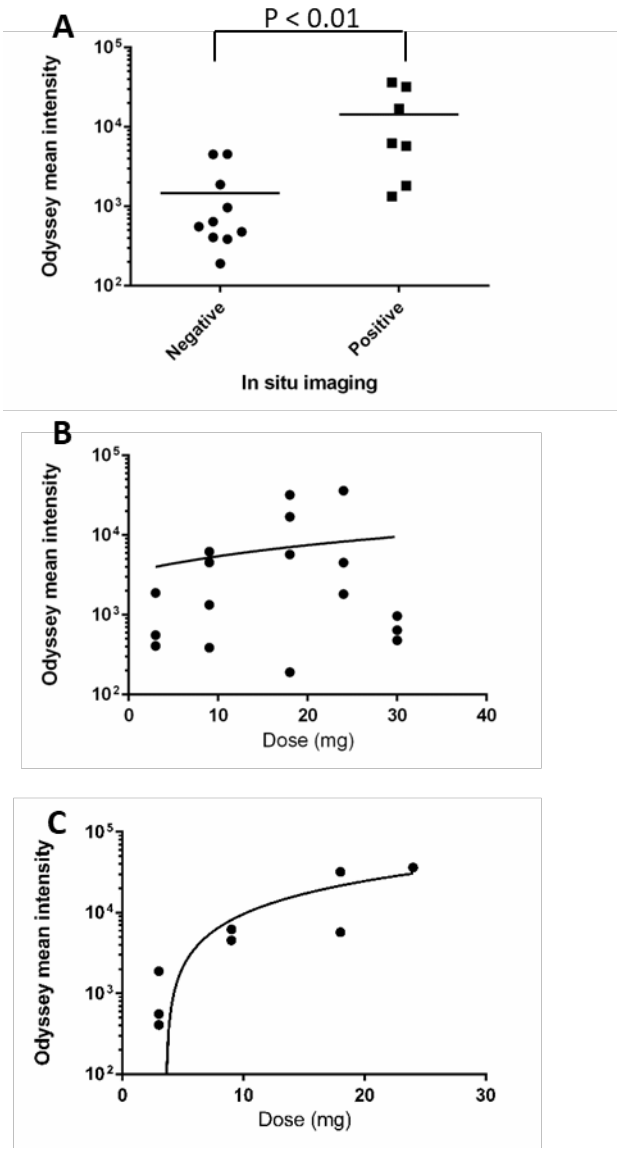


Figure 2 Analysis of Odyssey Intensity Data

(A) Cases were grouped according to *in situ* imaging score, with weak and strong cases combined and shown as "positive." The mean intensity of the fluorescence signal in the highest-intensity section analyzed for each case is shown on the y axis (log_{10} scale). (B) Mean intensity values plotted by dose for all cases. Dose (mg) is shown on the x axis and mean intensity is shown on the y axis (log_{10} scale). (C) Relationship between dose and intensity for high-grade glioma. Dose (mg) is shown on the x axis, and mean intensity is shown on the y axis (log_{10} scale). The curve is significantly non-zero (linear regression, $P < 0.01$).

1.4.3.2 BB-004

1.4.3.2.1 Efficacy/Imaging summary: BB-004

The SIRIS was used to detect tozuleristide fluorescence during surgery in the BB-004 study. The SIRIS is an investigational device for open field imaging of NIR fluorescence that is free-standing; it displays fluorescence, white light, and overlay images on a monitor; and it has laser power, digital gain, and exposure time adjustments that allow for detection of a range of fluorescence intensities. Because the SIRIS is an open-field free-standing imaging device, acquisition of *in situ* images of the surgical field (typically when tumor was first exposed and then again at completion of resection) was

done during pauses in the standard of care surgical procedures, to avoid interference between the imaging equipment and the surgical microscope. To the extent possible, ambient lighting was subdued.

The extent to which tumors were exposed for imaging in the BB-004 study varied and was dependent on when during the procedure the surgeon could pause for imaging and the anatomic location/depth of the tumor. *In situ* images were collected of exposed tumor and tumor bed. A variety of images were taken in each *in situ* setting, with a range of NIR exposure times to enable imaging of a range of observed fluorescence intensities. The available images for each case were subjectively scored as negative (no difference in fluorescence between tumor and normal brain), positive-weak (some fluorescence contrast apparent, but not well-defined), or positive-strong (well-defined fluorescence contrast that is visually obvious). Surgeons also qualitatively assessed the fluorescence during surgery and recorded their observations (see below). Semi-quantitative region of interest (ROI) analysis was also conducted using the *in situ* images to define tumor intensity and to calculate contrast between tumor and normal brain.

Tumor fluorescence generally increased with dose from 1.7 to 17.3 mg/m^2, with body surface area (BSA)-based dosing considered appropriate in this heterogeneous population (e.g. body weights ranged from ~6 to 115 kg). *In situ* tumor fluorescence was considered optimal between the 13.9 to 17.3 mg/m^2 doses, with the intermediate, expansion dose of 15 mg/m^2 (N=17) demonstrating positive *in situ* tumor fluorescence in 11 of 15 evaluable cases (tumor depth precluded effective *in situ* imaging in 2 cases). An IV bolus dose of 15 mg/m^2 was therefore considered the near-optimal dose for studies in subjects with pediatric CNS tumors.

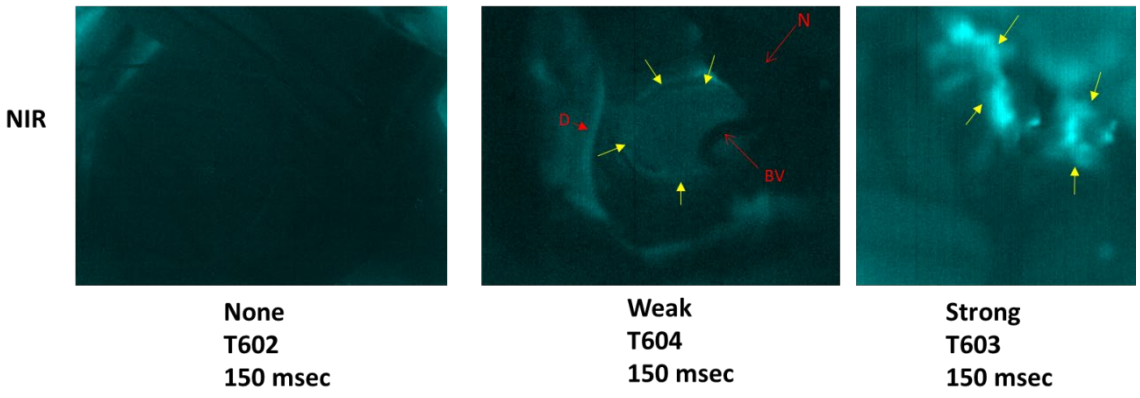
The following imaging summary includes subjects dosed with 13.9, 15, and 17.3 mg/m^2 and imaged with the SIRIS (n=18). Subject T613, the first case imaged with the Canvas system (15 mg/m^2 dose) is also presented.

1.4.3.2.2 Qualitative image assessment: BB-004

Surgeons assessed fluorescence qualitatively in real time, with tumor exposed and again after excision was complete. Their assessments were binary (fluorescence is present, yes or no) for tumor and normal tissue, and a qualitative assessment of the distinction between tumor and normal tissue using fluorescence (none/poor, moderate, or very good). The imaging operator recorded any other observations that might affect imaging; for example, cases in which the tumor was deep within the brain were noted.

Following each case, images acquired during the surgery were evaluated for fluorescence intensity. Example images of tumors in each of the qualitative categories (none, weak, or strong) are shown in Figure 3.

Figure 3 NIR images of exposed tumors representing qualitative categories of tozuleristide fluorescence relative to surrounding normal brain tissue



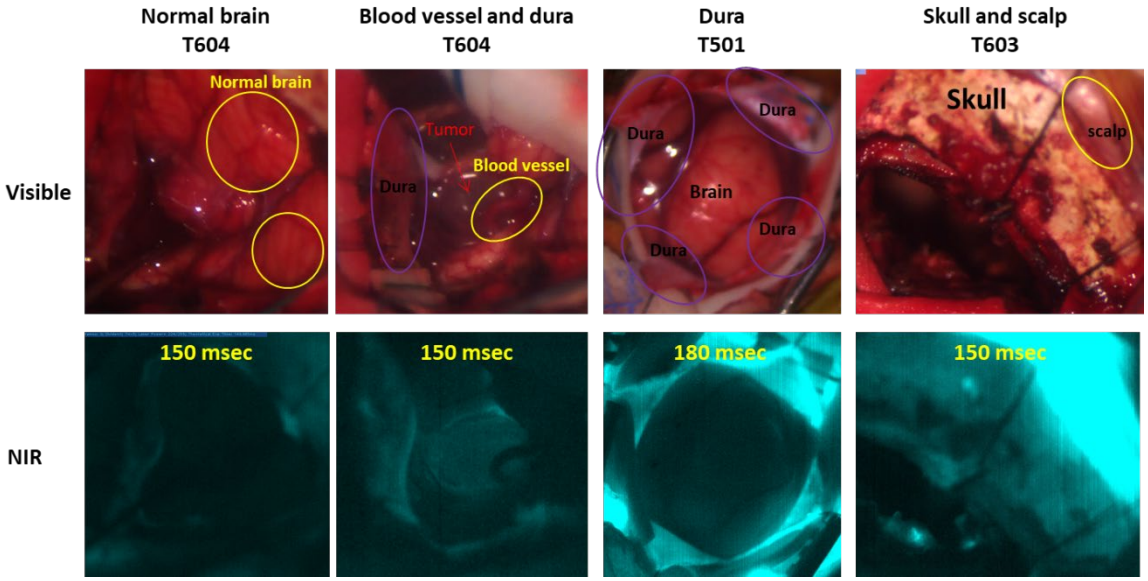
The SIRIS settings were the same for each image. T602 is an example of a negative case; the fluorescence at the edges of the image is coming from dura, and there is no signal in the central

area that includes the exposed tumor. T604 is an example of weak fluorescence. Tumor edges are indicated with yellow arrows. BV=blood vessel, D=dura, N=normal brain. T603 is an example of strong fluorescence. Tumor areas are saturated at this exposure (yellow arrows). NIR = near infrared

1.4.3.2.3 In Situ Imaging: BB-004

Intraoperative images captured during the BB-004 study included normal tissues and structures within the field of view. Normal brain parenchyma showed consistently low background fluorescence throughout the study. Some non-tumor tissues and structures (dura and skin, for example) fluoresce after tozuleristide administration, and some do not (skull).

Figure 4 In situ images showing the appearance of normal brain and other structures likely to be present in the surgical field



Structures of interest are labeled on the visible light images (top row). NIR = near infrared

The *in situ* images from BB-004, scored for fluorescence were used to assess positive and negative results on a subject level, and to determine the effect of tumor type, pre-surgery MRI contrast enhancement, new vs recurrent tumor, and time between dose and imaging on fluorescence. *Ex vivo* results (see Section 1.4.3.2.5 below) were used where supportive data were needed. The *in situ* imaging results and select subject details are summarized in Table 5. There was a total of 18 subjects enrolled in cohorts 4, 5, and 6 (near-optimal dose groups). Of these, 3 tumors were too deep to image *in situ* and are not shown in Table 5 (T503, T601, T610). Of the remaining 15 tumors, *in situ* images for 3 were scored fluorescence “none” (T510, T602, T606). All 3 were also negative for fluorescence *ex vivo* by SIRIS imaging (see *Ex Vivo* Imaging Section 1.4.3.2.5 below). These 3 tumors were scored as false negatives. The 3 deep cases were all positive for fluorescence *ex vivo* and were all tumor; these are true fluorescence positive tumors that were not adequately exposed to enable imaging *in situ* with the SIRIS. Taken together, on a subject level there were 15 true positives and 3 false negatives.

Table 5 BB-004 Summary of *in situ* imaging results. The table includes those cases for which SIRIS images of exposed tumor were acquired. Tumors too deep to image (N=3) are excluded (subjects T503, 601, and 610)

Subject ID	Tumor type	WHO Grade	Recurrent disease?	Pre-surgery MRI contrast enhanced?	Time from dose to surgery (hr)	Surgeon's assessments of fluorescence and distinction between tumor and normal tissue				Sponsor assessment of images		
						Tumor fluorescent (Y/N)	Distinction			Qualitative score	Tumor intensity[1]	Contrast[2]
							No/poor	Moderate	Very good			
T401	PXA	2	yes	yes	23.5	Y			X	strong	1077	5.1
T402	PXA	2	yes	yes	21	Y		X		strong	993	3.5
T403	PXA	2	yes	yes	24	Y			X	strong	1107	2.6
T603	MB	4	no	yes	18.5	Y		X		strong	697	2.3
T609	MB	4	no	yes	3.5	Y		X		strong	513	2.4
T607	Embryonal	4	no	hetero-geneous	7.5	Y		X		strong	1833	2.75
T611	Ependymoma	NA	yes	yes	3.5	Y		X		strong	700	1.2
T612	Myxopapillary ependymoma	NA	yes	yes	19	Y			X	strong	1134	1.5
T604	MB	4	no	yes	4	Y		X		weak	263	1.7
T605	MB	4	yes	no	18	Y		X		weak	318	1.5
T608	MB	4	no	hetero-geneous	20.5	Y		X		weak	422	1.7
T502	Pilocytic Astrocytoma	1	no	yes	17	Y		X		weak	96	1.3
T501	Ganglioglioma	1	no	yes	5.5	N	X			negative	1[3]	none
T602	Embryonal	4	no	yes	18	N	X			negative	1[3]	none
T606	Diffuse midline glioma	4	no	yes	18.5	N	X			negative	1[3]	none

MB = medulloblastoma; NA = not available; NIR = near infrared; PXA = pleomorphic xanthoastrocytoma

The table includes those cases for which SIRIS images of exposed tumor were acquired. Tumors too deep to image (N=3) are excluded (cases T503, T601 and T610).

[1]Mean pixel intensity per second of NIR exposure, as measured in tumor region of interest (ROI) using ImageJ software

[2]Tumor intensity divided by the intensity in an ROI from normal brain within the same image, computed in the same way

[3]A value of 1 was assigned to negative cases to facilitate graphing of the data

Pre-operative diagnostic criteria are of particular interest, since correlations might enable selection of cases that are more likely to be fluorescence positive and therefore benefit from tozuleristide imaging. Although this data set is small, it contains several different pediatric tumor types, including examples of low and high grade tumors, recurrent and newly diagnosed disease, and a few cases with heterogeneous or no contrast enhancement on MRI. For the newly diagnosed cases, there were examples of negative, weak, and strong fluorescence, whereas all of the recurrent cases were positive. Contrast enhancement on MRI appears to not be predictive of fluorescence, since tumors without contrast enhancement or heterogeneous cases were all positive for fluorescence, and the 3 fluorescence negative cases were all contrast-enhancing. The larger data set from the BB-006 pivotal study may identify pre-surgical diagnostic criteria that will enable exclusion of patients who are less likely to benefit from tozuleristide imaging.

