Study Protocol

Protocol Title: Utilization of Confocal Microscopy during Cardiac Surgery

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Utilization of Confocal Microscopy during Cardiac Surgery

A. Specific Aims/Objectives

This is a pilot study, exploring the safety and feasibility of using diluted fluorescein sodium with fiberoptic confocal microscopy during cardiac surgery.

- The primary aim of this study is to evaluate the safety of using diluted fluorescein sodium during cardiac surgery.
- A secondary aim of this study is to evaluate the feasibility of using this imaging modality. Feasibility will be evaluated in two ways: logistically and technologically.
    - Logistical feasibility will be evaluated by measuring increased time on bypass, rate of enrollment, and the ability to incorporate this imaging modality into standard OR procedures.
    - Technological feasibility will center on the ability to capture quality image files. A quality image file is defined as an image file where a rater can visualize the distinguishable features of the tissue type (cardiac tissue, conductive tissue) in order to classify that tissue type correctly.

B. Background and Significance

Congenital heart disease is the most common type of birth defect. Approximately 32,000 new cases of congenital heart disease occur in the US every year, and there are approximately 1.5 million new cases annually worldwide.[1, 2] More than 20,000 pediatric cardiac operations are performed each year in the US.[3] Over the past two decades, emphasis has been on the complete repair of the congenital cardiac defects in infancy. This paradigm shift has improved the surgical outcomes and neurodevelopmental progress of these infants. Without surgical intervention, these defects are uniformly lethal.[4] However, there are several complications associated with surgical intervention, including dysfunction of sino-atrial and atrio-ventricular conduction pathways. Despite the complexity and individual variations in cardiac conductive pathways, tissue discrimination during surgery is currently limited to the use of anatomic landmarks and measurements of local electrical activity, so accurate surgical intervention is therefore challenging and risky. In addition, there are only sparse data on the location and development of these pathways in pediatric hearts with congenital defects.[5] One of the perioperative complications that can be induced during these procedures is complete heart block purportedly associated with interruption of cardiac conduction pathways, which can occur due to damage to conductive tissues from clamping, suturing, or other procedures. Complete heart block contributes significantly to increased morbidity and mortality.[6] Once complete heart block has occurred and there has been no recovery by the patient, a permanent cardiac pacemaker must be implanted.[7] This creates a lifetime dependence on the pacemaker along with the associated lead and generator changes.[8] A study involving 497 patients over a 22-year period showed that lead failure occurred in 155 leads (15%) and 115 patients (23%), with a higher incidence of lead failures in pediatric patients.[9] With more complex procedures, the risk of complete heart block or sinus node dysfunction increases accordingly. Lifetime costs and morbidity associated with pacemaker implantation in pediatric patients are very high.[8] The economic burden of this rhythm management therapy is significant, especially when considering the average lifespan of these young patients.[10] There is also a societal burden, as evidence points to long-term morbidity associated with surgical correction of congenital heart defects.[11] Despite the significant improvement in surgical results, there is still a critical barrier that needs to be overcome, which is to avoid preventable causes of conduction defects.

The Cellvizio 100 Series System with Confocal Miniprobes is a confocal laser system with fiber optic probes that has been approved by the FDA to allow real-time imaging of the internal microstructure of tissues in anatomical tracts. This system is capable of acquiring and displaying microscopic images

directly after tissue contact with a rate of up to one image every 5 milliseconds (200 images per second). Preliminary studies (described in more detail below) suggest that image data obtained by this system allows for discrimination of the cardiac conduction system. We hope to develop a system for intraoperative identification of tissue of the conduction system, which could potentially decrease the incidence of preventable causes of conduction delays in the operating room. Reducing the damage to the conduction system tracts may preserve autonomic nervous activity and lead to improved short and long term outcomes for these patients.[12] Furthermore, this technology could be utilized not only for reconstructive pediatric heart surgery, but also for other corrective surgeries where detailed tissue discrimination would improve patient outcomes.

C. Preliminary Studies

In a recently published study, we established an approach based on fluorescent labeling, conventional confocal microscopy, and image analysis to characterize cardiac tissue types.[13] Tissue preparations from adult rat hearts were dissected using anatomical landmarks such as the superior vena cava and coronary sinus. The tissue preparations were labeled with 4',6-diamidino-2-phenylindole (DAPI) to identify nuclei, wheat germ agglutinin (WGA) conjugated Alexa 488 to define the extracellular space, antibodies for hyperpolarization-activated cyclic nucleotide-gated potassium channel 4 (HCN4) to identify nodal myocytes[14-17] and sarcomeric α-actinin to identify cardiomyocytes. Conventional confocal microscopy of the tissue preparations yielded 3D distributions of the four labels at a high resolution.

