Project Title: Sodium Fluorescein-guided resection of pediatric neurosurgical tumors
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I. Hypotheses and Specific Aims:

Hypothesis: Sodium fluorescein, which has been shown to be useful for intraoperative guidance regarding the resection of adult neurosurgical tumors, can aid the resection of pediatric neurosurgical tumors.

Specific Aims: To study the utility of sodium fluorescein when used in conjunction with a surgical microscope equipped with fluorescent filters in the resection of pediatric central nervous system tumors (i.e. brain tumors and spinal tumors).

II. Background and Significance:

Several techniques using fluorescent biomarkers have been investigated as means to identify and delineate brain tumors from critical neural and vascular structures, and to facilitate resection of multiple types of adult brain tumors. In certain tumor types, such as adult gliomas, extent of resection is a primary determinant of outcome and is associated with longer life expectancy [1]. Unfortunately, glioma heterogeneity and the diffuse infiltrative nature of the tumors make complete resection difficult. Thus, in glioma as well as other types of nervous system tumors, improving the intraoperative detection of residual tumor while preserving normal tissue is crucial. Consequently, methods that can aid in achieving gross total resection while minimizing the risk of damaging normal adjacent tissue are of clinical significance and direct utility to patients. Currently, multiple surgical adjuncts exist, including intraoperative ultrasound, frameless sterotaxy, and intraoperative MRI.

Recent research in fluorescein-guided microsurgery has emerged as an additional adjunct to these tools. The two most commonly used and researched agents are 5-ALA and sodium fluorescein. 5-ALA is a metabolic precursor protein to the heme biosynthesis pathway that leads to the accumulation of a fluorescent protein, protoporphyrin IX, in gliomas when administered exogenously [2]. In their study, Stummer et al. examined the use of 5-ALA to determine if its use would lead to better tumor visualization, more complete resection, and potentially increased survival. They concluded that in addition to age, residual solid fluorescence and absence of contrast enhancement on post-operative MRI were independent explanatory factors for survival. The authors concluded that 5-ALA-induced tumor fluorescence was useful for guiding tumor resection. 5-ALA however is very expensive and currently not FDA approved for surgical resection of central nervous system tumors.

Sodium fluorescein is an exogenous green fluorescent contrast agent whose use has been mostly limited to ophthalmologic angiography for the diagnosis of malignancies and vascular lesions. Sodium fluorescein was first used in neurosurgery in 1948 and has been found to be safe for use in neurosurgery [3,5,7]. The use of systemically-delivered intra-operative fluorescein has been evaluated in gliomas (low-grade and high-grade), metastatic tumors, and various skull base lesions including schwannoma, meningioma, craniopharyngioma, and pituitary adenoma [6-13]. Koc et al. compared the influence of fluorescence guided resection in gliomas on gross total resection and survival in patients versus those who underwent standard microsurgical resection and found that the extent of fluorescence had a powerful effect on the rate of gross total resection (83% vs. 55%) despite no statistically significant difference in survival between the groups. Okuda et al., found that the use of fluorescein-guided resection may reduce the rate of local recurrence in patients with metastatic lesions, and da Silva et al., introduced the possibility of using fluorescein-guided

resection in skull base surgery for various lesions [11,14]. Recent groups have taken the concept of fluorescein-guided resection further by developing a microscope capable of emitting excitatory light and detecting fluorescence from sodium fluorescein in an effort to further maximize resection rates [12].

Significantly, systemically delivered sodium fluorescein has been found to be useful in guiding the resection of adult central nervous system tumors and has been found to be safe and effective as an intraoperative tool. Thus far, there has been a paucity of research exploring the usefulness of sodium fluorescein in the surgical resection of pediatric tumors [15].

Particular advantages may exist in the resection of spinal tumors, for which other operative adjuncts (e.g. neuronavigation) are not available. Spinal cord anatomical and physiological composition is very similar to the composition of the brain. The blood brain barrier is also present in the spinal cord of adults and pediatric patients and is composed of astrocytes. Tumors within the spine cause disruption of the spinal cord blood brain barrier and permit for extravasation of both contrast as well as sodium fluorescein [5]. In the absence of tumors, sodium fluorescein does not non-specifically extravasate into the parenchyma and this has been shown in spinal vascular applications [5,11,16]. Our institution has demonstrated safe administration and use of sodium fluorescein in adult spinal populations and we plan to apply this to our pediatric patient population as well. [17,18] We will utilize a phased method of enrollment and enroll patients with spinal lesions into the second phase of our study. Pediatric patients have a relatively high incidence of spinal tumor and would benefit from the use of sodium fluorescein which may prove to be crucial in the maximal safe resection of these lesions.