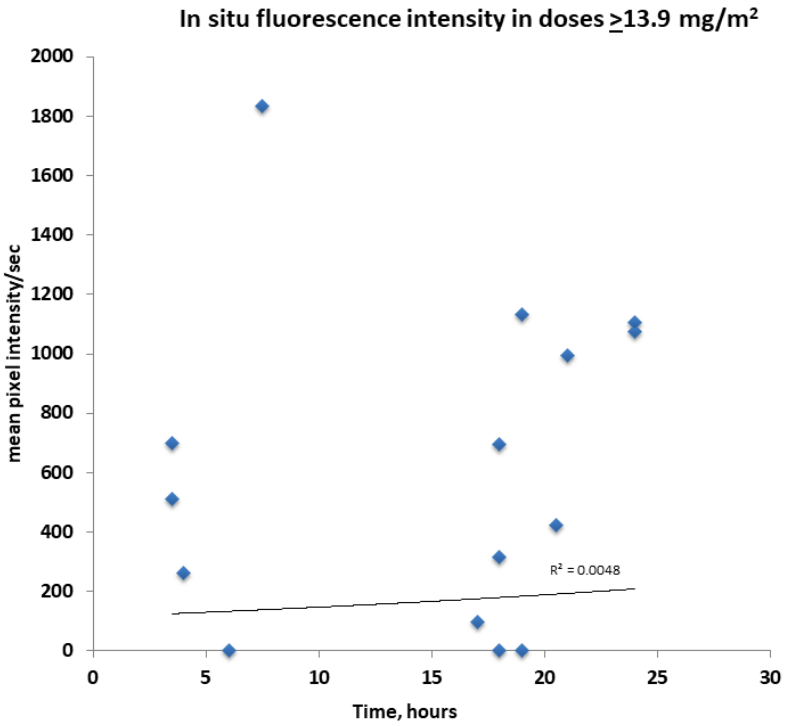
1.4.3.2.4 Semi-quantitative analysis: BB-004

In order to assess tumor fluorescence intensity and contrast between tumor and normal brain *in situ*, images were selected in which tumor fluorescence was sub-saturated and in which at least some normal brain tissue could be identified. The images were analyzed using ImageJ (version 1.49v, National Institutes of Health, USA) ROI tool. Although the analysis software quantitates fluorescence intensity in each pixel accurately, this analysis is only semi-quantitative because of variation in camera settings, distance from the field of view, levels of ambient lighting, and angle of imaging.

The numerical contrast between tumor and normal brain for each case that could be imaged *in situ* is shown above (Table 5). In general, cases with contrast >2 were also qualitatively scored as strong fluorescence, while those with contrast <2 were scored as weak fluorescence.

To assess the potential influence of tozuleristide administration time on fluorescence, the tumor fluorescence intensities from *in situ* images were plotted as a function of time between dose and surgery for fluorescence-positive cases from cohorts 4, 5, and 6 (Figure 5). The data show no influence of time on intensity within the range for these cohorts (3.5 – 24 hours). These data support flexible scheduling of dosing and surgery.

Figure 5 ***In situ*** **tumor fluorescence intensity plotted according to the time from tozuleristide dosing to the start of surgery for the high-dose cohorts: BB-004**

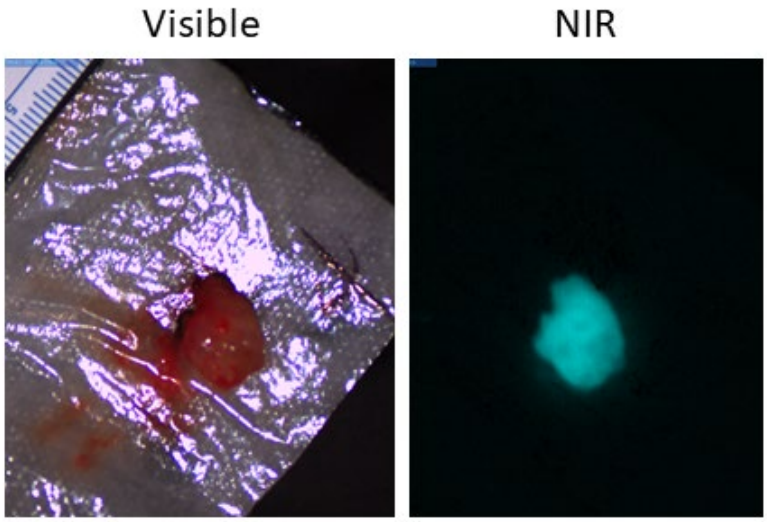


1.4.3.2.5 Ex vivo Imaging: BB-004

SIRIS Imaging

Following excision, tissue samples were imaged in the surgical suite using the SIRIS before they were sent to pathology (Figure 6). This enabled tissues to be classified as fluorescence-positive or -negative (supporting the *in situ* results and adding data for tumors that were too deep to image *in situ*). These data are used to estimate sensitivity at the sample level and are considered predictive of the results that will be obtained with biopsy specimen fluorescence and pathology in this BB-006 study.

Figure 6 Example of samples imaged *ex vivo* with the SIRIS: BB-004



The sample was imaged immediately after excision in the operating room (OR) using a 30 msec exposure setting.

Generally, each case had multiple surgical specimens/tissues with fluorescence assessment and histopathology results; therefore, sensitivity and specificity were calculated on a sample level. The samples used for this analysis are described in Table 6. Only samples with definitive images and histopathology are included. For this analysis, the presence of viable tumor >1% of the specimen resulted in a call of “tumor” for the sample. Fluorescence positivity was determined by whether the fluorescence was visible at an exposure of at least 150 msec.

Table 6 Summary of *ex vivo* imaging results for fluorescence-positive tumors from all dose groups: BB-004

ID	TP (FI+, tumor)	TN (FI-, not tumor)	FP (FI+, not tumor)	FN (FI-, tumor)
T401	2	1		
T402	4	2		
T403	3			
T502	1			1
T503	2			
T601	3			
T603	1			
T604	7			2
T605	1			
T607	4			
T608	2			2
T609	3			
T610	5			
T611	1			
T612	2			
Total	41	3	0	5

TP = true positive; TN = true negative. FP = false positive; FN = false negative; FI = fluorescent

On the sample level, sensitivity for fluorescence detection of tumor tissue is ~89% (41/46). Specificity was not calculated due to the small number of specimens for this calculation.

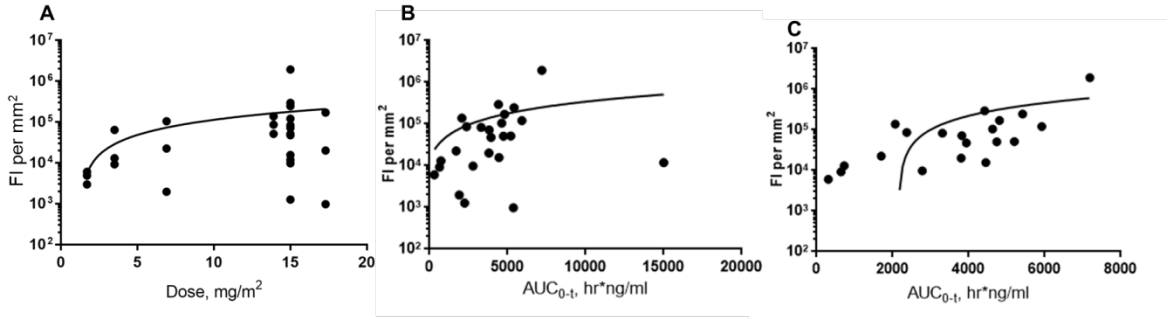
This analysis also indicates that some tumors may show heterogeneity in fluorescence intensity (for example, T502, T604, and T608 in this study had both fluorescence-positive and fluorescence-negative tumor samples). Some of the false negative samples did show fluorescence at longer exposure times, suggesting that using more sensitive camera settings may help surgeons identify regions of infiltration.

Odyssey Imaging: BB-004

The Odyssey NIR scanner (Li-Cor) was used to directly quantify fluorescence intensities in frozen sections derived from the excised tissues. The Odyssey is a flat-bed scanner with a light-tight cover, which controls for variables such as camera distance and ambient light. Since the tissues are sectioned, it also controls for tissue thickness. Hematoxylin and eosin (H&E) staining is performed on the same or serial sections, allowing correlation of areas of viable tumor with areas of fluorescence. However, it is susceptible to sampling artifacts like any microscopic specimen analysis.

Odyssey data were analyzed using ImageStudio software (Li-Cor) and recorded as fluorescence intensity (FI) per mm^2. To determine the impact of tozuleristide dose on viable tumor fluorescence, the area of highest fluorescent intensity was plotted against dose for each case (Figure 7A). Overall, tumor fluorescence was considered optimal between the 13.9 to 17.3 mg/m^2 doses, with the intermediate, expansion dose of 15 mg/m^2 demonstrating positive *in situ* tumor fluorescence in 11 of 15 evaluable cases.

Figure 7Tumor fluorescence intensity by tozuleristide dose (A) and exposure (B and C): BB-004



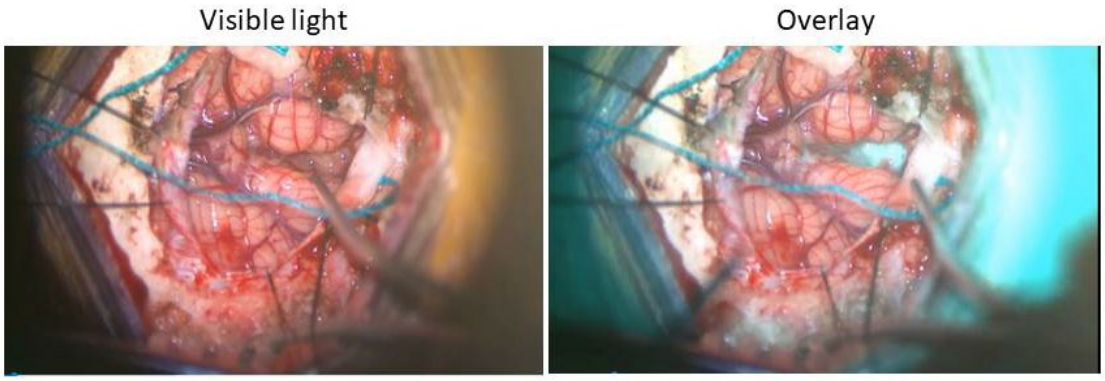
Fluorescence intensity (FI) is plotted on a log scale. Linear regression results are shown. All cases are included in panel A and B; fluorescence-negative tumors were excluded in panel C. $AUC_{0\text{-}t}$ was not evaluable in 3 cases; these were excluded from the graphs in panels B and C.

Odyssey intensity data were also analyzed to look for relationships between tumor fluorescence intensity and factors such as recurrent vs newly diagnosed disease, tumor grade, pre-surgery MRI contrast enhancement, and time between dosing and surgery. None of these factors appeared to influence tozuleristide fluorescence. Analysis of PK parameters demonstrated a relationship between tozuleristide exposure and tumor fluorescence intensity (Figure 7B, Figure 7C). Increasing exposure shows a positive trend with tumor fluorescence intensity. Subjects with low dose/low exposure had tumors with weak or no fluorescence. However, there were subjects with adequate or high exposure to tozuleristide who had no fluorescence in their tumors, demonstrating that the negative cases in the high dose cohorts are not explained by lack of exposure to drug. This suggests that tumor biology plays a strong role in whether a tumor is capable of tozuleristide uptake, however, in tumors that take up tozuleristide, fluorescence intensity increases with increased drug exposure, expressed either on a dose or AUC basis. The fluorescence-positive tumors in the high dose cohorts had fluorescence intensities that were sufficient for robust intraoperative detection.

1.4.3.2.6 Example of Intraoperative Imaging Results with the Canvas System: BB-004

Subject T613 received a 15 mg/m^2 dose of tozuleristide 13.5 hours prior to the start of surgery. The subject was diagnosed with an atypical teratoid rhabdoid tumor (ATRT) in the posterior fossa/brain stem. The Canvas system was attached to the Zeiss Pentero surgical microscope along with two eyepieces prior the start of surgery. After the tumor was exposed, the Canvas system was initialized and used continuously throughout the surgery with little to no interference with normal operating procedures. Tozuleristide fluorescence was observed in the exposed tumor (Figure 8).

Figure 8 Example of intraoperative imaging with the Canvas system



Subject T613, ATRT in the posterior fossa/brain stem imaged with the Canvas system

1.5 Rationale

1.5.1 Dose Rationale

Clinical data from Phase 1 studies support evaluation of doses ≥ 24 mg for adult primary CNS tumors administered between 1 and 24 hours before the initiation of surgery.

- As summarized above (Section 1.4.3.1), there was an apparent association between tumor grade and fluorescence in adult subjects with primary CNS tumors treated with tozuleristide (Study BB-002). In the 10 subjects treated with 18, 24, or 30 mg tozuleristide, 3 of 4 high grade tumors but only 1 of 6 low grade tumors exhibited positive fluorescence *in situ*, and the 3 subjects in the 30 mg cohort had low grade tumors. Therefore, it is not known if tozuleristide doses >24 mg in adult glioma patients with high grade tumors or a broader range of tumor types will lead to greater incidence and/or intensity of tumor fluorescence
- Tozuleristide imaging data (intensity and contrast) indicate enough drug remains in tumor tissue for fluorescence at least 30 hours post-administration
- Pharmacokinetic data indicate the half-life of tozuleristide is approximately 30 minutes, such that background fluorescence from circulating tozuleristide is expected to be minimal even for surgeries on the same day as dosing. In studies to date, fluorescence from blood has not been noted
- Clinical safety data in subjects receiving tozuleristide at doses up to 30 mg appear well tolerated.

Rationale for Dose Escalation in non-gadolinium enhancing primary brain tumors:

- In cohort 1 of this study, a fixed dose of 24 mg of tozuleristide was given to adult patients with high grade CNS tumors as a single intravenous injection between 1-24 hours before surgical

resection. This dose was considered appropriate for these subjects based on results from Study BB-002 (Section 1.4.3.1). However, given the observed association between tumor grade and fluorescence, it is possible that tozuleristide doses >30 mg may be needed to provide acceptable tumor fluorescence in subjects with low grade CNS tumors. While 3 subjects in Study BB-002 received 30 mg tozuleristide, an additional 28 subjects in Studies BB-004 and BB-006 with pediatric CNS tumors that were older and larger (ages 12 - 22 years; body weight 116 – 279 lbs) received tozuleristide doses ranging from 24.5 to 38 mg, with no Grade 2 or higher treatment-related AEs (Blaze Bioscience, unpublished data).