Using this method, we were able to characterize the microstructure of various cardiac tissue types. Atrial working myocardium (AWM) was characterized by a dense and aligned arrangement of myocytes that were covered by an epicardial layer with a thickness of 8.26±3.60 μm. Sino-atrial node (SAN) was characterized by three distinct layers comprised of epicardium, SAN cells, and subjacent myocytes of the AWM. The epicardial and nodal layers had thicknesses of 10.38±4.79 μm and 10.26±3.33 μm, respectively. SAN cells formed a highly irregular reticulum and had notably smaller diameters than myocytes of the AWM. The extracellular space in the SAN layer was also more prominent than in AWM and was comprised of oval shaped clearings of variable size. Atrio-ventricular node (AVN) and Bundle of His tissue presented a microstructural arrangement similar to SAN. It exhibited major differences from AWM tissue, in particular, an irregular reticulum of cells with ample extracellular space. The epicardial layer of AVN tissue had a thickness of 2.84±1.25 μm.

Following the investigations using conventional confocal microscopy, we investigated the hypothesis that fiberoptic confocal microscopy (FCM) technology could be used to identify the differences in tissue microstructure between nodal tissue and working myocardium in living Langendorff perfused rat hearts. An aqueous solution of 10 kDa dextran-conjugated Alexa 488 was pipetted to the tissue surface. It has previously been shown that dextran-conjugates of this molecular weight penetrate endothelial endocardium, endothelial epicardium, and myocardial capillary endothelium in rat, but do not penetrate intact cell membranes, and thus label specifically the extracellular space.[18] We found that FCM images from the dextran-labeled AWM and nodal regions resembled images obtained using conventional confocal microscopy.

The FCM images were of high enough quality to visually distinguish tissue types. We also explored approaches for automated image analysis of the conventional microscopy and FCM images. We evaluated the ability of two methods of 2D texture analysis to discriminate between AWM and nodal tissue using images of the extracellular space. The first method measured the texture orientation from Fourier transformed images.[19] The second method characterized texture orientation from second order image moments of image regions.[19] Both methods of 2D texture analysis yielded a larger magnitude of our defined measure of orientation in AWM in comparison to SAN and AVN tissue.

In further studies, we demonstrated that fluorescein sodium (Fluorescite®) is an equally reliable label of extracellular space. Fluorescite® is approved by the FDA for intravenous administration for use in ophthalmic angiography, and its toxicity has been extensively studied.[20] Fluorescite® is also used in gastrointestinal endoscopic imaging. The incidence of adverse reactions following intravenous administration of fluorescein is 1.1-4.8% for retinal fluorescein angiography and 1.4% for gastrointestinal endoscopic imaging.[21-23] In these studies, adverse reactions including dizziness, nausea, vomiting, and transient hypotension have been reported. However, there were no serious adverse reactions or deaths. For our purposes, we determined that localized, topical delivery of the dye would allow us to use a smaller amount and be equally effective.[24] Human examiners were able to classify FCM images obtained using fluorescein delivered by dye carrier as either AWM or nodal tissue with high degrees of sensitivity (99.2%±0.3) and specificity (98.0%±0.7). In further studies, we showed that topical delivery of the dye by pipette or surgical swab was equally effective to the dye carrier method, and allowed us to use a concentration of dye that is 1000 times more dilute than what is approved by the FDA.

Our research team is currently conducting experiments in sheep, but the data have not been published. In these studies, 3 sheep have been anesthetized and placed on cardiopulmonary bypass. Between 5-7mLs of 1:1000 diluted Fluorescite® was applied to the epicardium and endocardium, and images were obtained using the Cellvizio Imaging system. After the images were obtained, the animals were taken off bypass. ECGs were obtained at baseline, after re-perfusing the hearts, and after the animals were taken off bypass. The ECGs confirmed that after the procedure, the hearts resumed normal baseline cardiac activity. Visual examination of the hearts also confirmed normal ventricular function and vigorous activity. Furthermore, preliminary imaging data from these studies confirms that we are able to discriminate conduction tissue from the images taken.