III. Preliminary Studies/Progress Report:

To date, there are no published data using sodium fluorescein for the resection of central nervous system (CNS) tumors in children. Our group has considerable experience with the use of sodium fluorescein in adult tumors [19-20].

The neurosurgeons at Children's Hospital Colorado have extensive experience with pediatric brain and spine tumor surgery. They perform approximately 100 tumor surgeries each year, making them one of the busiest groups in the country. Their relevant research experience is formidable, having published 25 research articles related to brain and spine tumors in the last two years alone. Two of these papers were specifically about the use of sodium fluorescein in guiding adult brain tumor resections.

IV. Research Methods

A. Outcome Measure(s):

The primary outcome measure will be extent of tumor resection. For all patients, pre-operative imaging and post-operative imaging will be used to analyze the extent of resection of the tumors. In intracranial lesions, the use of volumetric imaging tools will be used to calculate the extent of tumor resection. In spinal lesions, manual measurement will be used to determine the extent of resection.

Secondary outcome measures include the subjective utility of fluorescein per the operating surgeon, as well as positive predictive value, sensitivity and specificity for extent of tumor resection. Additional outcome measure(s) will vary depending on the neurosurgical tumor removed. Intraoperative videos and images will be collected from the surgical case. Images obtained will be correlated to tissue biopsy and will aid in the analysis of fluorescein

extravasation in pediatric neurosurgical tumors. All tissue samples collected will be taken for histopathological analysis.

B. Description of Population to be Enrolled:

Potential study participants include patients between 31 days and 21 years old, who are being seen at Children's Hospital Colorado for a central nervous system tumor (brain and spine tumor) and are undergoing neurosurgical resection. Enrollment will include 2 subsequent phases with the following tumor types in each phase:

First Phase of Study:
- High Grade Glioma
- Pilocytic Astrocytoma

Second Phase of Study:
- Medulloblastoma
- Ependymoma
- Contrast Enhancing Spinal Tumors

Inclusion Criteria:
- Age 31 days through 21 years on date of surgery
- Undergoing resection of a central nervous system tumor at Children's Hospital Colorado
- Parent/legal guardian (or adult subject) willing and able to complete the informed consent process

Exclusion Criteria:
- Tumor in functionally eloquent cortex that precludes maximal surgical resection
- Severe renal dysfunction
- Preoperative serum creatinine level > than normal range and GFR < 30.
- Severe liver dysfunction
- History of asthma or pulmonary spasm
- Known allergy to sodium fluorescein or any other contrast dye
- Previous administration of sodium fluorescein within the last 72 hours
- Pregnant or nursing mother
- Other unspecified reasons that, in the opinion of the investigator, make the subject unsuitable for enrollment.
- Tumors with primary differential diagnosis not included in the inclusion groups

C. Study Design and Research Methods

Study Design:

This study will be a prospective non-randomized cohort study with patients presenting to Children's Hospital Colorado for resection of a central nervous system tumor. Due to heterogeneity of the pediatric central nervous system tumors and the evidence available, we will have a planned initial cohort (first phase) that will include tumor pathology that will allow us to test the feasibility of the study and the preliminary activity of fluorescein in the pediatric population. After completion of this initial cohort, we will expand our study to include tumors that are more specific to the pediatric population.

Currently, there exist many tumor types where there is robust literature on the use of fluorescein [3,4,6-15,21-26,27-29]. We plan to enroll patients based on a phased system and after completion of the first phase, we will perform a safety analysis as outlined below.

After safety analysis is completed, we will either proceed to the next phase, or in the event there is a safety concern, the DSMC will terminate our study. Terms for termination are detailed below.

Enrollment of ten patients is planned for each tumor type in each of the phases. The phases are designed to assess patients where evidence is available and proceed to tumor types where pediatric evidence is limited and the tumors are more unique to the pediatric population. This will allow us to safely assess patients and allow us to expand our trial in a controlled fashion. After completion of each phase of enrollment, we will continue enrolling the prior phase patients for a maximal total of 10 patients per each pathology group. Thus, maximal enrollment will be 20 patients for the first phase pathologies and 30 patients for the second phase pathologies, for a total enrollment of 50 treated patients and 150 historical controls

First Phase of Study:

- High Grade Glioma
- Pilocytic Astrocytoma

Second Phase of Study:

- Medulloblastoma
- Ependymoma
- Contrast Enhancing Spinal Tumors

Study Drugs and Devices:

This study will employ the use of sodium fluorescein and an FDA approved operative microscope equipped with excitation and barrier filters for monitoring with sufficient fluorescent enhancement and contrast. Fluorescein sodium is a small organic salt with a molecular weight of 376 and excitation maximum at 494 nm and emission maximum at 521 nm. When injected intravenously, sodium fluorescein has a urine clearance of 24-72 hours. This agent accumulates in areas of diminished blood-brain barrier integrity and allows for determination of the margins between tumor and normal brain parenchyma [13].