- To date in this study, 16 patients in Cohort 1 have received a 24 mg dose of tozuleristide. No drug-related SAEs have been noted. All patients that had pre-operative gadolinium enhancement of the tumor on T1 weighted MRI imaging have demonstrated highly reliable and accurate tumor labelling, and no obvious evidence of false negative labelling. However, 1 patient in which tumor did not demonstrate gadolinium enhancement on pre-operative imaging demonstrated no evidence of fluorescence at the time of surgery. The tumor was identified as high grade (WHO Grade III) glioma on histopathology. Based on the Phase 1 data, in which it was evident that approximately 20% of tumors failed to demonstrate any fluorescence, there was a concern that lack of uptake could be attributable to tumor grade, with grade II tumors (that tend to be non-enhancing) not having adequate receptor density on the cell surface to allow adequate tozuleristide uptake and subsequent imaging. This is despite the fact that immunohistochemical data has reliably demonstrated cell surface binding for all grades of glioneuronal tumors(21). However, this 1 case from the present IST, as well as unpublished data from a pediatric trial with WHO Grade I enhancing tumors demonstrating reliable uptake suggest that tumor grade may not be the critical factor, but rather the presence or absence of gadolinium enhancement. Gadolinium enhancement indicates at least a partial break down in blood brain barrier vascular permeability, allowing the gadolinium to become deposited in the extra-vascular spaces and detected on MRI imaging. This observation suggests that the 24 mg dose may be inadequate to visualize tumors that do not enhance in MRI imaging due to insufficient amounts of drug penetrating the blood brain barrier.

- To address this potential concern, we propose a modification to the current protocol for a subset of 10 subjects (Cohort 2) with tumors that do not demonstrate any appreciable gadolinium enhancement on pre-operative MRI imaging, regardless of known or suspected tumor grade. For these cases, a tozuleristide dose of 36 mg is anticipated to potentially provide greater levels of tumor fluorescence. The dose of 36 mg has safety margins of ~20 to 400-fold based on predicted serum tozuleristide exposures relative to exposures at the No-Observed-Adverse-Effect-Level (NOAEL) of 11 mg/kg in the GLP repeat-dose toxicology study of tozuleristide in monkeys (Table 8).

Table 8. Serum tozuleristide exposures in monkeys at the repeat-dose toxicology study NOAEL, estimated exposures in adults (36 mg), and safety margins

Exposure parameter	Monkey repeat-dose toxicology study [1]	Estimated in adults at 36 mg [3]	Safety Margin [4]
C_0 (ng/mL)	198,000	9,330	21
AUC (ng*hr/mL)	2,534,000 [2]	6,040	419

C_0 = extrapolated initial (highest) concentration after IV bolus dosing
AUC = area under the serum concentration-time curve
[1] Xenometrics Study 12078; NOAEL 11 mg/kg QDx7
[2] Estimated cumulative AUC for 7 doses
[3] Based on dose (3-30 mg) vs. exposure relationships in Studies BB-001 and BB-002
[4] Exposure at monkey repeat-dose NOAEL vs. estimated clinical exposure at 36 mg

Planned Trial Modification:

This modification is limited to 10 patients with pre-operative MRI imaging demonstrating no appreciable gadolinium enhancement on T1 weighted MRI images. 10 patients will be given a dose of 36 mg of tozuleristide prior to surgery. These patients will otherwise undergo an identical treatment paradigm, as currently outlined in the IST. If 3 or more ≥ grade 3 AE are note that are deemed probably related or related to the study treatment (tozuleristide and/or Canvas) are observed after enrolling 5 patients, further enrollment in Cohort 2 will stop. If cohort 2 is stopped, then the protocol may be revised to enroll remaining patients at lower doses as described below.

- If any drug related SAEs are noted at 36 mg the dose will be reduced to 30 mg. This dose scheme should allow us to better determine if tozuleristide dose impacts tumor fluorescence for non-enhancing glioneuronal tumors regardless of grade. Further it will inform additional and larger Phase III studies.

1.5.2 **Device Rationale**

The NIR fluorescence signal from tozuleristide is not visible to the human eye. As such, an imaging device is needed to capture and display fluorescence. Additionally, while currently marketed surgical microscopes, such as the Zeiss Pentero, have features capable of detecting ICG, they are not sensitive enough to detect fluorescence from tozuleristide. In the Phase 1 pediatric CNS cancer study (BB-004) and the phase 1 adult study (BB-002), intraoperative tumor imaging for tozuleristide was performed with the Fluobeam 800® or SIRIS prior to start of tumor excision and after completion of excision. Additional imaging was obtained at other time points at the discretion of the neurosurgeon. All clinical decision-making was made based on the routine neurosurgical practices with tissue resection taking place under normal light conditions in line with best clinical practices.

The Fluobeam and SIRIS devices were hand-held or mounted to a stand and required the surgical microscope to be moved to access the surgical site. Additionally, ambient light needed to be reduced or blocked for high quality images to be obtained. The Canvas, an investigational device based on the SIRIS, will be used for intraoperative imaging in this study to allow the surgeon the ability to use a fluorescence detection camera that is mounted to the optical head of the surgical microscope. No alterations in ambient light conditions are needed for the Canvas system to capture NIR images. The Canvas was employed in the Pacific Pediatric Neuro-oncology Consortium (PNOC) sponsored BB-006 trial in over 9 sites in the US. It has been incorporated into the IND filed by Blaze for that trial and has been used successfully in over one hundred cases to date. There have been no safety issues and surgeons have been generally pleased with the ease of use and imaging. The trial has completed enrollment and study close-out is ongoing.

The Canvas imaging system is an intraoperative viewing system of tissue fluorescence and is designed to detect the NIR fluorescence of tozuleristide in the intraoperative setting of neurosurgery. The Canvas is comprised of an Imaging Head (which mounts on a Zeiss Pentero 800 or 900 surgical microscope), an Imaging Station (which contains hardware and software for image acquisition and display), and a Clinical Display (which displays in real-time the NIR signal overlaid on the visible light image).

The Imaging Head mounts underneath the optical head of the surgical microscope, integrating with the optics for an interference-free field of view that is coaxial to the microscope. This design does not alter the standard operation of the microscope; however, the surgeon or imaging operator must view the Canvas images on the Clinical Display – they are not presented in the oculars of the surgical microscope.

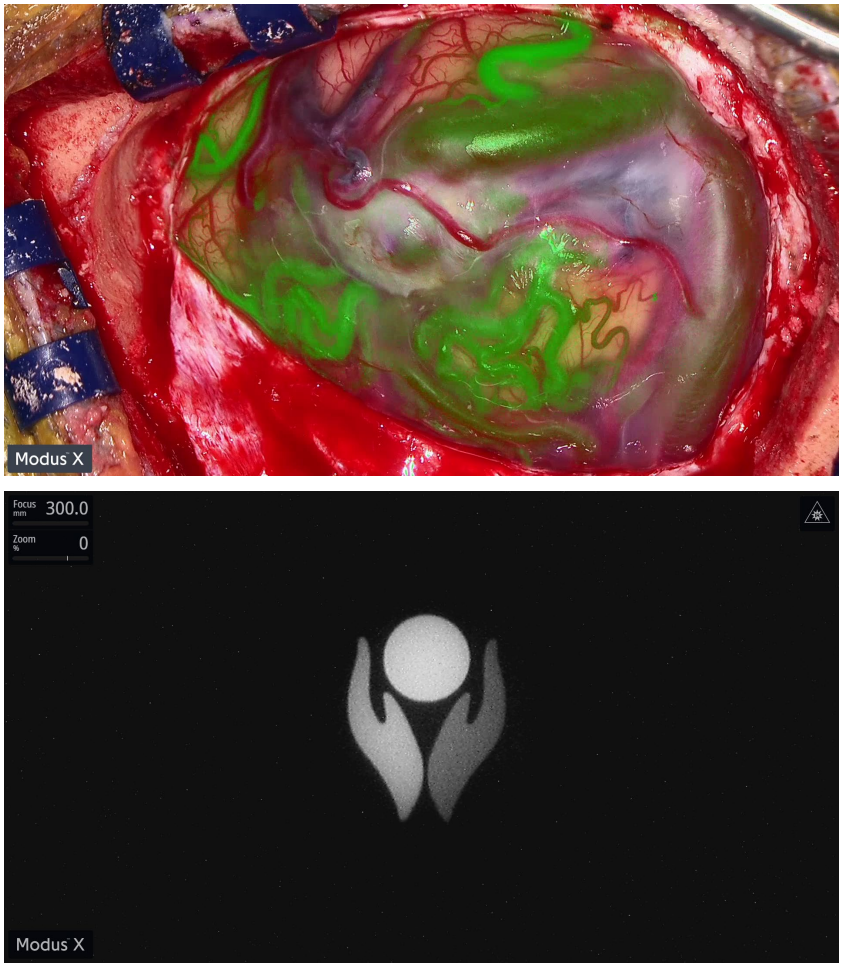
Modus X is an intraoperative viewing system with Modus IR optionally included as an accessory designed to detect the NIR fluorescence of ICG. Modus IR is FDA cleared (K231986) as of March 25, 2024. Modus IR provides surgical staff with a means to visualize vessels and blood flow

during surgical procedures that may not be visible under white light conditions. When used with the appropriate imaging agent, light output at a specific wavelength excites the imaging agent, which emits light at a specific wavelength that is detected by the optical system, thereby allowing the user to differentiate the structure that the imaging agent has concentrated in.

The indications for use are that Modus IR used with the Synaptive Modus X Surgical Exoscope is indicated for fluorescence imaging in conjunction with ICG to aid in the visualization of vessels (micro- and macrovasculature) and blood flow in the cerebrovasculature before, during, and after neurosurgery, plastic, and reconstructive surgeries (Figure 9). The excitation/detection wavelengths are 748 - 802 nm / 820 - 1000 nm, respectively.

The fluorophore component of tozuleristide is a conjugatable form of ICG. Following excitation, it has an emission wavelength higher than 800 nm which is within the range for detection by Modus IR. Bench testing has shown that tozuleristide is detectable with Modus IR with FDA cleared hardware and software and recommended settings (figure 9B). For this study, additional settings contained within the FDA cleared version of Modus IR may be used at the discretion of the neurosurgeon. In addition, settings can be further explored on excised tissue to further optimize the fluorescence imaging.

Figure 9. Example of imaging with Modus IR (A) of ICG intra-op (top), and (B) of tozuleristide during bench testing (bottom), demonstrating both Fusion and Monochrome modes of Modus IR, respectively.



1.5.3 **Study Rationale**

There is an urgent unmet need for a tumor-targeted imaging agent to enable real-time intraoperative distinction between malignant and normal tissue to improve neurosurgical outcomes, either as increased chance of a complete total resection or decreased risk of inadvertent normal brain resection or both.

This is a novel and rapidly emerging technology that may help surgeons visualize the extent of tumor or residual disease more precisely during surgery. If the study successfully meets the proposed sensitivity and specificity thresholds, and provides preliminary data on which tumors demonstrate detectable fluorescence, the results would provide the basis for development of a larger trial with an endpoint of improving the extent of resection.

Rationale for Modification to add 5 patients with vestibular schwannoma:
Despite decades of experience, the use of intraoperative neuromonitoring, and a variety of surgical approaches, safe removal of acoustic schwannomas remains one of the most challenging of all brain tumor surgeries. Acoustic schwannomas are benign tumors that arise from the Schwann cells lining the 8th (vestibulo-cochlear) cranial nerve, most commonly from the superior vestibular component of the nerve. These slow growing tumors typically follow the course of the 8th nerve along the brain stem and into the porous acousticus, a bony canal in the skull base that leads to the cochlea. The seventh (facial) nerve runs in parallel with the 8th nerve into the porous acousticus, and is typically stretched, thinned, and compressed by these tumors. The most common morbidity from acoustic neuroma surgery is injury to the facial nerve. Even in expert and highly experienced hands the 7th nerve can be extremely difficult to identify, and can run in a variable course round, under or even through the tumor mass at times. The 7th nerve controls movement of the face muscles on the ipsilateral side, including eyelid closure, facial animation, and muscles for chewing. Thus, the loss of facial function is both functionally limiting and aesthetically disfiguring. Avoiding facial nerve injury is therefore a primary goal of acoustic neuroma surgery, that has proven to be remarkably illusive. Current methods to identify the facial nerve involve electrical stimulation of the nerve with a probe during tumor dissection. This technique is user and experience dependent, and not entirely reliable. For tumors greater than 2.5 – 3 cm in diameter, injury to the facial nerve occurs in upwards of 40% of cases and is often permanent (22). Therefore, any method to identify the course of the facial nerve early and accurately in surgery and during dissection is likely to have an important impact on functional outcomes. Tozuleristide imaging may offer a novel approach to identifying the nerve.

The current IST is limited to primary glioneuronal tumors. Benign lesions such as meningioma, adenoma, or schwannoma are excluded. However, immunohistochemical data suggest that both meningioma and schwannomas take up chlorotoxin (21). Further, these tumors tend to avidly enhance, suggesting that tozuleristide is likely to have very good uptake. Considering the impressive intraoperative results that have been so far observed, we propose a modification to the IST to allow dosing of up to 5 patients with enhancing acoustic schwannomas undergoing surgery. Trial protocols will be similar (primary tumor and 4 other biopsies will be taken) but no negative fluorescence biopsies will be taken to avoid injury to brainstem. Surgeons and an imaging operator will observe tozuleristide fluorescence during surgery to determine if the facial nerve can be differentiated due to lack of uptake relative to the tumor (i.e. fluorescence negative). Standard electrical stimulation methods will be used to definitively identify the facial nerve, and this will be compared to fluorescence findings with the surgeon providing a subjective estimate of degree of fluorescence of the tumor and the nerve (strong, weak, or none) based on intraoperative observations. Tozuleristide will not be used to guide extent of resection. At completion of the case, the surgeon will complete a Likert scale-based rating of how useful the fluorescence was for aiding in surgical resection (Appendix Section 11.4).

A modification of the IST to include 5 cases of acoustic schwannoma will not achieve statistically meaningful data on the utility of this method for facial nerve identification. Nonetheless it may provide critical preliminary qualitative data to support a larger scale trial assessing the utility of this method to differentiate cranial nerves from tumor in a variety of skull base lesions.

2.0 STUDY OBJECTIVES

2.1 Primary Objectives

2.1.1 To evaluate preliminary efficacy of 24 mg tozuleristide administered intravenously and imaged with the Canvas imaging system during surgical resection to demonstrate fluorescence among a homogeneous population of primary enhancing/high grade CNS tumors.

2.1.2 Among tumors that demonstrate tozuleristide fluorescence, to preliminarily estimate the true positive rate and true negative rate of fluorescence in tissue biopsies from peripheral regions of primary CNS tumors, as well as sensitivity and specificity of tozuleristide fluorescence for distinguishing tumor from non-tumoral tissue.

2.2 Secondary Objectives

2.2.1 To evaluate the safety of tozuleristide and Canvas imaging system to provide fluorescence guidance for removal of primary brain tumor.