D. Design and Methods

(1) Study Design

We propose a descriptive pilot study to assess the safety and feasibility of using fiber-optic confocal microscopy during cardiac surgery to image the endocardium and epicardium. 6 patients undergoing surgery for closure of atrial septal defect will be enrolled in this study.

All enrolled subjects will receive standard of care for cardiac surgery and the operation will be conducted according to routine procedure. After the sternotomy, the subject will be placed on cardiopulmonary bypass. After cardioplegic infusion and cardiac arrest, the right atrium will be opened to visualize the atrial septal defect.

At this juncture, fluorescein sodium at a concentration of 100ug/ml in sterile saline will be applied topically to the myocardium, where it will diffuse into the endocardium and epicardium. A total volume of 5ml will be applied. The surgeon will confirm there are no visual indicators of an allergic reaction to the fluorescein study drug. As long as there is no appearance of tissue damage, the imaging will proceed. If any signs of allergy or intolerance occur, the dye will be immediately flushed out and no images will be obtained.

The imaging probe of the Cellvizio system will then be applied in direct contact to the region of interest with minimal pressure for approximately 90 seconds. During this period, Cellvizio Software and Viewer will be used to obtain images. The total additional time on bypass will be approximately 3 minutes.

Once the imaging procedure has been completed, the heart surface will be washed with saline and evacuated using a suction apparatus, to ensure all fluorescein has been removed and will

not enter the circulatory system. After that, the remainder of the operation will proceed in the usual fashion. As per standard of care for patients undergoing cardiac surgery, an intraoperative echocardiogram will be performed after the subject is separated from bypass.

The expected hospitalization for the Atrial Septal Defect (ASD) is 3-5 days post-operation. It is not expected that participation in this study will increase the post-operative hospital stay or post-hospitalization recovery. The principal investigator (PI) and research team will monitor clinical labs, electrocardiograms (EKGs), and echocardiograms for any evidence of adverse events. No additional follow-up testing, outside of standard clinical monitoring, are planned. In accordance with standard of care, all subjects will receive a pre-discharge EKG and trans-thoracic echocardiogram.

Image files will be exported out from the Cellvizio system, de-identified, and provided to the blinded raters. The image files will be assessed for quality and the ability to discriminate tissue types.

(2) Patient Selection and Inclusion/Exclusion Criteria

Patients undergoing cardiac surgery for ASD repair at Boston Children's Hospital will be enrolled in this study. Surgery for ASD repair exposes the conduction system of the heart, allowing access to the AWM and nodal tissue for image capture by the Cellvizio system. Congenital heart defects such as ASD are most often diagnosed and repaired in infants and children, which is why conducting this study in a pediatric population is appropriate. Additionally, the FCM has a pre-defined field of focus, with a depth ranging from 55 to 65 µm. Therefore, this technology is more suited to pediatric hearts, as adult hearts are thicker and the conductive tissue may be too deep to be identified using this method.

Inclusion criteria:

- Males and females between 30 days and 21 years old
- Undergoing elective surgery for closure of isolated secundum atrial septal defect
- Both parents attend pre-operative clinic appointment, to provide 2 parent consent, or an adult participant (age 18-21) may provide consent for him/herself

Exclusion criteria:

- Prior history of adverse reaction to fluorescein sodium
- Prior history of renal failure or abnormal renal function
- Baseline PR interval > 220 msec or 98% for age
- Baseline HR > 98% for age
- Underlying genetic syndrome associated with progressive AV block of sinus node dysfunction (e.g. Holt-Oram or NKX2.5)

(3) Description of Study Treatments or Exposures/Predictors

(3.1) Investigational Agent

Fluorescite® (fluorescein injection, USP) 10% contains fluorescein sodium (equivalent to fluorescein 10% w/v) (Alcon). Fluorescite® is commercially available, a supply of which will be purchased for the sole purpose of this study. The Boston Children's Hospital (BCH) research pharmacy will be responsible for the receipt and accountability of study agent. The pharmacy will maintain accountability logs with quantities received and dispensed, specifying the corresponding dates, and the identification code of the study subject.

The research pharmacy will dilute 1mL of the study drug into 1L of saline. A 5mL syringe of the 1:1000 diluted solution will then be dispensed to the OR. Any unused prepared study drug will be recorded, and then discarded or destroyed according to Boston Children's Hospital policy. The vial of undiluted Fluorescite® will also be discarded or destroyed according to BCH policy.

