The Efficacy of Intravenous Hydroxocobalamin Versus Methylene Blue as Treatment for Intraoperative Vasoplegic Syndrome in Liver Transplant Patients

NCT04054999

11DEC2019

The Efficacy of Intravenous Hydroxocobalamin versus Methylene Blue as Treatment for Intraoperative Vasoplegic Syndrome in Liver Transplant Patients

Regulatory Sponsor Principal Investigator:	Sher-Lu Pai, MD
Funding Sponsor:	Mayo Clinic
Study Product:	Hydroxocobalamin (Cyanokit) Methylene Blue (PROVAYBLUE™)
Protocol Number: (IRBe)	18-006247
IND Number:	N/A

Initial version:[17Aug2018] Version **(1.0)**
Revision: [30Oct2018] Version **(1.1)**
Revision: [11Apr2019] Version **(1.2)**
Revision: [15Jul2019] Version **(1.3)**
Revision: [23Jul2019] Version **(1.4)**
Revision: [11Dec2019] Version **(2.0)**

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List of Abbreviations

LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
CFR	Code of Federal Regulations
cGMP	Cyclic Guanosine Monophosphate
CRF	Case Report Form
CTSA	Center for Translational Science Activities
D5W	5% Dextrose in Water
DSMB	Data and Safety Monitoring Board
HER	Electronic Health Record
FDA	Food and Drug Administration
G6PD	Glucose-6-Phosphate Dehydrogenase
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
IND	Investigational New Drug Application
IRB	Institutional Review Board
IV	Intravenous
LAR	Legally Authorized Representative
LT	Liver Transplantation
MAOI	Monoamine Oxidase Inhibitor
Non-UPIRTSO	Non-Unanticipated Problems Involving Risk to Subjects or Others
PHI	Protected Health Information
PI	Principal Investigator
SAE	Serious Adverse Event/Serious Adverse Experience
SNRI	Serotonin and Norepinephrine Reuptake Inhibitor
SOC	Standard of Care
SOP	Standard Operating Procedure
SSRI	Selective Serotonin Reuptake Inhibitor
SVR	Systemic Vascular Resistance
VS	Vasoplegic Syndrome
UPIRTSO	Unanticipated Problems Involving Risk to Subjects or Others

Study Summary

Title	The Efficacy of Intravenous Hydroxocobalamin versus Methylene Blue as Treatment for Intraoperative Vasoplegic Syndrome in Liver Transplant Patients
Running Title	Methylene Blue vs Cyanokit
Protocol Number	18-006247
Phase	n/a
Methodology	Randomized, Open-Label
Overall Study Duration	12 months
Subject Participation Duration	Less than 12 hours
Single or Multi-Site	Single Center
Objectives	The primary aim of this study is to describe the efficacy of hydroxocobalamin vs methylene blue
Number of Subjects	20
Diagnosis and Main Inclusion Criteria	Patients undergoing liver transplant surgery and experiencing vasoplegia intraoperatively
Study Product, Dose, Route, Regimen	Hydroxocobalamin (Cyanokit): 5g IV infusion over 15 minutes
Duration of Administration	Single dose intraoperatively
Reference therapy	Methylene blue (PROVAYBLUE™), 2 mg/kg IV bolus administered over 15 minutes
Statistical Methodology	This pilot study is meant to be descriptive and it aims to collect basic data for future larger study

1 Introduction

This document is a protocol for a human research study. This study will be carried out in accordance with the applicable United States government regulations and Mayo Clinic research policies and procedures.

1.1 Background

Vasoplegic syndrome (VS) with hypotension, increased cardiac output, and low systemic vascular resistance (SVR) is a common complication during liver transplantation (LT) surgeries. Refractory hypotension often occurs after reperfusion with attenuated response to vasopressors and adequate fluid resuscitation. The development of vasoplegia greatly contributes to morbidity and mortality following LT. If the patient survives the intraoperative period, vasoplegia with prolong hypotension is associated with end-organ damages ranging from stroke, myocardial infarction, to acute renal failure. It also increases the risk of graft dysfunction after LT. Finding effective treatments for intraoperative vasoplegic syndrome my greatly improve outcomes and survival rate in patients undergoing LT.

The current clinical practice uses IV methylene blue to treat intraoperative vasoplegic syndrome in LT patients. Methylene blue has been found to increase SVR and decrease vasopressor requirements by inhibiting nitric oxide synthase. The synthase limits the generation of nitric oxide, a gasotransmitter contributing to vasoplegic syndrome. Methylene blue is also a guanylyl cyclase inhibitor, blocking the release of cyclic guanosine monophosphate (cGMP), thereby preventing vascular smooth muscle relaxation. However, methylene blue would cause inaccurate pulse oximetry reading immediately after IV administration. It also may cause a hemolytic response in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency or cause serious serotonergic syndrome when used in combination with serotonergic drugs such as selective serotonin reuptake inhibitors (SSRIs), serotonin and norepinephrine reuptake inhibitors (SNRIs), and monoamine oxidase inhibitors (MAOIs).