Schedule of Events:

Table 1: Schedule of Events	**Preop Assessment**	**Surgery and Postop Inpatient Stay**		**Outpatient Follow-Up**
Visit Type (Standard of Care)	**Neurosurgery Visit/Consult/H&P**	**Day of Surgery**	**Prior to Discharge**	**[1]Follow-Up Visit(s) Discharge through Month 2**
Informed Consent/Assent	X			
Eligibility Assessment	X			
[Δ]Demographics	X			
[Δ]Medical History	X			
[Δ]Neurologic Exam	X		X	X
[Δ]Medical Exam	X		X	X
[Δ]Imaging (MRI/CT) of the brain and/or Spine	X		X	X
[Δ]Serum Creatinine	X			
[Δ]Pregnancy Test	X			
Sodium Fluorescein Administration		X		

[Δ]Tumor Resection Surgery		X		
[Δ]Intraoperative Photography/Videography		X		
[Δ]Tumor Biopsy/Pathology		X		
Adverse Events		X	X	X

[Δ]Standard of care procedures, data will be collected via chart review.
[1]See Follow Up section below for more detail

Research Methods:

Study Recruitment: The primary neurosurgeons who will perform the resections will be investigators on the study and will identify eligible patients from their established practice. At the time surgical intervention is recommended, they will inform patients and parents/guardians of the existence of the study. Only those subjects with a clinical relationship with the study investigators will be recruited.

Informed Consent and Assent: Patients (and parents/guardians of child patients) will be informed of the risks and possible benefits of the study, and informed consent will be obtained before any research procedures are initiated. Those who are interested in participating in the study will be informed of the study details by a member of the research team. Ample time will be provided to answer any questions and concerns regarding the study during the consenting process and patients will be given the option to take the consent form home to review. Additionally, patients will be provided with contact information in the event that questions should arise. Patients who undergo emergent surgery in which there is not sufficient time to obtain informed consent will be excluded from the study. In children 7-17 years, informed assent will also be obtained. Patients 18 years and older will consent for themselves.

Eligibility Assessment: All inclusion/exclusion factors that result from procedures (imaging or laboratory tests) will already be clinically documented as part of the standard of care, so will be evaluated via chart review for the study. There are no study-specific screening tests or screening procedures.

The exclusion criteria “preoperative serum creatinine level > normal range and GFR < 30” will be evaluated through chart review (creatinine level is included in standard of care preoperative lab results). The reference ranges used by Children’s Hospital Colorado’s clinical lab will be used to determine the normal range based on the patient’s age and gender. If a patient’s serum creatinine is greater than the normal range, GFR will be calculated using the following equation. If the GFR is < 30, the patient will be excluded.

Table 2: GFR Calculation

Serum Creatinine Reference Range

Age	Normal Range
31 to 364 Days	0.14 to 0.52 mg/dL
1 to 10 Years	0.23 to 0.61 mg/dL
11 to 17 Years	0.42 to 0.90 mg/dL
Female: 18 to 21 Years	0.52 to 0.99 mg/dL
Male: 18 to 21 Years	0.71 to 1.18 mg/dL

Calculation for GFR

$$GFR = \frac{0.41 * Height\ in\ cm}{Serum\ Creatinine\ in\ mg/dL}$$

The exclusion criteria “pregnant or nursing mother” will be evaluated for females of childbearing potential. We will utilize Children’s Hospital Colorado’s perioperative definition of childbearing potential as females who have reached menarche or are 12 years of age or older. Pregnancy testing is standard of care for these patients and pregnancy test results will be evaluated through chart review.

Pre-operative Evaluation: A standard of care neurosurgical *a*ssessment will occur at admission: standard clinical evaluation for tumor including pertinent history, physical and neurologic exam data, laboratory findings, and initial imaging studies will all be documented clinically and recorded via chart review for the study.

Imaging: Patients will have pre- and post-operative imaging in accordance with the standard clinical procedures at Children's Hospital Colorado. Patients in this study will not require any study-specific imaging outside the standard of care. All post-operative images are reviewed by fellowship trained pediatric specific neuroradiologists. Neuroradiologists are not informed on the administration of sodium fluorescein and will conduct review of the post-operative imaging per standard protocol. No imaging evidence of sodium fluorescein is visible on post-operative MR or CT imaging and the independent neuroradiologist is blinded to the operative course of the patient.

Sodium Fluorescein Administration: Patients enrolled in this study will receive 3 mg/kg sodium fluorescein following induction of anesthesia by administration into a peripheral venous line over 10 seconds. This dose has been previously reported for use in a pediatric population and has been studied in adult tumor population [15]. Previous studies have used doses ranging from 500mg standing to 20mg/kg with minimal side effects [7]. The FDA-approved dosage for children is 7.7 mg/kg intravenously. A lower dose and earlier administration reduces the incidence of fluorescent contamination of normal brain. Furthermore, the advent of new neurosurgical microscopes with fluorescein filters decreases the dosage required to observe fluorescence.