(3.2) Device

The Cellvizio 100 Series System and the Gastroflex UHD probe (Mauna Kea Technologies) will be used for imaging in this study. This instrument has been approved by the FDA for gastrointestinal, urologic, gynecologic, and pulmonary applications. This device will be provided by the manufacturer for the purposes of this study, along with the associated control and acquisition software. The PI has been thoroughly trained on the use of this hardware and software. The cardiac surgery operating room will be responsible for the receipt, accountability, and return of the microscope tower and imaging console. The imaging console will be stored in the cardiac operating room. The microprobes will be sterilized according to standard protocol and stored in a sterile pouch in the operating room. The imaging console is password protected and will only be accessed by the PI.

The probe will be used in direct contact with the cardiac tissue with a pressure of approximately 0.7 mmHg, which is less than 1% of diastolic blood pressure. The maximum laser energy that the system is capable of delivering is 15 mW at 480 nm. This power and wavelength has been approved by the FDA for direct in-vivo imaging for various body tissues, including gastrointestinal, urogenital, and pulmonary. The proposed application is approximately 80% less than the maximum allowable retinal exposure, according to the IEC 60825-1 standard.

(4) Definition of Primary and Secondary Outcomes/Endpoints

Primary Outcome: All adverse events will be collected and relatedness to study participation will be determined. Complications will be compared to average complication rates for ASD repairs.

Hypothesis: Subjects will not experience a higher rate of adverse events than is expected in a clinical setting (<3-4% for all types of adverse events).

Secondary Outcome: The logistical feasibility of conducting FCM imaging during cardiac surgery.

Hypothesis: The study procedure will be logistically feasible in terms of added time on bypass and the ability to incorporate FCM imaging into standard cardiac surgery OR procedures. The rate of enrollment will be evaluated separately.

Secondary Outcome: The quality of the FCM image files collected, defined as the ability of observers to distinguish features and discriminate between working myocardium and nodal tissue.

Hypothesis: Blinded raters will be able to use the FCM image files to distinguish features and discriminate between working myocardium and nodal tissue.

(5) Data Collection Methods, Assessments, Interventions and Schedule (what assessments performed, how often)

Standardized demographic and clinical assessments of all study participants will be performed via medical record review prior to and after surgery until the subject is discharged from the hospital. Images will be obtained during surgery and saved using Cellvizio Software and Viewer. The image files will be de-identified and provided to blinded raters for analysis. Medical records of enrolled subjects will be reviewed post-surgery while the subject is inpatient in the hospital. Adverse events will be abstracted from the record and recorded on a CRF and input into an InForm database.

(6) Study Timeline

With several patients eligible weekly, it should be feasible to complete subject recruitment, enrollment, and follow-up within 6 months after study activation. Lead time will be needed to secure FDA and BCH Institutional Review Board (IRB) approval.

E. Adverse Event Criteria and Reporting Procedures

(1) Specification of Safety Parameters

Comprehensive assessments of any apparent toxicity experienced by the study subject will be performed throughout the course of the study. All safety events experienced by study subjects for the duration of the study will be documented. The types of adverse events that must be reported and procedures for appropriately recording, grading and reporting are outlined in this section.

(2) Definition of an Adverse Event (AE) or Medical Event

According to FDA regulations, an adverse event (AE) is any undesirable, noxious, or pathological change compared to preexisting conditions occurring to or in a study subject enrolled in a clinical trial, whether or not the event is considered related to the test drug. This includes the time periods during which no medication is administered to a study subject, such as run-in or follow-up periods. An adverse event is further defined as any change in structure (signs) or function (symptoms) of the body that occurs while the study subject is receiving study drug/placebo. AEs include the following types of changes:

- Suspected adverse drug reactions, which are any adverse events for which there is a reasonable possibility that the drug caused the adverse event. A reasonable possibility implies that there is evidence that the drug caused the event.

- Other medical experiences regardless of their relationship to the test drug, such as injury, surgery, accidents, extensions of symptoms or apparently unrelated illnesses, and significant abnormalities in clinical laboratory values, physiological testing, or physical examination findings.

(3) Definition of Serious Events (Serious Adverse Events, Serious Suspected Adverse Reactions or Serious Adverse Reactions)

A serious adverse event including a serious suspected adverse reaction or serious adverse reaction as determined by the Investigator or the sponsor is any event that results in any of the following outcomes:

1. Death. This includes any death that occurs during the conduct of a clinical study, including deaths that appear to be completely unrelated to the test drug (e.g., a car accident). If a study subject dies during the study and an autopsy is performed, the autopsy results will be attached to the study subject's Case Report Form (CRF). Possible evidence of organ toxicity and the potential relationship of the toxicity to the test drug are of particular interest. The autopsy report should distinguish the relationship between the underlying diseases, their side effects, and the cause of death.