2.2.2 To preliminarily determine if the presence of residual fluorescence at the time of surgery corresponds to residual tumor evident on post-operative MRI images, or if the absence of fluorescence corresponds to evidence of no gross residual tumor on post-operative MRI.

2.3 Exploratory Objectives

2.3.1 To explore the presence of fluorescence with administration of 36 mg of tozuleristide among a cohort of 11 patients with non-gadolinium enhancing primary brain tumors.

2.3.2 To explore the presence of fluorescence with administration of 24 mg of tozuleristide among a cohort of 5 patients with vestibular schwannomas and to determine whether fluorescence aids in distinguishing tumor from the cranial nerve to which it is adherent.

2.3.3 To explore the ability of the Modus IR device to detect tozuleristide fluorescence in situ and ex vivo in subjects undergoing resection of primary gadolinium-enhancing CNS tumors or in subjects undergoing resection of vestibular schwannoma.

2.3.4 To explore the extent of resection in subjects receiving tozuleristide and undergoing maximal safe resection as compared to historical controls.

2.3.5 To explore the size and intensity of the fluorescence in the images captured by the microscope and exoscope in subjects receiving tozuleristide.

2.4 Endpoints

2.4.1 Primary Efficacy Endpoints

Fluorescence positivity is defined as “weak” or “strong” by the independent imaging operator (see Appendix 11.3) based on displayed fluorescence signal and exposure setting.

Any specimens that have pathology confirmed viable tumor (>10% tumor cellularity) by the pathology assessment will be considered tumor positive. The co-primary efficacy endpoints are:

1. Percentage of patients with fluorescence-positive primary tumor biopsy
2. True positive rate (TPR) of fluorescence, defined as the percentage of tumor-positive and fluorescence-positive tissue biopsies among all tumor-positive tissue biopsies. This endpoint will be met if the TPR is 79% or higher.
3. True negative rate (TNR) of fluorescence, defined as the percentage of tumor-negative and fluorescence-negative tissue biopsies among all tumor-negative tissue biopsies. This endpoint will be met if the TNR is 84% or higher.

2.4.2 Secondary Efficacy Endpoints

1. Positive predictive value (PPV) of fluorescence, defined as the percentage of tumor-positive and fluorescence-positive tissue biopsies among all fluorescence-positive tissue biopsies.
2. Negative predictive value (NPV) of fluorescence, defined as the percentage of tumor-negative and fluorescence-negative tissue biopsies among all fluorescence-negative tissue biopsies.
3. Extent of residual tumor measured on post-operative MRI scans among all patients with evidence of residual fluorescence at the time of surgery.

2.4.3 Exploratory Endpoints

1. Percentage of patients with tumors that are fluorescence positive with dose 36 mg of tozuleristide among non-gadolinium enhancing primary brain tumors.
2. Percentage of patients with vestibular schwannomas that are fluorescence positive and of these, the percentage of patients where the positive fluorescence aided in distinguishing tumor from the cranial nerve.
3. Percentage of primary tumors visualized in situ with the Canvas that are fluorescence positive that are also fluorescence positive when visualized with Modus IR.
4. Correlation of ex vivo fluorescence of tissue specimens visualized with the Modus IR and percent tumor cells by histopathology.
5. Comparison of extent of resection of subjects receiving tozuleristide to historical controls of CSMC patients with similar resections for relevant subgroups to minimize bias. We will compare the historical controls’ pre and post operative MRI images to calculate tumor dimensions and volume, as well as length of hospital stay, neurological complications as detailed in the patient's chart, additional therapies before or after the surgery of interest,

mortality rate, readmission status, infection rate, demographic information (age, sex), Karnofsky Performance Status, and pathology report for tumor grade and subtype. The historical control group will be identified through data pull request from the Cedars Sinai electronic medical record database to identify patients who had undergone surgery for high and low grade gliomas from 2012-2023.

6. Size and intensity of the fluorescence in the images will be measured and compared by the open-source ImageJ image analysis software.

2.4.4 Safety Evaluations

Incidence of adverse events (AEs) observed up to 30 days after surgery, or until receiving additional therapy for their CNS tumor (whichever comes first), recorded and graded according to the National Cancer Institute's Common Terminology for Adverse Events (NCI CTCAE, Version 5).

3.0 STUDY DESIGN

3.1 Study Design

This is an open label single-center Phase 2 study of a single dose of tozuleristide in subjects with primary CNS tumors; intraoperative imaging will be performed with the Canvas NIR imaging system. In a subset of subjects the Modus IR will also be used for intra-operative in situ imaging and ex vivo imaging of excised specimens (Cohorts 1 and 3).

Futility analysis and stopping rules are described in Sections 8.4-8.5.

There will be 3 cohorts that will receive tozuleristide administered as a single IV bolus dose at least 1 hour and no more than 24 hours before start time of surgery:

- Cohort 1: Primary gadolinium enhancing (high grade) CNS tumors: 24 mg
- Cohort 2: Primary non-gadolinium enhancing CNS tumors: 36 mg
- Cohort 3: Primary vestibular schwannoma: 24 mg
- Note: Cohort 1 and Cohort 3 subjects that consent and enroll under Protocol v5 may undergo imaging with Modus IR in addition to Canvas. To minimize interruptions to the workflow, the timing of Modus IR imaging is at the discretion of the neurosurgeon.
- Note: Canvas will be used for fluorescence assessment of regions of interest/ tissue specimens. Modus IR fluorescence assessments will contribute to exploratory endpoints (3 and 4) only.

During the surgical procedure, and once the tumor has been accessed, the Canvas, 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. Regions of interest for biopsy collection will be identified before fluorescence images are collected. The independent Canvas operator will ensure white light and fluorescent images are captured and will note fluorescent status (none, weak, or strong). The surgeon will be informed if the tumor demonstrates fluorescence and if so, to what extent (strong, weak). However, clinical decision-making regarding tissue resection should be made based on the routine neurosurgical practices.

A primary endpoint for analysis is the comparison of tissue biopsies demonstrating *in situ* tozuleristide fluorescence to histopathological slides of tumor biopsies, to determine the ability of fluorescence to distinguish tumor from normal brain. All biopsy specimens will be assessed by the imaging operator *in situ* at the time of biopsy for fluorescence status (strong, weak, or none) and these data will be referenced to pathologist determination of tumor status (tumor or non-

tumoral tissue). This will also determine which subtypes of tumor are likely or unlikely to demonstrate tozuleristide induced fluorescence. Pathologists will semi-quantitatively assess the extent of tumor present in each biopsy specimens, estimating extent of tumor as <10 10-25%, 25-50% and >50%. Analysis of pathology specimens that failed to demonstrate fluorescence in situ, compared to those that did will preliminarily determine what amount of tumor volume is required to generate a fluorescence signal across a variety of tumor types.

Tozuleristide and the Canvas device are intended as an aid to surgical decision-making. As such, when a region of interest is noted to be fluorescent, it is always the surgeon's decision whether to biopsy/resect or not. During the resection the surgeon will take a series of biopsies of tissue for each case the surgeon will biopsy 1 region of definite tumor, 4 regions of tissue near the periphery or margin of the tumor, and 1 biopsy of tissue that is known fluorescent negative (except for schwannoma cohort) as an internal control. At completion of the resection, the tumor cavity will be assessed for any residual fluorescence. The surgeon will be informed and may view the fluorescence images. He/she may extend the resection if it is deemed safe based on standard neurosurgical decision making under white light conditions. If there are any regions of interest with fluorescence identified by the surgeon *after* completion of resection, up to 5 additional biopsies of "other tissue" may be collected when feasible. Residual fluorescent areas not resected due to concern for patient safety will be indicated by the surgeon and noted by the imaging operator. After surgery post-operative MRIs with be obtained, and the site radiologists will determine the radiographic extent of tumor resection (gross total, near gross total, or sub-total). Extent of resection will be compared to in situ determination of the presence or absence of residual fluorescence.

Measures to minimize or avoid bias include:

- Fluorescence will be displayed on a separate monitor (not visualized through the microscope that is used for biopsy/tissue collection) and therefore fluorescence may highlight a region of interest but will not directly guide biopsy/tissue resection. However, the surgeon will be able to look at the monitor during the procedure.
- Standard of care/white light and fluorescence images of regions of interest for biopsy will be assessed by an independent imaging operator. The operating surgeon will assign presumed status of tumor (tumor/not tumor) and will point at that area using an instrument under the microscope. The imaging operator will capture Canvas images of those regions of interest and assess fluorescence (positive/negative) by looking at the Canvas monitor. The determination of fluorescence status will be made by the imaging operator alone, although the surgeon may look at the imaging system monitor intermittently during the case to assist in determining regions to biopsy based on surgeon clinical judgement.

3.2 Sample Size and Accrual Duration

The anticipated accrual rate will be 55 subjects over 36 months. Enrollment will include 39 subjects with gadolinium enhancing high-grade tumors for the primary analysis, and 11 subjects with non-gadolinium enhancing glioneuronal tumors and 5 subjects with Schwannoma tumors for the exploratory aims.

For patients with enhancing/high grade tumors, the analysis will be conducted in two stages. In the first stage, 21 high grade tumor subjects will be accrued. *Subject 22 cannot be enrolled until futility analysis has been completed among the first 21 subjects*. In the second stage, 18 subjects will be accrued.

There will be enrollment pauses at two timepoints throughout the study for safety analysis: after 10 and 21 subjects with high-grade tumors are enrolled. There will also be enrollment pauses for the non-enhancing and schwannoma cohorts throughout the study for safety analysis: after 5 subjects are enrolled in Cohort 2 and 2 subjects are enrolled in Cohort 3. Please see section 8.5 for more details on safety stopping rules.

4.0 PATIENT ELIGIBILITY

4.1 Inclusion Criteria

4.1.1 At least 18 years of age at the time of study enrollment

4.1.2 MRI obtained within 30 days of study enrollment documents a measurable lesion consistent with a primary malignant CNS tumor for which maximal safe resection is indicated, as determined by the treating physician or neurosurgeon.

OR

Cohort 3 only: MRI obtained within 1 year of study enrollment documents a measurable lesion consistent with a primary schwannoma enhancing tumor in the cerebellopontine angle for which maximal safe resection is indicated, as determined by the treating physician or neurosurgeon.

4.1.3 Adequate renal function defined as serum creatinine within normal limits for age. Subjects will be eligible if they have a calculated creatinine clearance of ≥ 60 mL/min/1.73 m^2 based on the CKD-EPI equation

4.1.4 Adequate liver function defined as bilirubin <2 x upper limit of normal, alanine aminotransferase (ALT [SGPT]) <5 x upper limit of normal, and aspartate aminotransferase (AST [SGOT]) <5 x upper limit of normal.

4.1.5 Prior therapy: Subjects with prior therapy are eligible provided they have recovered from any acute toxic effects of prior therapy and have sufficient time interval prior to enrollment.

a. Radiation therapy: Subjects must not have had radiation therapy to the area of tumor planned to be resected within 28 days of study enrollment

b. Chemotherapy: Subjects must not have had any myelosuppressive chemotherapy within 14 days of study enrollment (28 days if prior nitrosourea)

c. Biologic: Subject must not have had any anti-neoplastic biologic agent within 7 days of study enrollment

d. Immunotherapy: Subject must not have had any cellular immunotherapy, such as CAR-T cell therapy within 42 days of study enrollment

4.1.6 Prior surgery for CNS tumors is allowed.

4.1.7 The risks of treatment with tozuleristide during pregnancy have not been evaluated. Women of child-bearing potential must agree not to attempt to become pregnant or undergo in vitro fertilization and, if participating in sexual activity that could lead to pregnancy, must use 2 reliable methods of contraception simultaneously for 30 days after surgery. Male subjects must agree to use 2 reliable methods of contraception simultaneously for 30 days after surgery if their partner is of child-bearing potential.

4.1.8 Written informed consent obtained from subject and/or legally authorized representative and ability for subject to comply with the requirements of the study.

4.2 Exclusion Criteria

4.2.1 Subjects who are pregnant or breast-feeding or planning to conceive a child within 30 days

4.2.2 Subjects with on-going serious medical conditions (e.g., poorly controlled asthma, diabetes, heart disease) such that participation in the study could put the subject at increased risk of worsening their condition

4.2.3 Subjects planned to undergo only a diagnostic biopsy procedure, without intent to resect tissue for therapeutic purposes (e.g., stereotactic pontine biopsy)

4.2.4 Subjects who have previously received tozuleristide within 14 days of enrollment

4.2.5 Subjects for whom radiographic evidence suggest a non-intra-axial primary brain tumor (e.g., WHO Grade I-II meningioma, pituitary adenoma, osteoma, colloid cyst, craniopharyngioma) or a non-tumoral lesions (e.g., cortical migration defect, hamartoma, infection, abscess).

5.0 TREATMENT PLAN

5.1 Treatment Dosage and Administration

Tozuleristide will be administered as a single IV bolus dose of 24 or 36 mg at least 1 hour and no more than 24 hours before start time of surgery. Dosing will be based on cohort listed below:

- **Cohort 1 (n=39): Primary gadolinium enhancing (high-grade) CNS tumors:** 24 mg
- **Cohort 2 (n=11): Primary non-gadolinium enhancing CNS tumors**: 36 mg
- **Cohort 3 (n=5): Primary vestibular schwannoma tumors**: 24 mg

During the surgical procedure, and once the tumor has been accessed, the Canvas, 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. Regions of interest for biopsy collection will be identified before fluorescence images are collected. The independent Canvas operator will ensure white light and fluorescent images are captured and will note fluorescent status (none, weak, or strong). The surgeon will be informed if the tumor demonstrates fluorescence and if so, to what extent (strong, weak). However, clinical decision-making regarding tissue resection should be made based on the routine neurosurgical practices. After completion of resection, if there are any regions of interest with fluorescence identified by the surgeon, up to 5 additional biopsies of “other tissue” may be collected when feasible. The independent Canvas operator will ensure white light and fluorescent images of regions of interest for other tissue biopsies are captured and will note fluorescent status (none, weak or strong).

In a subset of patients (Cohorts 1 and 3) that consent and enroll under Protocol v5, the Modus IR may be used to capture intraoperative in situ images of the primary tumor and/or the tumor bed, and ex vivo images of excised tissue specimens. To minimize interruptions to the workflow, the timing of Modus IR imaging is at the discretion of the neurosurgeon.

5.2 Formulation

Tozuleristide is the stable conjugation product of the peptide KNT-03 with the NIR fluorescent dye ICG. KNT-03 is a synthetic, 36 amino acid long, folded peptide with a molecular weight of

approximately 4 kDa. The lysine residue at position 27 is conjugated with the ICG dye. The tozuleristide molecule contains four disulfide bonds, forming a compact three-dimensional structure.