Tumor Resection: Resection of the lesions will proceed as normal as if sodium fluorescein had never been given. Resection will proceed with the use of a surgical microscope equipped with excitation and barrier filters for monitoring with sufficient fluorescent enhancement and contrast. The surgeon will be capable of switching the filters so as to observe the ordinary microscopic image and the fluorescein image as necessary. This ensures that the safety and normal operating procedure are minimally disturbed during this study.

Fluorescein absorbs at a peak between 465-490 nm which is selected for from the light source by the excitation filter. The barrier filter will only permit fluorescence emitted by sodium fluorescein in the 500-530 nm range to the surgeon's eye. The margins of resection will be based on identifying the fluorescein stained tissue that indicates neoplastic change and breakdown of the blood brain barrier. The resection will be performed piece by piece until the primary surgeon feels that surgical goal is achieved. Samples of fluorescent tissue will be sent to pathology for evaluation of histology.

Utilizing the microscope's internal recording device, we will record or photograph the surgery. When possible, biopsy location will be marked on the patient's preoperative MRI with the neurosurgical navigation device and a screenshot of this localization will be taken from the device system. This will only be possible for intracranial lesion and will not be used for spinal lesions. Images will be stored on a password-protected encrypted computer on the Children's Hospital Colorado server and accessible only to the investigators. Biopsies will be sent to pathology for diagnosis as is customary in all tumor resections, and diagnosis will be followed up in the patient's medical record by authorized study personnel. Fluorescein staining will be correlated with biopsies/pathological samples based on samples collected per standard of care. Official pathology reports will be used to determine pathological correlations with fluorescein staining.

Follow-Up: A neurosurgical assessment will occur post-operatively prior to discharge, as well as at standard of care outpatient follow-up clinic visits that take place between discharge and Month 2 post-surgery. These clinic visits typically occur at one week (for incision check) and one month post- surgery. Standard clinical evaluation for tumor includes pertinent history,

physical, neurologic exam data, and may also include laboratory findings and post-operative imaging studies. These will all be documented clinically and recorded via chart review for the study. Patients will be followed for the study through Month 2 (or 8 weeks) post-surgery.

Furthermore, any adverse events occurring after surgery will be collected until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study intervention (administration of fluorescein). All adverse events will be followed for outcome information until resolution or through Month 6 post-surgery.

For example, if a patient has no adverse events following surgery, they will only be followed through end of study period which is 2 months post-surgery. If the patient had adverse events within the first 30 days, those adverse events will be followed through resolution or 6 months post procedure, whichever comes first.

Re-Enrollment: Patients who have previously been enrolled in this study and received prior administration of fluorescein sodium as a part of this study for guided tumor resection are eligible for re-enrollment into the study, should further surgical resection be clinically indicated. Patients will receive the same dosage as outlined in the protocol of 3mg/kg. Clearance of 500mg of fluorescein sodium is 24-72 hours after administration of a single dose [16]. Patients re-enrolled into the study will be eligible after 3 days given the clearance time necessary for fluorescein sodium. Patients will be enrolled with the same study number. Patients will be re-consented at time of re-enrollment.

D. Description, Risks and Justification of Procedures and Data Collection Tools:

Potential Risks: Studies have examined the risk of using fluorescein for multiple purposes with no serious side effects [21]. The FDA has already approved the use of sodium fluorescein in adults and children for diagnostic angiography of the retina and it is also widely used as a contrast agent. The dose and route of administration being used in this study are consistent with the FDA approval for use of this drug in children.

The use of intravenous sodium fluorescein in gastrointestinal procedures has been reported to be extremely safe. Side effects from these studies include nausea/vomiting, injection site erythema, rash, and epigastric pain. Other investigators have examined the safety profile of intravenous fluorescein use in angiography. In these studies, reported potential side effects of sodium fluorescein administration include, urticaria, syncope, respiratory reactions, cardiac reactions, and hypersensitivity reactions. Multiple mechanisms have been suggested such as vasovagal phenomenon, histamine release via a nonallergic pathway, and sympathetic discharge related to anxiety as the pathogenetic basis of these side effects. The most common symptoms (>20%) reported in these studies include malaise, headache, dizziness, and nausea/vomiting [22-24].

A reported side effect of Fluorescein is yellowish skin for 6-12 hours after administration and yellow urine for 24-36 hours after administration. Fluorescein is systemically cleared by 48 to 72 hours after administration.