2. Life-threatening adverse drug experience. This includes any AE during which the study subject is, in the view of the investigator, at immediate risk of death from the reaction as it occurred. This definition does not include any event that may have caused death if it had occurred in a more serious form. For example, drug-induced hepatitis that resolved without evidence of hepatic failure would not be considered a life-threatening event, even though drug-induced hepatitis can be fatal.

3. Persistent or significant disability or incapacity or substantial disruption of the ability to conduct normal life functions.

4. Inpatient hospitalization or prolongation of existing hospitalization, except for admission for elective surgery.

5. Important medical event that may not result in one of the above outcomes, but may jeopardize the health of the study subject or require medical or surgical intervention to prevent one of the outcomes listed in the above definition of serious event.

(4) Definition of Unexpected Adverse Events

An adverse event is "unexpected" when its nature (specificity) or severity is not consistent with applicable product information, such as safety information provided in the package insert, the investigational plan, the investigator's brochure or the protocol.

In addition, an adverse event is "unexpected" when not one of the most common complications that typically arise post-operation for ASD repair such as: rhythm disturbances, arrhythmia, pericarditis, cardiac tamponade, bleeding, residual holes requiring a re-operation, transfusion associated diseases, stroke, and brain damage. These adverse events will be considered expected and unrelated to the study drug.

(5) Toxicity Grading

We will assign toxicity grades to indicate the severity of adverse experiences and toxicities. Toxicity grading consistent with NCI-CTCAE v 4.03 will be used for this protocol. The purpose of using the NCI-CTCAE system is to provide standard language to describe toxicities and to facilitate tabulation and analysis of the data and assessment of the clinical significance of treatment-related toxicities.

Adverse events not included in the NCI-CTCAE listing will be recorded and graded 1 to 5 according to the General Grade Definition provided below:

Grade 1	Mild	Transient or mild discomforts (< 48 hours), no or minimal medical intervention/therapy required, hospitalization not necessary (non-prescription or single-use prescription therapy may be employed to relieve symptoms, e.g., aspirin for simple headache, acetaminophen for post-surgical pain).
Grade 2	Moderate	Mild to moderate limitation in activity, some assistance may be needed; no or minimal intervention/therapy required, hospitalization possible.
Grade 3	Severe	Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalization possible.
Grade 4	Life-threatening	Extreme limitation in activity, significant assistance required; significant medical/therapy intervention required, hospitalization, or hospice care probable.
Grade 5	Death	Death

For additional information and a printable version of the NCI-CTCAE v. 4.03 manual, consult the NCI-CTCAE website, http://ctep.cancer.gov/reporting/ctc.html.

(6) Guidelines for Determining Causality of an Adverse Event

The Investigator will use the following question when assessing causality of an adverse event to study drug: Is there a reasonable possibility that the drug caused the event? An affirmative answer designates the event as a suspected adverse reaction.

If there is any doubt as to whether a clinical observation is an AE, the event should be reported. All AEs must have their relationship to study product assessed using the terms related or not related. In a clinical trial, the study product must always be suspect. To help assess, the following guidelines are used:

- Related – Related means there is evidence to show a causal relationship between the study product and the adverse event.

- Possibly Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.

  Specific adverse events considered related or possibly related to study participation have been identified as: myocarditis or ventricular dysfunction, as measured by ejection fraction and fractional shortening. These will be reported to the FDA in an IND Safety Report, per discussion with the FDA.

  Specific ECG and rhythm abnormalities requiring DSMB review prior to enrollment of next patient:
  1. AV block > the a PR interval > 220 msec lasting more than 2 hours post completion of cardiopulmonary bypass. This does not include transient Wenkebach with sleep/rest/hypothermia.
  2. Need for atrial pacing or junctional rhythm lasting more than 6 hours post completion
  3. Hypotension or ventricular dysfunction requiring pressor support > 5mcg/kg/min of dopamine more than 4 hours post completion of cardiopulmonary bypass.

- Not Related – There is not a reasonable possibility that the administration of the study product caused the event, and/or there is a more plausible explanation for the adverse event than administration of the study product.