The drug product is provided as a sterile, lyophilized powder in a single use clear glass vial containing 6 mg tozuleristide for injection. After dissolution with 1.4 mL water for injection the formulation contains 5 mg/mL tozuleristide, 10 mM L-Histidine and 5% D-Mannitol, pH 6.8. All excipients are of USP/multi-compendial grade or equivalent. Study product will be shipped and stored at controlled room temperature (15-25°C). Tozuleristide will be administered by an IV bolus injection at least 1 hour (and no more than 24 hours) before the subject's planned surgical tumor excision.

NOTE: Tozuleristide is incompatible with saline.

Tubing used for tozuleristide administration should be DEHP-free PVC tubing. Only 5% Dextrose Injection, USP should be used to prime or flush tubing that will be used with tozuleristide.

Tozuleristide will be given at an IV bolus dose of 24 mg over approximately 1-5 minutes.

5.3 Method of Assigning Subjects to Treatment Groups

Following eligibility verification, subjects will be assigned to a cohort/treatment group based on their tumor type:

- Cohort 1 (n=39): Primary gadolinium enhancing (high grade) CNS tumors: 24 mg
- Cohort 2 (n=11): Primary non-gadolinium enhancing CNS tumors: 36 mg
- Cohort 3 (n=5): Primary vestibular schwannoma tumors: 24 mg

5.4 Toxicities and Dosing Delays/Dose Modifications

Dosing will be stopped if suspected adverse drug reactions, changes in vital signs, or clinical laboratory results are observed, and these changes pose a significant health risk.

No dosing modifications are allowed.

5.5 Warnings and Precautions

The two components of tozuleristide are a form of the CTX peptide and the NIR dye, ICG. Both of these components have been safely used in humans, with the ICG component having been FDA approved and in clinical use since 1959. A radiolabeled form of the CTX peptide has been studied in clinical trials and showed tumor-specific targeting of the peptide and good patient tolerance at the doses used.

For further information regarding warnings and precautions with the study product, please refer to the Investigator's Brochure for tozuleristide.

5.6 Risks for Women of Childbearing Potential or During Pregnancy

The risks of treatment with tozuleristide during pregnancy have not been evaluated. Women of child-bearing potential must agree not to attempt to become pregnant or undergo *in vitro* fertilization and, if participating in sexual activity that could lead to pregnancy, must use two reliable methods of contraception simultaneously for 30 days after receiving the study product. Male subjects must agree to use two reliable methods of contraception simultaneously for 30 days after receiving the study product if their partner is of child-bearing potential.

5.7 Concomitant Medications/Treatments

Radiation therapy: Subjects must not have had radiation therapy to the area of tumor planned to be resected within 28 days of study enrollment

Chemotherapy: Subjects must not have had any myelosuppressive chemotherapy within 14 days of study enrollment (28 days if prior nitrosourea)

1. If subject has had prior chemotherapy within the past year, subject must have an absolute neutrophil count recovery of ≥ 1000/mm3 following count nadir

Biologic: Subject must not have had any anti-neoplastic biologic agent within 7 days of study enrollment

Immunotherapy: Subject must not have had any cellular immunotherapy, such as CAR-T cell therapy within 42 days of study enrollment

All treatments that the investigator considers necessary for a subject's welfare may be administered at the discretion of the investigator in keeping with the community standards of medical care. All concomitant medications will be recorded on the case report form (CRF) including all prescription, over-the-counter (OTC), herbal supplements, and IV medications. Drug dosage, frequency, route, and date will be included on the CRF.

No other anti-cancer therapy (chemotherapy, hormonal therapy, radiation therapy, surgery, immunotherapy, biologic therapy, or tumor embolization) or investigational agent may be used while on-study until the end-of-study visit.

5.8 Estimated Duration of Participation

The overall duration of the study will be approximately 38 months from the time the study opens for accrual until completion. The on-study period per subject will be approximately 2 months from time of obtaining informed consent, including up to 30 days for the screening evaluations, and 1-month post-surgery for safety monitoring.

5.9 Removal of Patients from Protocol

Patients will be removed from the study when any of the criteria listed in Section 6.4 apply. Notify the Principal Investigator and document the reason for study removal and the date the patient was removed on the Case Report Form. The patient should be followed-up per protocol.

5.10 Subject Replacement

Subjects who withdraw from the study treatment prior to study agent administration and/or who do not undergo surgical excision will be replaced.

Subjects that are found to not have primary malignant CNS tumor after surgery, will not be replaced. They will be considered evaluable for the primary and secondary endpoints.

Subjects that fall under Cohort 2 and were previously dosed prior to protocol version 2 approval, will not be replaced for the exploratory endpoint.

5.11 Evaluable for toxicity

Any patient who receives at least one dose of investigational product is evaluable for toxicity and will be included in the safety analysis.

6.0 STUDY PROCEDURES

6.1 Screening/Baseline Procedures

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

All screening procedures must be performed within 30 days prior to registration unless otherwise stated. The screening procedures include:

6.1.1 Informed Consent

6.1.2 Review subject eligibility criteria

6.1.3 Demographics

Age (including date of birth), sex, race, ethnicity

6.1.4 Medical history and record review

Relevant medical history, including history of current disease and information regarding underlying diseases will be recorded at Screening.

6.1.5 MRI of tumor

MRI with and without contrast prior to treatment should be obtained within 30 days of enrollment, or within 1 year of enrollment for Cohort 3 only. MRI sequences should include T1 pre-contrast, T1 post-contrast, T2, FLAIR, and Diffusion weighted images. Images will be evaluated by site radiology for patient management and local assessment of EOR.

6.1.6 Physical examination

A complete physical examination will include the following clinical assessments: head, ears, nose and throat, respiratory, cardiovascular, gastrointestinal, genito/urinary (optional), neurological, hematological, endocrine and metabolic, musculoskeletal, neurological, dermatological and lymph nodes.

A modified examination will be performed to document physical findings of systems appropriate to assessing the clinical status of the subject relative to study product and their disease.

6.1.7 Vital Signs

- Body temperature (degrees Celsius [°C])
- Respiratory rate (breaths/min)
- Radial pulse rate (beats/min),
- Blood pressure (mm mercury [mmHg])

6.1.8 Weight and height measurement

Body weight (kilograms [kg], without shoes) and height (centimeters [cm], without shoes) will be measured and documented at the Screening visit only.

6.1.9 Karnofsky Performance Status assessment

6.1.10 Adverse event assessment

Baseline adverse events will be assessed. See section 7.0 for Adverse Event monitoring and reporting.

Duration (start and stop dates and times), severity/grade, outcome, treatment and relation to study drug will be recorded on the case report form (CRF).

6.1.11 Concomitant medications review

6.1.12 Laboratory Evaluations

Hematology (complete blood count with differential), chemistry (comprehensive metabolic panel), and coagulation parameters (PT, INR, aPTT)

6.1.13 Urine or serum pregnancy test (for females of childbearing potential)

All women of childbearing potential (defined as sexually mature women who have had menses within the preceding 24 months and have not undergone hysterectomy, bilateral oophorectomy, or tubal ligation) must have a negative urine or serum pregnancy test performed at Screening/Baseline.

Women of childbearing potential must agree not to attempt to become pregnant or undergo *in vitro* fertilization and, if participating in sexual activity that could lead to pregnancy, must use 2 reliable methods of contraception simultaneously for 30 days after receiving the study product. Male subjects must agree to use 2 reliable methods of contraception simultaneously for 30 days after receiving the study product if their partner is of child-bearing potential.

A combination of 2 of the following methods must be used:

- Condoms (male or female) with spermicide
- Diaphragm or cervical cap
- Intrauterine Device
- Hormonal-based contraception
- Vasectomy

Women (including pre-pubescent females) who are not of reproductive potential are not required to use contraception.

6.2 Procedures During Treatment

6.2.1 Day 1 (Day of Surgery)

- **Study Drug Administration:** Tozuleristide is to be administered at least 1 hour (and not more than 24 hours) before the planned start of the surgical excision of the tumor.
- **Vital signs** obtained pre-dose (within 15 minutes prior to start of study drug administration) and 30 minutes (+/- 5 minutes) after dosing. Blood pressure and pulse rate are to be recorded after the subject has been resting semi-supine for at least 3 min.
    - Body temperature (degrees Celsius [°C])
    - Respiratory rate (breaths/min)
    - Radial pulse rate (beats/min),
    - Blood pressure (mm mercury [mmHg])
- **Imaging and pathology during surgery (for all subjects) (See Appendix 11.3)**
- **Concomitant medications review**

- **Adverse events assessment**
- **Surgical excision of tumor**
- **Biopsy assignment and collection for pathology:** Biopsies from regions designated as regions of interest as determined by the surgeon are to be collected whenever safe and feasible, as determined by the surgeon
    - Pathology Assessments
        - Specimens from definite tumor and regions of interest will be required for pathology assessment of presence or absence of tumor.
        - Specimens with viable tumor (>10%) will be considered tumor positive.
        - Specimens with tumor will be further subclassified by percentage tumor (<10 %; 10-25%; 25-50%; >50%) by the pathologist.
- **Canvas Imaging:** Canvas images will be taken *in situ* after the surgeon has designated the regions of interest. Once Canvas fluorescence imaging is turned on, imaging operator will make assessment of “none,” “weak,” or “strong” fluorescence. At the end of the surgical procedure, an additional fluorescent Canvas image of the surgical bed will be collected to determine if residual fluorescence is present. The surgeon may use his/her own discretion at this point to determine if the resection can be extended.
- **Modus IR Imaging:** Modus IR images may be taken *in situ* after the surgeon has designated the regions of interest. As permitted by the surgical workflow, Modus IR images will be taken *in situ* to assess fluorescence of the primary tumor and/or the tumor resection bed. Excised surgical specimens may also be imaged with the Modus IR *ex vivo* (‘back table’). Modus IR imaging will not be used by the surgeon for the assessment of fluorescence in regions of interest or in the collection of tissue specimens. To minimize interruptions to the workflow, the timing of Modus IR imaging is at the discretion of the neurosurgeon.
- **MRI of tumor**: MRI with and without contrast should be obtained within 72 hours of surgery including the site of primary tumor. MRI sequences should include T1 pre-contrast, T1 post-contrast, T2, FLAIR, and Diffusion weighted images. Images will be evaluated by site radiology for patient management and assessment of EOR for this study.
- **Labs (hematology, chemistry, coagulation):** If drawn per standard of care, from Day of Surgery until the 30-days post-surgery visit, results will be reviewed for adverse events.

6.2.2 **30 days post-surgery (+/- 7 days)**

- Performance status
- Physical examination
- Vital signs
- Urine or serum pregnancy test
- Labs (hematology, chemistry, coagulation): If drawn per standard of care, from Day of Surgery until the 30-days post-surgery visit, results will be reviewed for adverse events
- Concomitant medications review
- Adverse events assessment
- Surgeon survey (for Schwannoma cohort only): Completed by surgeon after surgery.

6.3 Time and Events Table

	Screening Period (within 30 days prior to study enrollment)	Day 1 (Day of Surgery)	Day 30 post surgery (±7 days)
Informed Consent	X		
Eligibility Review	X		
Demographics	X		
Medical History and Record Review	X		
MRI of tumor	X[3]	X (within 72 hours after surgery)	
Physical Examination	X		X
Weight and height measurement	X		
Karnofsky Performance Status assessment	X		X
Adverse events assessment (continuous)		X	X
Concomitant medications review	X	X	X
Labs: hematology (CBC/diff), chemistry (CMP), and coagulation parameters (PT, INR, aPTT)	X	*If drawn per standard of care, results will be reviewed for adverse events.*	
Urine or serum pregnancy test (for females of child-bearing potential)	X		X
Study Drug Administration		X (1-24 hours prior to surgery)	
Vital signs	X	X[1]	X
Surgical excision of tumor		X	
Biopsy collection and pathology review		X	
Canvas Imaging		X	
Modus IR Imaging[4]		X	
Surgeon survey[2]			X

[1] Obtained pre-dose (within 15 minutes prior to start of study drug administration) and 30 minutes (+/- 5 minutes) after dosing. Blood pressure and pulse rate are to be recorded after the subject has been resting semi-supine for at least 3 min.
[2]Completed by surgeon for Schwannoma cohort only after surgery.
[3]MRI with and without contrast prior to treatment should be obtained within 30 days of enrollment, or within 1 year of enrollment for Cohort 3 only.
[4]Modus IR Imaging will only apply to a subset of patients in Cohorts 1 and 3, that consented and enrolled under protocol v5 and as surgical conditions permit.

6.4 Removal of Subjects from Study

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

6.4.1 Patient voluntarily withdraws (follow-up permitted);

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

6.4.3 Patient is unable to comply with protocol requirements;

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

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

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

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

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

6.4.9 Lost to follow-up. *If a research subject cannot be contacted after a period of 1 month, the subject may be considered "lost to follow-up." All attempts to contact the subject must be documented. This will be reviewed during an interim data monitoring visit.*

6.4.10 Initiation of anti-neoplastic chemotherapy, radiation therapy, or another investigational agent prior to 30 days post-surgery. In this case, the last day on study will be considered the day the subject initiated the new therapy. Subject should undergo all end-of-study required evaluations prior to initiation of new therapy if possible.

7.0 ADVERSE EVENTS (AE), SERIOUS ADVERSE EVENTS (SAE), UNANTICIPATED ADVERSE DEVICE EFFECTS (UADE), UNANTICIPATED PROBLEMS INVOLVING RISK TO SUBJECTS OR OTHERS (UPIRSO), AND SUSPECTED UNEXPECTED SERIOUS ADVERSE REACTIONS (SUSAR)

7.1 Definitions

7.1.1 Adverse Event (AE)

An adverse event is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

7.1.1.1 Laboratory test abnormalities

A laboratory test abnormality considered clinically relevant (e.g., causing the subject to withdraw from the study, requiring treatment or causing apparent clinical manifestations, result in a delay or dose modification of study treatment, or judged relevant by the investigator), should be reported as an adverse event.

7.1.2 **Serious Adverse Events (SAE)**

An adverse event is considered “serious” if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death
- A life-threatening adverse event
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

7.1.3 **Unanticipated Adverse Device Effect (UADE)**

An unanticipated adverse device effect (UADE) is defined as “any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety or welfare of subjects.”

7.1.4 **Unanticipated Problem Involving Risk to Subjects or Others (UPIRSO)**

UPIRSOs include any incident, experience, or outcome that meets all of the following criteria:

- Unexpected (in terms of nature, severity, frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- Suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known to an individual or group of individuals (including research subjects, research staff, or others not directly involved in the research).

7.1.5 **Suspected Unexpected Serious Adverse Reaction (SUSAR)**

Suspected unexpected serious adverse reactions (SUSARs) include adverse events that are deemed unexpected, serious, and as having a reasonable possibility of a causal relationship with the study drug.