The package insert for Fluorescein describes “care must be taken to avoid extravasation during injection as the high pH of fluorescein solution can result in severe local tissue damage.” To alleviate this risk, the medication will be given IV push over 10 seconds.

Confidentiality of Study Data: Data including enrollment, study drug orders, pre-operative and postoperative exams and adverse event reporting will remain identifiable. De- identification of this information is not possible as patient identifying information is necessary for continued

patient safety monitoring and follow-up while in the trial.

Data that is collected clinically and analyzed for this study will remain identifiable in the patient's medical record but will be de-identified in the research records. Identifying information will be replaced with a unique study-specific code. All documents will be handled in accordance with the HIPPA policy and the Children's Hospital Colorado "Research Data – Ownership, Use, and Retention" policy. Only members of the research team will have access to the study documents and they will be stored on a password protected, encrypted computer on the secure Children's Hospital Colorado server. Paper documents will be stored in a locked filing cabinet in the secure administration pavilion at Children's Hospital Colorado.

Privacy Protections: Every effort will be made to protect patient privacy. Patients enrolling in the study will be informed with whom their information will be shared and they will sign a combined consent/HIPAA authorization form. Patients will not be identified if the study results are published or presented at a scientific meeting.

E. Potential Scientific Problems:

One possible limitation of this study is the single-institution nature of the project. Because Children's Hospital Colorado is a high-volume center, we are confident that we will be able to enroll the number of subjects needed for successful data analysis. Once this pilot project is complete, one of the future directions will be to conduct a multi-site study that can account for inter-institutional variance.

Another possible limitation is the absence of randomization to a control arm. We plan to use resection rates reported in the literature, as well as historical controls from our institution to compare to tumor resection rates during this pilot study.

F. Data Analysis Plan:

Annually at the Children's Hospital of Colorado, roughly 80 patients present with tumors requiring neurosurgical interventions. Prior adult population studies had enrolled between 10 – 40 patients. Importantly, these studies have largely involved single tumor types. Our study involves multiple tumor types and locations and would warrant increased enrollment for statistical analysis. Additionally, extent of surgical resection is an important outcome in our project. Using data extrapolated from adult studies, such as the most recent Niera et al. study, 32 patients were enrolled and compared to historical controls for tumor resection. This study demonstrated differences in extent of resection. Given the population and nature of our study, about 1/10 of the patients whom qualify for this study will be spine patients and the rest will be cranial with varying types of tumor pathology. [19]

Furthermore, if we use the number from this study [Niera] patients with fluorescein use had an average resection rate of 93.1% versus 77.1% for non-fluorescein historical control patients [19] Power calculations using a one-way ANOVA with alpha=0.05 power at 70%, 80%, and 90%, a mean difference of 16% (93.1-77.1) and a standard deviation of 25% are shown in Table 3.

Table 3: Power Calculation

Power	N Total
70%	64
80%	80
90%	106

Our pediatric neurosurgery/ neuro-oncology program benefits from a robust tumor program with multiple historical patients with reliable clinical follow-up that assesses for post-operative neurologic reactions. However, we do not believe that randomization is feasible in our study. The overall prevalence of pediatric brain tumors and the nature of the pathology would not allow our center the ability to perform a randomized trial in a meaningful time-frame. Our trial is designed to be a multi-year trial and randomization would double the study time for enrollment and greatly decrease feasibility to perform this study.

Given this, we propose a 3:1 ratio of historical controls to fluorescein treated patients to match tumor location and histology. 150 historical controls will be pulled from the last ten years of Children's Hospital patients and matched to the 50 fluorescein treated cases on lesion location and pathology. This 3:1 ratio will strengthen our ability to objectively assess for adverse events related to sodium fluorescein as well as our extent of tumor resection analysis. Importantly, historical controls undergo stereotactic-guided tumor resection in a similar fashion to patients undergoing fluorescein administrations as outlined in our protocol.

All patients who undergo sodium fluorescein-aided resection will also have neuropathological analysis. We will analyze correlation of fluorescein staining to histopathology. Tissue obtained during the time of surgery will be used to correlate fluorescein staining to histopathological diagnosis. This data will be used to calculate sensitivity and specificity of fluorescein staining for tumors. Pre- and post-operative imaging will be used to assess the degree of the extent of resection in intraparenchymal tumors. Data collected from post-operative follow-up will be used to assess for potential complications/adverse reactions associated with sodium fluorescein administration.

For data analysis, extent of tumor resection will be compared as a continuous value across groups using a parametric ANOVA. Further calculations, such as sensitivity, specificity and positive predictive value will be calculated by contingency tables. Additionally, since group membership is not random we will run an adjusted analysis predicting the likelihood of tumor resection as a continuous outcome adjusting for demographic and co-morbidity factors. Significance will be assigned at $p < 0.05$.