(7) Recording and Reporting Procedures

Reporting Adverse Events and Serious Adverse Events

All AEs will be followed until the time the event is resolved or medically stable, or until subject is discharged from hospital, whichever comes first. AEs may be discovered through any of these methods:

- Observing the subject
- Questioning the subject, which should be done in an objective manner
- Receiving an unsolicited complaint from the subject
- Review of medical records/source documents

All AEs must be recorded accurately on the Adverse Event log for each study subject. The date of onset and duration of the AE, grade, whether it was expected or unexpected, action taken, date of resolution (if applicable) and outcome of the event must be recorded. The investigator will treat subjects experiencing adverse events appropriately and observe them at suitable intervals until their symptoms resolve or their status stabilizes or they are discharged from the hospital.

SAEs and unexpected problems will be reported promptly to BCH IRB according to local regulations and guidelines.

The PI and delegates are responsible for notifying the DSMB chair of all SAEs. DSMB panel will evaluate the relationship of the SAE to the underlying disease. The PI, in cooperation with the DSMB panel, will determine whether the SAE is unexpected in nature and make a recommendation for any further action. The primary consideration is the study subjects' safety.

(8) Regulatory Reporting

The PI will report events that are both serious and unexpected and that are associated with Study Agent/Intervention to the FDA and other applicable health authorities within the required timelines as specified in 21 CFR 312.32: fatal and life threatening events within 7 calendar days (by phone /fax/electronic communication) and all other serious adverse events in writing within 15 calendar days. All serious events designed as "not associated" to Study Agent/Intervention, will be reported to the FDA at least annually.

Given the low dose and method of delivery of fluorescein sodium, serious adverse events related to the study drug are not anticipated. Subjects will be monitored at the time of dye application to observe for signs of allergy or intolerance. If any signs of allergy or intolerance occur (as evidenced by discoloration of tissue or other signs of allergic reaction), the dye will be immediately flushed out and no images will be obtained. As per standard of care for patients undergoing cardiac surgery, subjects will receive an intraoperative echocardiogram after being separated from bypass, and will be monitored closely post-surgery. Before being discharged, all subjects will receive an electrocardiogram and trans-thoracic echocardiogram, in accordance with standard of care.

F. Data Management Methods

Study specific case report forms (CRFs) will be completed and the case report forms will be stored in individual subject binders. Data will be transcribed to a secure research data management system (InForm database). All hardcopies will be kept in a locked file cabinet accessible by authorized study staff only. Subject confidentiality will be maintained by recording subject data using a unique subject identifier. Identifiable linking data, such as contact information and medical record numbers, will be recorded and stored separately from the clinical study data.

FCM image files obtained for the study will be stored on a password-protected hard drive connected to the Cellvizio system. The image files will be exported, labeled only with the subject's study ID number, and provided to the blinded raters for analysis.

G. Quality Control Method

All study related tasks will follow Good Clinical Practice guidelines. A manual of operations (MOO) will be developed to detail the specific procedures to be conducted and data to be collected. Any significant deviations or exceptions to the protocol will be reported to the IRB and FDA in addition to being discussed among the investigators, and the PI will develop and implement a plan of action as needed (e.g. modification to MOO, study amendment, etc.). The PI will ensure the retention of all necessary records and reports for a minimum of 2 years after the discontinuation of the IND. The FDA will be allowed access to these records. A record of accountability of all investigational drug dispositions will be maintained, including batch and lot numbers and storage records.

H. Data Monitoring

Monitoring and auditing procedures as outlined in the Monitoring Plan will be followed to ensure that the entire study is conducted, documented, and reported in accordance with the IRB approved protocol, the International Conference on Harmonization (ICH) Good Clinical Practice (GCP) Guidelines, and applicable regulatory requirements of Boston Children's Hospital.

Monitors will review and verify protocol compliance with particular focus on AE/SAE reporting, CRF data, source documentation, informed consents, and any other study-related documentation, including review of hospital pharmacy procedures, drug accountability documentation, and drug storage facilities and records.

I. Data Analysis Plan

The FCM image files will be provided post-surgery to blinded raters, who will attempt to categorize the image files as either myocardium or nodal tissue. The raters will note if the image quality is not high enough to discriminate tissue types.

J. Statistical Power and Sample Considerations

This is a single site, descriptive, pilot study, so no power calculations have been performed. Six subjects will provide enough images to determine the feasibility of a larger randomized trial, to be performed in the future.

K. Study Organization

This is a single site pilot study. Dr. Kaza will perform all study drug administration and image acquisition. BCH research team members will assist in data abstraction and analysis.

L. References

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