7.2 Principal Investigator Responsibilities for Safety Monitoring

7.2.1 AE Reporting Period

The investigator or designee is responsible for ensuring that all adverse events (both serious and non-serious) observed by the clinical team or reported by the subject, which occur ***from start of first dose of study drug and until 30 days following surgery or until subject begins another cancer therapy (whichever occurs first)***, are fully recorded in the subject's medical records.

7.2.2 AE Documentation

The investigator, sub-investigator (treating physician if applicable), or study team (according to the responsibilities delegated per the DOA) will document the following for all AEs (both serious and non-serious):

- Event term, according to the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5. The CTCAE current version is available at http://ctep.cancer.gov/reporting/ctc.html
- Description of the event
- Date of onset and resolution
- Expectedness of the toxicity
    - Expected events are those that have been previously identified as resulting from administration of the agent. An adverse event is considered unexpected, for expedited reporting purposes only, when either the type of event or the severity of the event is <u>not</u> listed in:
        - the current known adverse events listed in this protocol;
        - the drug package insert; and/or
        - the current Investigator's Brochure
- Grade of toxicity, as per CTCAE criteria
- Attribution of relatedness to the investigational agent- (this must be assigned by an Investigator, sub-investigator, or treating physician)
    - Attribution categories are as follows:
        - Definite: The AE is clearly related to the study treatment.
        - Probable: The AE is likely related to the study treatment.
        - Possible: The AE may be related to the study treatment.
        - Unlikely: The AE is doubtfully related to the study treatment.
        - Unrelated: The AE is clearly NOT related to the study treatment.
- Action taken as a result of the event, including but not limited to; no changes, dose interrupted, reduced, discontinued, etc. or action taken with regard to the event, i.e. no action, received conmed or other intervention, etc.
- Outcome of event

Source documentation must be available to support all AEs.

7.2.3 Duration of AE monitoring

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

- the AE resolves or the symptoms or signs that constitute the AE return to baseline

- any abnormal laboratory values deemed an AE as per section 7.1.1.1, has returned to baseline
- there is a satisfactory explanation other than the study drug for the changes observed
- death, or
- until 30 days following surgery or until subject begins another cancer therapy (whichever occurs first)

7.3 Safety Reporting Requirements

7.3.1 Reporting to the Principal Investigator

The Principal Investigator must be notified by study staff or co-investigators within 24 hours of learning of any serious adverse events, regardless of attribution and expectedness, occurring during the study or within 30 days (refer to Section 7.2) of surgery.

Contact for Expedited Reporting:
Dr. John Yu, 310-248-6693, John.Yu@cshs.org

Alternate Contact for Expedited Reporting:
Dr. Ray Chu, 310-248-6693, Ray.Chu@cshs.org

7.3.2 Reporting to DSMC:

Serious Adverse Events deemed to be related to the protocol and on-study deaths, including death of a research subject unless the death is expected (e.g. due to disease progression) to be reported to the DSMC within 24 hours of awareness. Hardcopies or electronic versions of the MedWatch Form 3500A (Mandatory Reporting) or a narrative report, along with any other supporting documentation available, should be submitted to the DSMC Coordinator. The DSMC Coordinator will forward the information to the DSMC Chair, and/or medical monitor. The DSMC Chair will review all documentation upon receipt from the DSMC Coordinator and determination of whether the following actions are required: 1) takes action immediately, 2) convenes a special DSMC session (physical or electronic), or 3) defers the action until a regularly scheduled DSMC meeting. Reports to be emailed to the DSMC team at GroupSOCCICCTODSMCAdmin@cshs.org.

7.3.3 Reporting to Blaze Bioscience

Blaze Bioscience has requirements for the expedited reporting of SAEs that meet specific criteria of the appropriate regulatory authorities; therefore, all appropriate parties must be notified as soon as possible and within 24 hours of the investigator becoming aware of the event. The procedures for reporting all SAEs, regardless of causal relationship, are as follows:

In the case of a SAE, information should be recorded on a SAE Report Form, which will be provided by Blaze, and faxed or emailed to the contacts below.

Attention: Clinical Operations
Email: kristi.harrington@blazebioscience.com &
clinical.trials@blazebioscience.com
(206) 257-5924

When reporting safety events to the drug manufacturer, copy the PI and GroupCCTOSPIN@cshs.org.

For fatal or life-threatening events, also send copies of hospital case reports, autopsy reports, and other documents when requested and applicable.

Blaze Bioscience may request additional information from the investigator to ensure the timely completion of accurate safety reports.

The investigator must take all therapeutic measures necessary for resolution of the SAE.

An SAE may qualify for reporting to regulatory authorities if the SAE is considered to have a possible causal relationship to the study product and is unexpected/unlisted based upon the current Investigator's Brochure.

Where this is required by local regulatory authorities, and in accordance with the local institutional policy, the investigator should notify (in writing) the IRB of SAEs according to local guidelines.

7.3.4 **Reporting to Synaptive Medical**

Synaptive Medical has requirements for the expedited reporting of UADEs that meet specific criteria of the appropriate regulatory authorities; therefore, all appropriate parties must be notified as soon as possible and within 24 hours of the investigator becoming aware of the event. The procedures for reporting all UADEs are as follows:

In the case of a UADE, information should be recorded on a FDA Form 3500A (MedWatch), and faxed or emailed to the contacts below.

Attention: Quality and Regulatory Affairs
Michael McDonald
Email: michael.mcdonald@synaptivemedical.com

When reporting safety events to Synaptive Medical, copy the PI and GroupCCTOSPIN@cshs.org.

For fatal or life-threatening events, also send copies of hospital case reports, autopsy reports, and other documents when requested and applicable.

Synaptive Medical may request additional information from the investigator to ensure the timely completion of accurate safety reports.

The investigator must take all therapeutic measures necessary for resolution of the UADE.

7.3.5 **Reporting to the Institutional Review Board (IRB)**

As per the Cedars-Sinai IRB Reporting Possible Unanticipated Problems Involving Risks to Subject or Others (UPIRSO) Policy, the IRB must be notified of all UPIRSOs as soon as possible, but no later than 10 business days from when the study team learned of any of the following events:

1. Any internal SAE, AE or Research-Related Subject Injury (RRSI), which in the opinion of the Principal Investigator was unanticipated or unexpected, and has a reasonable possibility of relationship to the research.
2. Any actionable external SAE, AE, SUSAR, development safety update report (DSUR), or FDA MEDWATCH report deemed to be a UPIRSO. An event is considered "actionable" if it warrants a change to the conduct of the study.
3. Any internal or external UADE.
4. Any accidental, unintentional protocol or consent/HIPAA related deviation that may impact subjects' rights, safety, or welfare. See section 9.7.3.

5. Any planned protocol exception or eligibility waiver. See sections 9.7.2 and 9.7.3.
6. Changes to the research or protocol deviations made without prior IRB approval in order to eliminate apparent immediate hazard to a research subject. (Note: These must be reported to the IRB within 72 hours.)
7. Problems, events, unanticipated incidental findings, billing problems, or other events, outcomes, or new information related to the research (e.g., publication, safety monitoring report, interim findings, product labeling changes, findings generated from preclinical, animal studies) that may adversely affect the rights, safety, or welfare of the subjects or others, put subjects or others at increased risk, compromise the research data, or require/recommend changes to the study conduct.
8. Subject complaints or concerns that cannot be resolved by the research staff to the subject's satisfaction.
9. Breach or potential breach of confidential or sensitive information.
10. Incarceration of a subject who is enrolled in a study that is not approved by the IRB to include prisoners.

7.3.6 **Reporting to the Food and Drug Administration**

The sponsor-investigator of the IND, or designee at the Coordinating Center, must submit all reported **SUSARs** to the FDA on the FDA Form 3500A (MedWatch).

The sponsor-investigator of the IND must notify the FDA as soon as possible but no later than:

- **7 calendar** days after the sponsor-investigator's initial receipt of the information in a written IND safety report of any **fatal or life-threatening SUSARs.**
- **15 calendar** days after the sponsor-investigator's initial receipt of the information in a written IND safety report of any **non-fatal / non-life-threatening SUSARs**.

The sponsor-investigator of the abbreviated IDE must submit all reported **UADEs** to the FDA as soon as possible but no later than 10 working days after the investigator first learns of the effect.

Copies of all IND and IDE Safety Reports will be kept in the Trial Master File in the SOCCI CCTO.

7.3.7 **Procedures to be Followed in the Event of Pregnancy**

The subject must be instructed to inform the investigator IMMEDIATELY if she/his partner becomes pregnant during the study. The investigator should report all pregnancies to Blaze Bioscience within 24 hours of becoming aware of the pregnancy. The outcome of pregnancies in a subject enrolled in the study must be reported to Blaze Bioscience.

8.0 STATISTICAL CONSIDERATIONS

This is a single-arm study aimed at demonstrating preliminary efficacy of tozuleristide and the Canvas imaging system.

8.1 Sample Size Determination

8.1.1 **Two-stage Design**

Simon's two-stage design (Simon, 1989) will be used. The null hypothesis that the true percentage of subjects with fluorescence-positive primary tumor biopsy (one per patient) is < 60% will be tested. In the first stage, 21 subjects will be treated. If there are 13 or

fewer subjects with fluorescence-positive primary tumor biopsy among the 21 subjects, the study will be stopped for futility. Otherwise, 18 additional subjects will be treated for a total of 39. The null hypothesis will be rejected if 29 or more subjects with fluorescence-positive primary tumor biopsy are observed in 39 subjects. This design yields a one-sided type I error rate of 0.0434, power of 85% when the true response rate is 80%, probability of early termination of 65% under the null hypothesis. The sample sizes are calculated using Jung et al (2004).

8.1.2 **TPR and TNR**

This is a phase II study aimed at demonstrating preliminary efficacy of tozuleristide and the Canvas imaging system to guide resection of primary tumors. One component of this study is better quantifying which primary tumors fluorescent after tozuleristide administration, therefore the study included a broad population of primary tumors. Based on Phase I data, we estimate up to 20% of cases will show no fluorescence and will be excluded from further analysis. Assuming that 82% of 39 subjects will show fluorescence, this leaves 32 cases for further analysis. We estimate obtaining 1 primary tumor biopsy specimen, 4 other suspected tumor biopsy specimens and 1 non-fluorescent tissue biopsy from each patient on average, providing 192 histopathological specimens to compare with tozuleristide based imaging.

We assumed that biopsies in the same patient will be correlated, therefore we calculated Sidak-corrected 95% confidence interval width for TPR and TNR based on a simulation study: latent normal variables were generated with mean zero and covariance structure defined by variance 1 and correlation r. Then, latent variables were dichotomized to represent the fluorescence evaluation using normal quantiles for given values of TPR or TNR. Finally, a general estimating equation (GEE) logistic model is fitted with only the intercept and an exchangeable correlation matrix allowing us to obtain Sidak-corrected 95% CI for TPR and TNR separately.

Table 8: Sidak-corrected 95% CI width (%) for TPR as function of correlation (r)

TPR (%)	r		
	0.20	0.50	0.8
60	22	26	30
70	20	25	29
80	18	21	25
90	13	16	20

Assuming that each patient will contribute 4 tumor-positive biopsy specimens, a total of 128 (4x32) tumor-positive biopsy specimens will provide an Sidak-corrected 95% CI for the TPR with width from 13% to 30%. Table 8 shows 95% CI width as function of selected values of TPR and correlation (r) among biopsies. For example, if the TPR is estimated to be 90% with r = 0.5, its 95% CI is (82%, 96%).

Similarly, if each patient will contribute 1 tumor-negative biopsy specimens on average, a total of 32 (1x32) tumor-negative biopsy specimens will provide an exact 95% CI for the TNR with width as showed in Table 9.

Table 9: Sidak-corrected 95% CI width (%) for TNR

TNR (%)	Width (%)
60	40
70	38
80	33
90	26

8.2 Analysis Populations

8.2.1 **Efficacy Analysis**

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

- meet all eligibility criteria
- receive tozuleristide
- undergo neurosurgery
- have tumor specimens and *in situ* fluorescence status collected

Subjects in the full analysis population will be considered evaluable for efficacy.

8.2.2 Safety Analysis

The safety analysis population will consist of all subjects who receive any amount of tozuleristide. The frequencies of AEs by type, body system, severity and relationship to study drug will be summarized. SAEs (if any) will be described in detail.

8.3 Statistical Analysis Plan

8.3.1 General Considerations

In general, descriptive statistics will be provided for all study endpoints. Descriptive statistics will include number of subjects, mean, standard deviation, median, and range (minimum, maximum) for continuous variables, and frequencies and percentages for categorical variables. All CIs will be constructed at a two-sided 95% confidence level. All analyses will be presented by tumor grade (high and low) and overall.

8.3.2 Subject Disposition

Subjects who discontinue tozuleristide prematurely and subjects who withdraw from the study will be summarized. The reason(s) for tozuleristide discontinuation and study discontinuation will be summarized. The number of subjects who do not undergo surgery will also be summarized.

8.3.3 Demographics and Subject Characteristics

Demographic and other baseline characteristics will be summarized with descriptive statistics.

8.3.4 Efficacy Analyses

Image operator fluorescence assessments of Canvas images (positive or negative) and pathologist assessments (tumor positive or negative) will be tabulated. TPR, TNR, PPV and NPV will be estimated by tumor grade (high and low) and overall. General estimating equation (GEE) logistic models with an exchangeable correlation matrix and only the intercept will be fitted for either tumor-positive or tumor-negative biopsies. Then, Sidak-corrected 95% confidence intervals (CI) for TPR and TNR will be estimated based on the fitted model. Similar procedure will be employed for fluorescence positive and negative fluorescence images yielding Sidak-corrected 95% CI for PPV and NPV, respectively

Positive fluorescence rate from the primary tumor will be estimated with exact 95% CI by tumor grade (high and low) and overall. Tumor subtype will be determined by pathologist. If the primary tumor biopsy specimen is non-tumor, their tumor subtype will be assigned as "No Tumor". Positive fluorescence rate for each tumor subtype will be estimated with exact 95% CI.

An exploratory analysis will be performed to evaluate the extent of tumor required in a biopsy specimen to yield fluorescence positive imaging. A logistic regression on fluorescence positivity with percent tumor cellularity, cohort and their interactions as covariates. Predicted probability of positive fluorescence will be plotted against percent tumor cellularity with 95% CI.

Surgeon qualitative assessment of the utility of the Canvas imaging system, and the utility of tozuleristide and the Canvas imaging system to guide surgical decisions regarding resection in situ will be summarized descriptively.

Exploratory analyses of efficacy include:

1. Association between the radiographic extent of resection (gross total resection, near total resection or subtotal resection) and the residual fluorescence in situ (presence or absence) at the end of a surgical resection will be analyzed using contingency table.