Furthermore, adverse reactions and population/demographic data will be characterized using relative risk and odds ratios. Overall survival and progression free survival analysis will be characterized using Kaplan-Meier curves and compared across groups using a log rank test.

G. Adverse Events and Unanticipated Problems:

1. Adverse Events and Serious Adverse Events

a. Definition of Roles:

- Sponsor: person listed in field 1 of form FDA 1571
- Site Principal Investigator: person listed in field 1 on form FDA 1572
- Licensed Independent Practitioner (LIP): MD with current Colorado Medical License

b. Definition of Adverse Events (AE)

Adverse event means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related (21 CFR 312.32 (a))

c. Definition of Serious Adverse Events (SAE)

An adverse event (AE) or suspected adverse reaction is considered "serious" if, in the view of either the Site Principal 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, or
- 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 participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

d. ***Classification of an Adverse Event***

i. Severity of Event:

A study team member, who is a Licensed Independent Provider (LIP) will be responsible for determining the severity of each AE.

For adverse events (AEs), the following guidelines will be used to describe severity:

- **Mild** – Events require minimal or no treatment and do not interfere with the participant's daily activities.
- **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
- **Severe** – Events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term "severe" does not necessarily equate to "serious".]

ii. Relationship to Study Intervention:

A study team member, who is a Licensed Independent Provider (LIP) will be responsible for determining the relationship of each AE to the study intervention.

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition.
- **Potentially Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", or downgraded to …, as appropriate.
- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- **Not Related** – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the

iii. Expectedness:

A study team member, who is a Licensed Independent Provider (LIP) will be responsible for determining whether an AE is expected or unexpected.

An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

e. Time Period and Frequency for Event Assessment and Follow Up

21 CFR 312.64(b) requires that the Site Principal Investigator records non-serious adverse events and reports them to the Sponsor according to the timetable for reporting specified in the protocol.

A properly delegated study team member will record all adverse events with start dates occurring any time after administration of fluorescein until 7 (for non-serious AEs) or 30 days (for SAEs) after the last day of study intervention. All events will be followed for outcome information until resolution or stabilization or through Month 6 post-surgery.

All AEs occurring while on study will be documented regardless of their relationship to the study intervention, and whether or not they meet the definition of an SAE. AEs will be captured, reviewed and assessed by an LIP member of the study team. The outcome of this review and assessment will be documented by a properly delegated study team member on an Adverse Event Log. Information to be collected includes event description, time of onset (start date) and time of resolution/stabilization of the event (end date), and the LIP's assessment of expectedness, severity and relationship to study intervention.

Any medical condition that is present at the time the participant is screened will be considered as baseline medical history, and will not be recorded as an AE. However, if the study participant's condition deteriorates at any time during the study, the event will be recorded as an AE.

Changes in the severity or frequency of an AE will be documented as separate events to allow an assessment of the duration of the event at each level of severity or frequency.

AEs characterized as intermittent will be documented with onset and duration of each episode.

At each study visit, a properly delegated study team member will document all occurrences of AEs and SAEs since the last visit. This information will be gathered through direct observation (e.g. rash), elicited from or spontaneously volunteered by the participant, documentation in Medical Records, laboratory or other clinical reports or notes, or identified in patient-reported outcome tools (e.g. diaries, surveys, questionnaires), or any other means.

f. Adverse Event Reporting

Adverse Event Reporting will be performed in accordance with the study protocol, any contractual agreement, and all IRB requirements.

21 CFR 312.64(b) requires that the Site Principal Investigator records non-serious adverse events and reports them to the Sponsor according to the timetable for reporting specified below.

Disease-Related Events (DREs): The following disease-related events (DREs) are expected and common with brain tumor surgery, and will not be reported per the standard process for adverse event reporting: bleeding in the brain, injury or swelling to the brain, infection in the brain or at wound site, meningitis, seizures, hydrocephalus, cerebral spinal fluid leakage, the need for

additional surgery, tearing of the eyes, damage to the eye, blood clots, stroke, dizzy spells, recurrence or tumor growth, postoperative pain and allergic reactions to anesthesia.

AEs will be reported as follows (check the arrangement to be used for this protocol):

i. To Safety Monitoring entity:

☐ Study Principal Investigator is an LIP and provides safety oversight

- Review of AEs and SAEs will be conducted in regularly occurring research team meetings that at scheduled at pre-determined frequency. The review and any follow-up action will be documented in meeting minutes.

☐ Safety Officer/Monitor is an LIP and provides safety oversight

- Review of AEs and SAEs will be conducted at predetermined intervals in accordance with the Safety Monitoring Plan developed for this study
- Safety officer/monitor may be independent or part of study team, as appropriate to risk and phase of study

☒ Data Safety Monitoring Board/Committee (DSMB/C)

- Review of AEs and SAEs will be conducted in accordance with the DSMB Charter and Data Safety Monitoring Plan developed for this study

ii. **To IRB:**

Within 5 days of becoming aware of them, a properly delegated study team member will report to the IRB any AE that meets the definition of an unanticipated problem (UAP), which means the event is are unexpected, related to the research procedures, and caused harm or risk of harm.