1.2 Investigational Agent

IV hydroxocobalamin, a form of vitamin B12, is currently approved for cyanide poison treatment. Its ability to bind and inhibit the 3 main gasotransmitters (nitric oxide, carbon monoxide, and hydrogen sulfide) contributing to vasoplegic syndrome has made it a new treatment for intraoperative vasoplegic syndrome. It is currently used as a rescue medication in methylene blue-resistance vasoplegic syndrome. In most vasoplegic cases, hydroxocobalamin is only used after methylene blue administration.

Both medications used in this study are IV liquids. methylene blue appears as clear dark blue solution. Hydroxocobalamin is a dark red crystalline powder and after reconstitution it appears as clear dark red solution.

1.3 Clinical Data to Date

A comprehensive search of the terms "hydroxocobalamin," "vitamin B12," "Cyanokit," "vasoplegia," "vasoplegic syndrome," and "liver transplantation" within MEDLINE and Embase was performed. Only 3 case reports with a total of 4 individual patients receiving B12 for the treatment of VS during LT surgeries were yielded from the MEDLINE search from 1946 to 2018 and Embase from 1988 to 2018. Although all 4 patients were reported to have favorable responses after B12 administration consistent with improved hemodynamics and reduced vasopressor requirements, no statistically significant conclusion could be drawn from the limited number of case reports.

A retrospective chart review was conducted for LT surgeries performed at our institution between January 2016 and June 2018. For patients with post-reperfusion VS treated with vasopressors, MB, and B12, hemodynamics variables and vasopressor requirements were reviewed. Fourteen of 401 LT patients during the study period presented with intraoperative VS and received vasopressors and MB as the first-line VS treatment due to the institutional protocol. Nine out of 14 patients receiving MB had an ineffective response defined as failure to improve hemodynamics or to reduce pressor requirements and were subsequently administered B12. Favorable changes in hemodynamic parameters with minimal averaged change of vasopressor requirements were observed after B12 administration. No major side-effect, medication associated complication, and post-operative mortality within 6 months of LT was observed. The institutional protocol required the usage of MB before B12 for VS treatment, causing the retrospective chart review to have clear comparison on the effectiveness of the 2 medications.

1.4 Dose Rationale

Hydroxocobalamin 5g IV infusion had been administered to effectively treat hypotension attributable to vasoplegia common in LT and cardiac surgery. Most of the case reports described using 5g IV dose in the operating room.

Intravenous methylene blue 1-2 mg/kg had been described as a therapeutic dose to treat vasodilatory shock. 2 mg/kg was found to be the most commonly used dosage from a literature review in the treatment of vasoplegic and septic shock. As the goal of this study is to find the efficacy of intravenous methylene blue as a treatment for intraoperative vasoplegic syndrome, the dosage period will be limited to a single bolus during the LT surgery. In addition to the dosage recommendation from literature search, 2 mg/kg dosage is selected due to this specific patient population. Patients undergoing LT surgery commonly experience massive blood loss and multiple blood product transfusions. A higher dosage would ensure adequate medication effect if some may be lost due to surgical blood loss.

1.5 Risks and Benefits

- The benefits of methylene blue:
  Methylene blue causes vasoconstriction by inhibiting nitric oxide synthase and preventing subsequent activation of guanylyl cyclase in smooth muscle cells. This reaction increases systemic blood pressure and decreases vasopressor requirements for

patients with vasoplegic syndrome. In LT surgery patients, hemodynamic stability without the requirements of vasopressors may allow immediate extubation at the end of the surgery and bypass the intensive care unit in the postoperative period, allowing faster recovery and improving outcomes. Advocates for early extubation have argued that it may be beneficial for the new organ graft to limit the exposure to mechanical ventilation. Kaisers et al reported the deleterious effects of positive end expiratory pressure (PEEP) on liver graft hemodynamics.