2. Univariate and multivariable analyses will be performed to evaluate which tumor characteristics (e.g. tumor grade, tumor type, immunohistochemical markers) correlate with the tozuleristide fluorescence in situ. Logistic regression on tozuleristide fluorescence will be used.
3. Percentage of primary tumors visualized in situ with the Canvas that are fluorescence positive that are also fluorescence positive when visualized with Modus IR will be estimated with exact 95% confidence intervals.
4. Correlation of ex vivo fluorescence of tissue specimens visualized with the Modus IR and percent tumor cells by histopathology will be assessed using univariate Spearman correlation coefficient and multivariable regression models.
5. Comparison of extent of resection of subjects receiving tozuleristide to historical controls of CSMC patients with similar resections for relevant subgroups to minimize bias will be assessed using Mann-Whitney tests.
6. Size and intensity of the fluorescence in the images will be measured and compared by the open-source ImageJ image analysis software.

8.3.5 **Study Product Exposure**

The dose of tozuleristide administrated in volume (mL) and amount (mg) will be summarized.

8.3.6 **Safety Analyses**

All safety summaries will be tabulated for subjects who receive tozuleristide.

Adverse events will be coded using MedDRA 20.1. Adverse events will be defined as treatment-emergent if they are newly occurring or worsen following treatment with tozuleristide. Adverse events will be scored for severity using NCI CTCAE version 5.0 if applicable, or the protocol specified severity stated in Section 7.3.

All AEs will be reported from time of tozuleristide dosing and until 30 days post-surgery. Treatment-emergent AEs, SAEs, severity of AEs, tozuleristide-related AEs, Canvas-related AEs, and AEs leading to withdrawal from study will be summarized by MedDRA preferred term and system organ class. 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. All SAEs occurring within 30 days post-surgery will also be reported.

8.4 Futility Analysis

A futility analysis will be performed using a Simon's two-stage design (Section 8.1.1). In the first stage, 21 subjects will be treated. If there are 13 or fewer subjects with fluorescence-positive primary tumor biopsy among the 21 subjects, the study will be stopped for futility. Otherwise, 18 additional subjects will be treated for a total of 39. If there are at least 29 subjects with fluorescence-positive primary tumor biopsy, further investigation of the study treatment is warranted. The probability of early termination is 0.65 under the null hypothesis.

8.5 Stopping Rules

We expect the probability of grade 3 or greater AE deemed probably related or related to the study treatment to be less than 10%. Let P1, P2 and P3 be the true probability of grade 3 or greater AE deemed probably related or related to the study treatment for the cohort 1, 2 and 3 respectively. The trial will stop if there is statistical evidence that P1 is greater than 10%, i.e., $Pr(P1 > 0.10 \mid data) > 0.972$ for cohort 1, $Pr(P2 > 0.10 \mid data) > 0.971$ for cohort 2, $Pr(P3 > 0.10 \mid data) > 0.948$ for cohort 3. The trial safety stopping

rules were calculated using exact binomial tests of proportion in a Bayesian sequential trial design with a single arm. A noninformative prior distribution for P1, P2 and P3, will be used. **Table 10** shows the stopping rules for the design at each look for the cohorts 1, 2 and 3. In column 2, the maximum number of patients with undesirable events in order for the trial to proceed is presented. For example, in Table 10, if 4 or more patients with undesirable events are observed after enrolling 10 patients in cohort 1, the trial stops. The third column gives the probability of stopping the trial at each look when in fact, the true P = 0.10. This is the equivalent of the Bayesian type I error probability, which was set at 0.0557 for cohort 1; 0.009 for cohort 2, 0.01 for cohort 3. Note: If cohort 2 is stopped, then the protocol may be revised to enroll remaining patients at lower doses.

Table 10. Testing the hypothesis of grade 3 or greater AE deemed probably related or related to the study treatment to be greater than 10%

Number of Patients	Maximum number of patients with undesirable events at which trial will continue	Probability to Stop	Cumulative Probability to stop
Cohort 1			
10	3	0.01280	0.01280
21	4	0.05565	0.05565
Cohort 2			
5	2	0.00856	0.01000
Cohort 3			
2	1	0.01000	0.01000

Table 11 shows the design operating characteristics under selected values of the true probability *P* for the cohorts 1, 2 and 3. It indicates the probability of stopping the trial under the alternative hypothesis, the expected sample size, and the average sample size given that the trial stopped. For example, in Table 11, if the true value of *P* is 0.15 for cohort 1, then there is 20.4% chance that the trial is stopped early and the average expected sample size is about 19. On the other hand, there is a small chance of stopping the trial if *P* is low; 0.38% chance of stopping the trial for cohort 1 when in fact *P* = 0.05.

Table 11. Design operating characteristics under selected values of the true probability P.

True Value P	Probability to Stop	Expected N	Expected N given that We Stopped
Cohort 1			
0.05	0.0038	38.92	18.01
0.1	0.0557	37.86	18.47
0.15	0.2042	34.78	18.31
0.2	0.4216	30.08	17.85
Cohort 2			
0.05	0.012	10.99	5.00

0.10	0.0086	10.95	5.00
0.15	0.0266	10.84	5.00
0.20	0.059	10.65	5.00
Cohort 3			
0.05	0.0025	4.99	2.00
0.1	0.0100	4.97	2.00
0.15	0.0225	4.93	2.00
0.2	0.0400	4.88	2.00

9.0 STUDY MANAGEMENT

9.1 Conflict of Interest

Any reportable conflict of interest will be disclosed to the local IRB and will be outlined in the Informed Consent Form.

9.2 Institutional Review Board (IRB) Approval and Consent

It is expected that the IRB will have the proper representation and function in accordance with federally mandated regulations.

Any documents that the IRB may need to fulfill its responsibilities (such as protocol, protocol amendments, Investigator's Brochure, consent forms, information concerning patient recruitment, payment or compensation procedures, or other pertinent information) will be submitted to the IRB. The IRB's written approval of the study protocol and the informed consent form will be in the possession of the Investigator before the study is initiated and prior to the shipment of study supplies to participating sites, if applicable. This approval must refer to the study by exact protocol title and number and should identify the documents reviewed and the date of review.

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

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

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

9.3 Registration/Enrollment Procedures

All patients will be tracked following written informed consent. Those patients who are consented to participate in the clinical trial but do not meet one or more criteria required for participation during the screening phase will be listed as screen failures on the master

list of consented subjects. Eligible subjects, as determined by screening procedures and verified by a treating investigator, will be registered on study at Cedars Sinai Medical Center by the Study Coordinator.

Issues that would cause treatment delays after registration should be discussed with the Principal Investigator (PI). If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The Study Coordinator should be notified of cancellations as soon as possible.

Assignment of Subject ID: The study team will track all subjects who sign consent using OnCore. Subjects found to be ineligible will be recorded as screen failures. Subjects found to be eligible will be registered using a three-digit numeric ID that follows the standard SOCCI format (001, 002, etc.).

A) Eligibility Verification

Prior to registration/enrollment, all subjects must undergo eligibility verification by the SOCCI Cancer Clinical Trials Office (CCTO) Quality Management Core (QMC). The following documents will be organized into an eligibility packet, scanned as a pdf, and emailed to GroupSOCCICROQMC@cshs.org for review:

- QMC approved Eligibility Checklist signed by investigator and 2 members of the study team (or equivalent)
- Source documents substantiating eligibility that cannot be located in the subject's CS Link medical record
- Signed consent form with Subject's Bill of Rights, HIPAA authorization form, consent progress note and any optional consent forms, as applicable, if not available in CS Link

B) Registration

After eligibility is verified, each site will assign the subject a study number and site staff will then register the patient in OnCore®.

Registration is completed as follows:

- Assign a patient study number
- Assign the patient a dose/treatment arm as determined through communication with Biostatistics and the principal investigator, if applicable
- Enter the patient in OnCore
- Notify the investigational pharmacy and treating physicians that a subject has gone on study and anticipated treatment start date

Oversight by the principal investigator is required throughout the entire registration process.

9.4 Data Management, Quality Assurance, Quality Control and Reporting

REDCap is the Cedars-Sinai Cancer institutional choice for the electronic data capture of case report forms for SOCCI Investigator Initiated Trials. REDCap, a HIPAA-compliant database, will be used for electronic case report forms in accordance with institutional requirements, as appropriate for the project. The Study Staff will be responsible for data processing, in accordance with procedural documentation. Database lock will occur once quality assurance procedures have been completed. See also Section 9.5.2, Monitoring.

All procedures for the handling and analysis of data will be conducted using good computing practices meeting FDA guidelines for the handling and analysis of data for clinical trials.

9.5 Data and Safety Monitoring

9.5.1 **Safety Oversight**

Adherence to the protocol, Good Clinical Practices (GCP), and institutional policy will be monitored by the PI during the course of the study through routine Disease Research Group (DRG) meetings (or equivalent). The PI will maintain continuous safety monitoring for the duration of the study by reviewing subject/study data. It is the responsibility of the principal investigator to adhere to the Data Safety Monitoring Plan throughout the life of the study.

In addition, safety oversight and efficacy data will be reviewed by the SOCC Data and Safety Monitoring Committee (DSMC). The DSMC will review this trial commensurate with the assigned risk class as categorized by the Protocol Review and Monitoring Committee (PRMC). The DSMC membership and responsibilities are governed by the committee charter. The DSMC findings and recommendations will be reported in writing to the Principal Investigator as a summary letter which will be forwarded by the Principal Investigator or designee to the CS-IRB. The DSMC outcome letters will be furnished to the FDA, as applicable. Refer to the DSMC Charter for details of the DSMC review.

9.5.2 **Monitoring**

The SOCC Cancer Clinical Trials Office (CCTO) Quality Management Core (QMC) will conduct internal monitoring visits and audits to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with International Conference on Harmonisation Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s).

Refer to the DSMP for details pertaining to the type, frequency, and extent of monitoring that will be performed.

9.6 Record Retention

Study documentation includes all Case Report Forms, data correction forms or queries, source documents, monitoring/auditing logs/letters, records of study drug receipt, dispensation, destruction, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms). Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study. Government agency regulations and directives require that the study investigator must retain all study documentation pertaining to the conduct of a clinical trial. Study documents should be kept on file in accordance with all applicable federal guidelines and local guidelines.

Investigators must make study data accessible to the monitor, other authorized representatives of the Sponsor (or designee), IRB, and Regulatory Agency (e.g., FDA) inspectors upon request.

9.7 Adherence to Protocol

It is the responsibility of the Investigator-sponsor to ensure that patient recruitment and enrollment, treatment, follow-up for toxicities and response, and documentation and reporting at SOCCI are all performed as specified in the protocol. Except for an

emergency situation in which proper care for the protection, safety, and well-being of the study patient requires alternative treatment, or a protocol exception request approved by the IRB of record, the study shall be conducted exactly as described in the approved protocol.

9.7.1 Emergency Modifications

Investigators may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior IRB approval. For any such emergency modification implemented, the IRB must be notified as soon as possible, but no more than 72 hours from the investigator's awareness of the event.

9.7.2 Protocol Exceptions and Eligibility Waivers

A protocol exception is an anticipated or planned deviation from the IRB-approved research protocol, as described in the IRB Policy, *Reporting Possible Unanticipated Problems Involving Risks to Subject or Others (UPIRSO) Policy: Institutional Review Board/Research Compliance and Quality Improvement.* A protocol exception most often involves a single subject and is not a permanent revision to the research protocol. Protocol exceptions that extend beyond a single subject should result in a protocol amendment to avoid serial violations.

Planned exceptions to the protocol that are more than logistical in nature and/or affect timing of study drug administration, or the investigator assesses the event may impact subject safety and/or study integrity, may not be implemented without prior approval from the IRB. The PI or her/his designee is responsible for submitting a protocol exception and its supporting documents for IRB review.

Study team should refer to the IRB *Reporting Possible Unanticipated Problems Involving Risks to Subject or Others (UPIRSO) Policy: Institutional Review Board/Research Compliance and Quality Improvement* guidelines to determine which deviations and exception requests meet reporting guidelines. The deviation or exception request must be submitted to the IRB for review and approval prior to implementation.

Special considerations for Eligibility Waivers (EW)
Subjects who do not meet the eligibility requirements should not be enrolled. Eligibility waivers are not permitted.

9.7.3 Other Protocol Deviations

Logistical deviations from the protocol (e.g., minor changes to the study schedule for an individual subject) do not require prior IRB approval unless the deviation has the potential to affect the subject's safety or study integrity. Such planned deviations that do meet this definition and do not affect the subject's safety or study integrity should be noted in the subject's research record or deviation log as described in the SOCCI CCTO's Standard Operating Procedure 12: Deviation and Noncompliance Reporting.

Unintentional deviations from the protocol that might affect subject safety or study integrity should be reported to the IRB within 10 days from when the investigator becomes aware that such a deviation has occurred, as outlined in the SOCCI CCTO's Standard Operating Procedure 12: *Deviation and Noncompliance Reporting*. In this case, a Protocol Deviation report must be submitted in CS-IRB, per IRB policy, *Reporting Possible Unanticipated Problems Involving Risks to Subject or Others (UPIRSO) Policy: Institutional Review Board/Research Compliance and Quality Improvement*. All submissions should include a description of the plan to avoid similar deviations or exceptions in the future.

9.7.4 Amendments to the Protocol

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

The written amendment, and if required the amended consent form, must be sent to the IRB for approval prior to implementation. Repeat exceptions or deviations to the protocol may suggest a protocol amendment is needed.

9.8 Obligations of Investigators

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

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

9.9 Publications

The preparation and submittal for publication of manuscripts containing the study results shall be in accordance with a process determined by mutual written agreement among the sponsor-investigator and participating institutions. The publication or presentation of any study results shall comply with all applicable privacy laws, including, but not limited to, the Health Insurance Portability and Accountability Act of 1996.