SAEs that do not meet the criteria for prompt reporting will be tabulated on an aggregate reporting form for submission at the time of continuing review.

Note: If there is an independent safety monitor for the study (e.g., Safety Officer, DMC, or DSMB), aggregate reports do not need to be submitted at the time of continuing review. Instead, a summary report from the monitor should be submitted at the time of continuing review.

iii. To FDA:

A narrative or tabular summary showing the most frequent and most serious adverse events by body system will be provided by the Sponsor to the FDA with each annual report in compliance with 21 CFR 312.33(b)(1).

g. Serious Adverse Event Reporting

The Site Principal Investigator will immediately report to the Sponsor any serious adverse event, whether or not considered study intervention related, including those listed in the protocol or investigator brochure, and will include the study team LIP's assessment of whether there is a reasonable possibility that the study intervention caused the event.

Study endpoints that are serious adverse events (e.g. all-cause mortality) will be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between

the study intervention and the event (e.g., death from anaphylaxis). In that case, the Site Principal Investigator must immediately report the event to the Sponsor.

All SAEs will be followed until satisfactory resolution or until the study team's LIP deems the event to be chronic or the participant is stable.

Serious adverse events that are considered related (i.e., determined to be possibly, probably, or definitely related) to the investigational product by the Sponsor will be followed until the event resolves or stabilizes. Any SAE that occurs after treatment completion, and is considered by the Sponsor to be related to the investigational product, will be documented and reported as appropriate.

i. To FDA:

The Sponsor is responsible for notifying the Food and Drug Administration (FDA). Events meeting the following criteria will be submitted to the FDA as expedited IND Safety Reports according to the following guidance and timelines:

7 Calendar Day Telephone or Fax Report
The Sponsor will notify the appropriate review division at the FDA of any fatal or life-threatening adverse event that is unexpected and assessed by the Sponsor to be possibly related to the use of the investigational product. An unexpected adverse event is one that is not already described in the investigational product's Investigator Brochure. Such reports are to be telephoned or faxed to the appropriate review division at FDA and to the manufacturer of the investigational product within 7 calendar days of first learning of the event.

The report will be completed using Form FDA 3500A (Mandatory Reporting).

15 Calendar Day Written Report
The Sponsor will notify the appropriate review division at the FDA, in a written IND Safety Report, of any serious, unexpected AE that is considered reasonably or possibly related to the use of the investigational product. An unexpected adverse event is one that is not already described in the investigational product's investigator brochure.

Written IND Safety reports will include an Analysis of Similar Events in accordance with regulation 21 CFR § 312.32. All safety reports previously filed by the Sponsor with the IND concerning similar events will be analyzed and the significance of the new report in light of the previous, similar reports commented on.

Written IND safety reports with Analysis of Similar Events will be submitted to the appropriate review division at the FDA, and the manufacturer of the investigational product within 15 calendar days of first learning of the event, using Form FDA 3500A.

Written IND Safety Reports will be submitted to the IRB(s) of record in accordance with IRB Guidelines.

IND Annual Reports
The Sponsor will provide annual reports to the appropriate review division at the FDA within 60 days of the IND's anniversary date, until the IND is withdrawn or terminated.

ii. To Safety Monitoring entity:

SAEs will be reported to the safety monitoring entity at the next scheduled research team meeting or as defined in the Safety Monitoring Plan, which ever applies to this study.

2. Unanticipated Problems

a. Definition of Unanticipated Problems (UAP)

An unanticipated problem involving risk to participants or others includes any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the Institutional Review Board (IRB)-approved research protocol and informed consent document; and (b) the characteristics of the participant 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 participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

b. Unanticipated Problem Reporting

The Site Principal Investigator will report unanticipated problems (UAPs) to the reviewing Institutional Review Board (IRB) per IRB policy.

c. Reporting Unanticipated Problems to Participants

Any event which may relate to subjects' willingness to continue participation will be provided to the subjects, per IRB policy.

H. Data and Safety Monitoring

The PI will be responsible for monitoring the trial, in addition to overseeing the safety and efficacy of the trial, executing the DSM plan and complying with all reporting requirements to the local and federal authorities. This oversight will be accomplished through additional oversight from the Data and Safety Monitoring Committee (DSMC) at the University of Colorado Cancer Center (CU Cancer Center).