- The risks of methylene blue:
  Common side effects include hypertension, discoloration of skin, hyperhidrosis, nausea, dizziness, headache, pain in limb, and abnormal urine color, hypersensitivity, hemolytic anemia
  Medications which may accumulate with methylene blue due to inhibition of cytochrome P450 isozymes: Alfentanil, cyclosporine, digoxin, fentanyl, phenytoin, sirolimus, tacrolimus, warfarin.
  Methylene blue is contraindicated in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency as it can result in a hemolytic response in patients with G6PD deficiency. The intravascular hemolysis typically occurs two to five days after drug administration and is typically self-limited.
  Methylene blue may cause serotonin syndrome when used in combination with serotonergic drugs such as Selective serotonin reuptake inhibitors (SSRIs), serotonin and norepinephrine reuptake inhibitors (SNRIs), and monoamine oxidase inhibitors (MAOIs).
- The benefits of hydroxocobalamin:
  IV hydroxocobalamin is currently approved for cyanide poison treatment. Its ability to bind and inhibit the 3 main gasotransmitters (nitric oxide, carbon monoxide, and hydrogen sulfide) contributing to vasoplegic syndrome has made it a new treatment for intraoperative vasoplegic syndrome. Unlike methylene blue, it does not have the same implications in serotonin syndrome. Similar to the benefits of methylene blue on treating vasoplegic syndrome, hydroxocobalamin may play a major role to allow hemodynamic stability and early extubation in this particular LT population.
- The risks of hydroxocobalamin:
  Common side effects include increased blood pressure, erythema, rash, nausea, decreased lymphocyte count, headache, and abnormal red urine color.

2 Study Objectives

Primary Objective
To assess the efficacy of hydroxocobalamin as compared to methylene blue on improving hemodynamic stability as measured by SVR, systemic blood pressure, and amount of vasopressors administered.

Secondary Objective
The secondary end point will examine the patient outcomes in the postoperative period as measured by early tracheal extubation after surgery, length of hospital and intensive care unit stay, perioperative complications (stroke, myocardial infarction, and acute renal failure), graft function, and survival rate.

3 Study Design

3.1 General Description

This randomized, prospective, open label, pilot study will involve 20 LT patients with intraoperative vasoplegic syndrome. If vasoplegic syndrome is identified via SVR lower than 500 dynes-sec/cm^{-5}, the patients will be randomized to receiving either IV methylene blue or hydroxocobalamin (10 patients in each group). The administration of assigned medication will only take place once during surgery if patient is exhibiting vasoplegia after the reperfusion of the new organ as demonstrated by SVR lower than 500 dynes-sec/cm^{-5} sustained for a period of more than 5 minutes despite the administration of vasopressors. The primary end point will examine the efficacy of the two medications on treating intraoperative vasoplegic syndrome. The SVR, blood pressure, and amount of administered vasopressors at 0, 15, 30, 60, 90, 120, 150, and 180 minutes after the medication administrations will be recorded. .

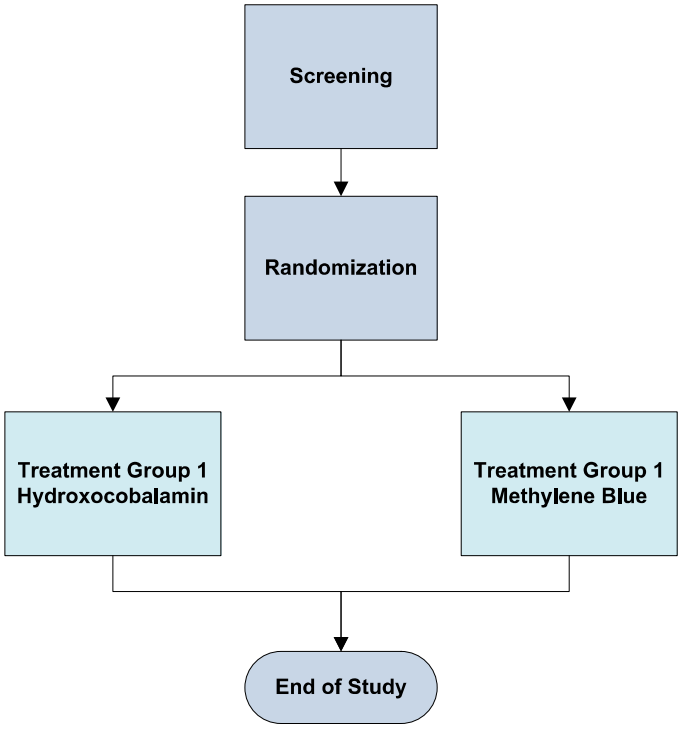
If the patients still present with vasoplegic syndrome 30 minutes after receiving IV methylene blue or hydroxocobalamin during LT surgery, the vasoplegia treatment which the patients have not received in the operating room would be utilized as life-saving measures per standard of care practice. The patients will be excluded from the comparison study, but the data of frequency of failure to effectively treat vasoplegia via certain medication may be utilized in understanding the rate of failure to provide effective treatment.

3.2 Number of Subjects

Twenty

3.3 Duration of Participation

Intraoperatively during LT surgery



3.4 Primary Study Endpoints

The primary endpoint of the study will be the improvement in hemodynamic values as measured by SVR, systemic blood pressure, and amount of vasopressors administered. The monitoring and documentation of hemodynamic values is part of SOC practice during LT surgery.