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11.0 APPENDICES

11.1 SUMMARY OF CHANGES

Protocol Amendment 4 (Protocol version 5 dated 04SEP2024)

- Cover page: addition of agent sources and abbreviated IDE required
- Study Summary: additional exploratory objectives to include Modus IR imaging, historical controls, and size and intensity of fluorescence in the images; clarification of number of evaluable subjects to account for subject replacement
- Sec. 1.0 Background and rationale updated to include Modus X/IR information
- Secs. 2.3.3 & 2.4.3 additional exploratory objective and endpoints to include Modus IR imaging and assessment of fluorescence in situ and ex vivo in subjects undergoing resection of primary gadolinium-enhancing CNS tumors or in subjects undergoing resection of vestibular schwannoma;
- Secs. 2.3.4 & 2.4.3 additional exploratory objective and endpoint to compare extent of resection against historical controls of similar resections.
- Secs. 2.3.5 & 2.4.3 additional exploratory objective and endpoint to explore the size and intensity of the fluorescence in the images captured by the microscope and exoscope in subjects receiving tozuleristide.
- Secs. 3.0, 5.1 clarification of subset of patients (Cohorts 1 and 3) that consent and enroll under Protocol v5, the Modus IR may be used to capture intraoperative in situ images of the primary tumor and/or the tumor bed, and ex vivo images of excised tissue specimens.
- Secs. 6.2.1, 6.3 additional procedure details of Modus IR imaging to capture intraoperative in situ images of the primary tumor and/or the tumor bed, and ex vivo images of excised tissue specimens.
- Sec. 7.1.3 addition of unanticipated adverse device effect (UADE) definition
- Sec. 7.3.4 addition of Synaptive Medical reporting requirements
- Sec. 7.3.6 addition of UADE FDA reporting requirements
- Sec. 8.3.4 efficacy analyses updated to include exploratory endpoints of Modus IR imaging, historical control comparison, and ImageJ measurement of fluorescence in images
- Sec. 11.3 addition of Modus IR imaging for subset of patients (Cohorts 1 and 3) that consent and enroll under Protocol v5

Protocol Amendment 3 (Protocol version 4 dated 13MAR2024)

- Cover page: Removal of co-investigator Serguei Bannykh, MD; Biostatistician reassigned to Mourad Tighiouart
- Secs. 4.1.2, 6.1.5, 6.3 clarification of Cohort 3 screening/baseline MRI should be obtained within 1 year of enrollment.
- Sec. 6.3, 7.2.1 clarification of start of AE reporting period
- Secs. 7.3.3 & 9.0 Study management language updated with institutional template language

Protocol Amendment 2 (Protocol version 3 dated 16AUG2023)

- Cover page: Addition of co-investigator Xuemo Fan, MD
- Sec. 3.1 & 5.1 Addition of up to 5 additional biopsies of “other tissue” may be collected after surgical resection
- Sec. 7.3.3 updated to include additional Blaze contact for SAE reports
- Secs. 7.3.4 & 9.0 Study management language updated with institutional template language
- Sec. 11.3 Removal of #13 and replaced with addition of up to 5 additional biopsies of “other tissue” collection instructions

Protocol Amendment 1 (Protocol version 2 dated 17MAR2023)

- Cover page: Removal of co-investigators Surasak Phuphanich and Ravi Prasad
- Study Schema updated to include all 3 cohorts and co-primary endpoints

- Study Summary: updated primary objective, addition of exploratory objectives, updated number of subjects (n=55) and doses to include 36 mg.
- Section 1.0 Background and Rationale updated throughout to include current safety data
- Sec. 1.5.1 addition of increased dose rationale for non-gadolinium enhancing primary brain tumors.
- Sec. 1.5.3 addition of rationale for modification to include vestibular schwannoma cohort
- Sec. 2.1.1 Primary objective updated from heterogenous to homogenous population of primary high grade CNS tumors
- Sec. 2.3 & 2.4.3 Addition of two exploratory objectives and endpoints for non-gadolinium enhancing tumors and vestibular schwannomas.
- Sec. 3.1 & 3.2 Study design and sample size clarified to include 3 different cohorts
- Sec. 4.0 removal of high- and low-grade enrollment plan for first stage.
- Sec. 4.1.2 inclusion criterion updated to include MRI documentation for primary schwannoma tumor
- Sec. 5.1 updated to include 3 cohorts and 36 mg dosage in cohort 2
- Sec. 5.3 addition of method of assigning subjects to treatment groups for 3 cohorts
- Sec. 5.10 Subject replacement clarification of evaluable subjects found to not have primary malignant CNS tumor after surgery and clarification of cohort 2 subjects previously dosed in protocol version 1 will not be replaced.
- Sec. 5.11 Evaluable for toxicity language moved from Sec. 7.0
- Sec. 6.2.2 & 6.3 addition of Surgeon survey for Schwannoma cohort only
- Sec. 7.0 AE language reorganized with updated institutional template language throughout
- Sec. 7.3.3 revised email contact for safety reporting to Blaze Bioscience
- Sec. 8.5 Stopping rules: Addition of safety rules for non-enhancing and schwannoma cohorts
- Sec. 9.0 Study management language updated with institutional template language
- Sec. 10.0 References updated
- Sec. 11.4 Addition of Surgeon Survey

11.2 Karnofsky Performance Status Scale

The Karnofsky Performance Scale Index allows patients to be classified as to their functional impairment. This can be used to compare effectiveness of different therapies and to assess the prognosis in individual patients. The lower the Karnofsky score, the worse the survival for most serious illnesses.

KARNOFSKY PERFORMANCE STATUS SCALE DEFINITIONS RATING (%) CRITERIA

Able to carry on normal activity and to work; no special care needed.	100	Normal no complaints; no evidence of disease.
	90	Able to carry on normal activity; minor signs or symptoms of disease.
	80	Normal activity with effort; some signs or symptoms of disease.
Unable to work; able to live at home and care for most personal needs; varying amount of assistance needed.	70	Cares for self; unable to carry on normal activity or to do active work.
	60	Requires occasional assistance, but is able to care for most of his personal needs.
	50	Requires considerable assistance and frequent medical care.
Unable to care for self; requires equivalent of institutional or hospital care; disease may be progressing rapidly.	40	Disabled; requires special care and assistance.
	30	Severely disabled; hospital admission is indicated although death not imminent.
	20	Very sick; hospital admission necessary; active supportive treatment necessary.
	10	Moribund; fatal processes progressing rapidly.
	0	Dead

References:

Crooks, V, Waller S, et al. The use of the Karnofsky Performance Scale in determining outcomes and risk in geriatric outpatients. J Gerontol. 1991; 46: M139-M144.

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Hollen PJ, Gralla RJ, et al. Measurement of quality of life in patients with lung cancer in multicenter trials of new therapies. Cancer. 1994; 73: 2087-2098.

O'Toole DM, Golden AM. Evaluating cancer patients for rehabilitation potential. West J Med. 1991; 155:384-387.

Oxford Textbook of Palliative Medicine, Oxford University Press. 1993;109.

Schag CC, Heinrich RL, Ganz PA. Karnofsky performance status revisited: Reliability, validity, and guidelines. J Clin Oncology. 1984; 2:187-193.

11.3 Imaging and Pathology Review Manual

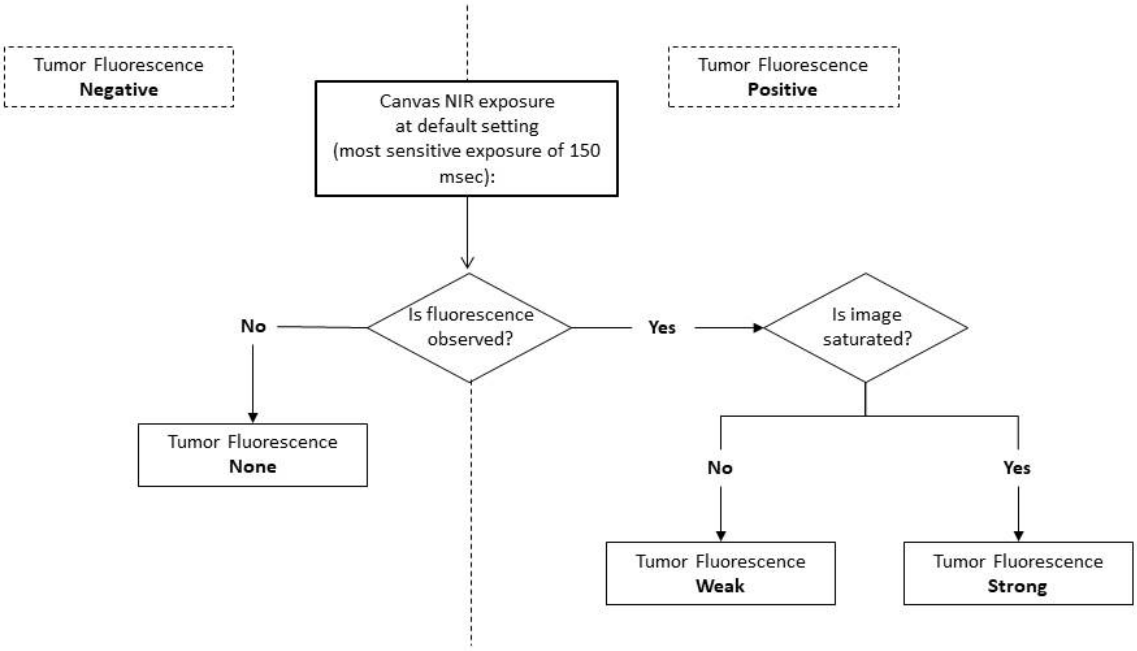
- Canvas set-up and ongoing video recording will be turned on once tumor has been exposed, but prior to any biopsy collection.
- Surgery will be conducted according to standard of care procedures using the Zeiss Pentero microscope with Canvas attached and routine lighting conditions. All clinical decision making should be made based on routine neurosurgical practices and tissue resection will take place under normal light conditions in line with best clinical practice.
- Only in a subset of patients (Cohorts 1 and 3) that consent and enroll under Protocol v5, the Modus IR may be used to capture intraoperative in situ NIR light images of the primary tumor and/or the tumor bed, and ex vivo images of excised tissue specimens.
- Modus IR Imaging: Modus IR images may be taken in situ after the surgeon has designated the regions of interest. As permitted by the surgical workflow, Modus IR images will be taken in situ to assess fluorescence of the primary tumor and/or the tumor resection bed. Excised surgical specimens may also be imaged with the Modus IR ex vivo ('back table'). Modus IR imaging will not be used by the surgeon for the assessment of fluorescence in regions of interest or in the collection of tissue specimens. To minimize interruptions to the workflow, the timing of Modus IR imaging is at the discretion of the neurosurgeon. Refer to the Modus IR User Manual for imaging instructions.

    1. The surgeon will designate the suspected nature of the region of interest (tumor or not tumor), document, and point to the planned area for biopsy collection.
    2. The imaging operator will look at the Canvas monitor and capture a white light image of the area.
    3. The imaging operator will turn on the NIR light on the Canvas and capture an NIR image to evaluate tissue fluorescence.
    4. The imaging operator will note fluorescence status from the Canvas monitor, and will classify the intensity of fluorescence by a 3-point scale (none, weak, or strong).
    5. The surgeon will take a biopsy from a portion of the tumor that is clearly identified as tumor by white light, which will be sent for frozen section. The pathologist will confirm the biopsied tissue contains tumor.
    6. The surgeon may now look at the monitor and document his/her fluorescence assessment on the neurosurgery survey and will be informed of the fluorescence status of the primary tumor biopsy.
    7. If the primary tumor biopsy is fluorescent and confirmed to be tumor, the surgeon will then proceed with the remainder of the surgical resection under white light conditions, but may look at the fluorescence imaging as desired.
    8. During the procedure the surgeon will biopsy tissue from 4 distinct regions ideally, preferably towards the peripheral margins of the resection. The surgeon will identify regions of interest under white light conditions but may look at the fluorescence image at the time of biopsy.
    9. If the surgeon identifies a region of interest with or without fluorescence that he/she decides is safe to biopsy, he/she may biopsy that tissue after looking at the monitor and the white light image. However, the extent of fluorescence determination for that biopsy will be made by the imaging operator independent of the surgeon decision.
    10. The surgeon collects the biopsy.
    11. Each tissue biopsy will be placed in a labeled and numbered container for blinded pathology to determine presence/absence of tumor.
    12. After completion of the resection the surgeon will look at the monitor and identify a region of non-fluorescent tissue that he/she feels is safe for biopsy, and this will be noted and collected as above. One (1) specimen of non-fluorescent tissue will be collected per patient (except for Schwannoma cohort) unless it is deemed unsafe to do so by the surgeon.

13. After completion of resection, if there are any regions of interest with fluorescence identified with the Canvas system by the surgeon, up to 5 additional biopsies of “other tissue” may be collected when feasible.
    a. The surgeon will designate the region of interest, reason for interest in capturing data and provide suspected nature (more likely tumor or less likely tumor). The imaging operator will note the surgeon’s designation. The surgeon may view fluorescence prior to designating region of interest for “other tissue.”
    b. The surgeon points to the planned area for biopsy/tissue collection for pathology.
    c. The imaging operator will look at the Canvas monitor and capture a white light and NIR image of the area.
    d. The imaging operator will note fluorescence status from the NIR image on the Canvas monitor, and will classify the intensity of fluorescence by a 3-point scale (none, weak, or strong).
    e. The surgeon looks at the monitor and documents his/her fluorescence assessment by the same 3-point scale (none, weak, strong) (Note: the surgeon’s assessment and the imaging operator’s assessment are two distinct assessments).
    f. The surgeon collects the biopsy.
    g. Each tissue biopsy will be placed in a labeled container as “other #1” to “other #5.”

Intraoperative fluorescence will be assessed as outlined in Figure .

Figure 10 Intraoperative fluorescence scoring



- Extent of resection by surgeon designation under white light conditions will be recorded as well as limitations to extent of resection at the conclusion of the case (e.g., resection of residual tumor tissue deemed to be unsafe for neurologic function of subject, such as in the event of brainstem invasion or involvement in eloquent cortex)
- At the end of the surgical procedure, an additional white light and fluorescent image of the surgical bed will be collected, and any residual fluorescence noted. If surgeons feel further resection of tissue (fluorescent or not fluorescent) he/she may proceed with resection based on best clinical judgement. A biopsy of the region where further resection is carried out will be sent to pathology for review.
- Local diagnostic pathology will be performed and used to record final diagnosis.
- Specimens with viable tumor (>10%) will be considered tumor positive.
- Intraoperative Fluorescence Measurement

  Refer to Section 1.5.2 for information on the Canvas Imaging Device. Detailed information is also provided in the current version of the Canvas IB.

  For every imaging procedure, the Canvas system collects a variety of data. A Procedure Record records data about the imaging procedure; e.g., location, operator, date-time. During the procedure, the surgeon or operator may collect a number of still-frame images. For each still-frame image, raw data image files are also captured to facilitate post-procedure analysis and image processing. Finally, the system can save real-time video recordings. These data files are stored in the Canvas system on a dedicated hard-

disk drive (HDD). The HDD has sufficient capacity to store all of the procedure data expected to be collected by the instrument during this clinical trial. Thus, no additional data management or migration tools or utilities are needed.

11.4 Surgeon Survey (Schwannoma cohort only)

Patient Study ID: _____

Surgeon Name: _____

Date completed: _____

Instructions: Below is a statement regarding the surgery you performed for your patient. Please circle or mark one number per line to indicate your response as it applies to the performed surgery.

	Strongly disagree	Disagree	Neither agree nor disagree	Agree	Strongly Agree
Tozuleristide associated fluorescence was useful during this surgical procedure.	(1)	(2)	(3)	(4)	(5)
Tozuleristide associated fluorescence improved the distinction between tumorous brain tissue and normal brain tissue.	(1)	(2)	(3)	(4)	(5)
Tozuleristide associated fluorescence improved the distinction between tumorous brain tissue and cranial nerves.	(1)	(2)	(3)	(4)	(5)
In the future, for a similar surgical indication, I would choose to use tozuleristide if it were available.	(1)	(2)	(3)	(4)	(5)