The DSMC is responsible for ensuring data quality and patient safety for all clinical studies at the CU Cancer Center. A summary of the DSMC's activities is as follows:

- Conduct internal audits
- Ongoing review of all serious adverse events (SAEs), unanticipated problems (UAPs) and reportable adverse events (AEs)
- Has the authority to close and/or suspend trials for safety or trial conduct issues
- May submit recommendations for corrective actions to the CU Cancer Center's Executive Committee

All SAEs, UAPs and reportable AEs are to be reported to the DSMC within 5 business days of receiving notification of the occurrence.

The PI will provide a DSM report to the CU Cancer Center DSMC on a 6-month basis. The DSM report will include a protocol summary; current numbers; summary of toxicity data to include specific SAEs, UAPs and AEs; any dose modifications; all protocol deviations; and protocol amendments. Results and recommendations from the review of this 6-month report by the DSMC will then be submitted by CHCO to COMIRB at continuing review.

Study audits conducted by the DSMC will consist of a review of the regulatory documents, consent forms, and source data verification. Documentation of the audit conducted by the DSMC will then need to be submitted to the IRB of record at the time of the IRB's continuing review of this trial.

Importantly, the DSMC is composed of personnel that includes persons with specialty training in pediatric oncology and pediatric surgery. The DSMC at our institution routinely monitors both adult and pediatric trails at our institution. The oversight of our study will include normal DSMC activities with an additional specific review of our study after the first phase of enrollment to evaluate the safety of our study. DSMC approval will be obtained prior to proceeding to the second enrollment phase of enrollment.

Study Stopping Criteria

The objective of the safety review is to decide whether the study should continue per protocol, proceed with caution, be further investigated, be discontinued, or be modified and then proceed. Suspension of enrollment is a potential outcome of the safety review.

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party (DSMC) to the PI, funding agency, and regulatory authorities. If the study is prematurely terminated or suspended, the PI will promptly inform the IRB and will provide the reason(s) for the termination or suspension.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants defined by reporting of AEs, SAEs and UAPs
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination of futility
- Study closure based on PI decision or sponsor/funder decision

The study may resume once concerns about safety, protocol compliance, data quality are addressed and satisfy the sponsor, IRB and/or FDA.

I. Summarize Knowledge to be Gained:

Sodium fluorescein for intraoperative neuronavigation using a neurosurgical microscope has been useful in the resection of adult nervous system tumors. Currently, there are no studies researching sodium fluorescein in pediatric brain and spine tumors. The application of this technology has the potential to: increase the fidelity of tumor tissue identification, increase potential to achieve safe maximal resection, and increase overall survival and prognosis in brain and spine tumor patients.

J. References:

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Person in Charge of the Study: Todd Hankinson, MD, MBA
COMIRB No: 18-0242
Version Date: March, 29, 2021

Assent Form for: Sodium Fluorescein-Guided Resection of Pediatric Neurosurgical Tumors

What is this study about?
You are being asked if you want to be in this research study. The goal of this study is to use a fluorescent dye called sodium fluorescein to help doctors see the tumor better during surgery.

Why are you asking me?
You are being asked to be in the study because you have brain tumor or a spine tumor.

What Do I Have to Do or What Will Happen to Me?
If you are in the study, you will be asked to:

- Have sodium fluorescein dye put through your IV while your asleep for surgery
- Allow the study doctor to video record and take pictures of your surgery
- Allow study staff to look at your medical records and images
- Allow a microscope company called Leica Microsystems to look at your video and images from surgery

The study will last for 2 months. The only time you will get the dye is during surgery.

You don't have to answer any questions that you don't want to, and you can stop at any time.

Will this Hurt?
The dye will be put through your IV while you are asleep for surgery. There will not be extra pokes for this study. Everything else will be part of your normal surgery care.
If you are in the study, you could have:

- Your skin and urine turn yellow
- A funny taste in your mouth
- Pain in your stomach and throwing up
- Pain in your head
- Dizziness
- Itchy or red skin

These things do not usually happen, but you could have:

- A skin injury where your IV was
- An allergic reaction
- Passing out
- Heart problems
- Unusual body movements

- Blood clots
- Lung problems
- Death

Can I ask Questions?
You can ask any questions that you have now about the study.

If you have a question later, you can ask and get an answer. If you want to, you can call Todd Hankinson at (720)-777-2985.

Do I Have to Do This?
You do not have to be in this study. No one will be mad at you if you say no. You can choose to stop at any time. Just tell the researcher if you want to stop.

Do you want to be in the study at this time? (Check one box)
☐ Yes ☐ No

You will get a copy of this form to keep.

Child's Signature:_____ **Date**:_____

Child's Printed Name:_____

Consent form explained by:_____
(Print name)

I have explained the research at a level that is understandable by the child and believe that the child understands what is expected during this study.

Signature of Person Obtaining Assent*:* _____

Date: _____