3.5 Secondary Study Endpoints

The secondary endpoints of the study are the patient outcomes in the postoperative period and 30-day mortality rate.

3.6 Primary Safety Endpoints

This is an exploratory efficacy study with no specific primary safety endpoint. Adverse events will be tracked and reported using Good Clinical Practice standards.

3.7 Identification of Source Data

The following source data will be directly recorded in the EHR and later retrieved by the study team members:

- Vital signs (including hemodynamic values)
- Intraoperative Events

- Postoperative Patient Outcomes

4 Subject Selection Enrollment and Withdrawal

4.1 Inclusion Criteria

- Age > or = 18 years old
- Patients scheduled to undergo liver transplantation
- Patients able to read and understand consent document (if patient is unable to provide an informed consent, the Legally Authorized Representative will be ask to consent on behalf of the patient).
- SVR lower than 500 dynes-sec/cm^{-5} sustained for more than 5 minutes despite the administration of vasopressors intraoperatively (this criterion must be met after consent intraoperatively)

4.2 Exclusion Criteria

- Patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency documented as a medical history
- Patients taking serotonergic drugs such as selective serotonin reuptake inhibitors (SSRIs), serotonin and norepinephrine reuptake inhibitors (SNRIs), and monoamine oxidase inhibitors (MAOIs)
- Patients with allergy to methylene blue or hydroxocobalamin

4.3 Subject Recruitment, Enrollment and Screening

Subjects will be enrolled from the Departments of Anesthesiology and Transplantation at the Mayo Clinic in Florida. Patients will be initially approached when they are evaluated and placed on a transplant waiting list. Full discussion about the study will take place at that time and the actual written consent will not be obtained until the day of surgery. The study has an accrual target of 20 patients. In the year 2017, 150 unique patients underwent LT at Mayo Clinic in Florida and thus no difficulties in accrual based on historical volumes are anticipated. Co-departmental participation aids in reaching accrual targets and minimizing referral bias. The initial accrual period will last at least 24 months and treatment of participants may not occur for all should they not experience intraoperative refractory vasoplegia. Patients will be provided with a Research Participant Consent and Privacy Authorization Form describing the study drug, protocol, inclusion and exclusion criteria, as well as risks and benefits of participation.

4.3.1 Capacity to consent assessment

All potential subjects should be recruited and informed of the study as outlined in the study protocol. To determine whether the subject has the capacity to provide consent, the study team

member will ask the following questions at the conclusion of the consent process:

Level of Understanding 5-Point Scale

1= None, 2= Poor, 3=Unclear, 4=Good, 5=Excellent

Assessment Questions	Level of Understanding (circle one)
Why is this study being done?	1 2 3 4 5
If you decide to participate in the study, what are some of the things you will be asked to do?	1 2 3 4 5
What parts of the study are being done as part of your regular care and what parts of the study are being done only for the research?	1 2 3 4 5
Describe some of the risks or discomforts that people may experience if they participate in this study.	1 2 3 4 5
What are the benefits of participating in this study?	1 2 3 4 5
Do you have to be in this study?	1 2 3 4 5
What will happen if you decide not to be in the study?	1 2 3 4 5
If you are in the study and stop your participation, will you still be able to receive regular care?	1 2 3 4 5
Who will pay for your medical care if you are injured while in this study?	1 2 3 4 5
Who should you contact if you have questions or experience a problem while in the study?	1 2 3 4 5

Individuals who achieve a demonstrated understanding (scoring a 4 or 5 on **all** questions) of the study are determined to have capacity to provide consent. However, if in answering these

questions, the potential subject is unable to demonstrate understanding, reasoning, or appreciation of the study, and the Investigator still wishes to enroll the subject, the consent should be reviewed with the Legally Authorized Representative.

Transplant team identifies the legally authorized representative at the time of patients' admission in order to obtain permission for necessary procedures/treatments. We will obtain consent for participation from a family member or legally authorized representative (LAR) proxy as recorded in the patient's medical record if an eligible patient is unable to participate in a lengthy consent process.

A member of the research team will approach the designated proxy LAR and explain the study and what is being asked of the family member if consent is provided to participate in the study. Each proxy will then read the consent form and be asked to re-phrase to a member of the research team what the study aims are, how long the patient will remain in the study, and if payment will be received. Opportunity will be provided for asking questions concerning the protocol.

If the LAR is not physically available, the participant will not be enrolled in the study and will proceed with SOC transplant surgery and medical care.

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

Patients are free to withdraw at any time and for whatever reason. No data will be collected for withdrawn subjects. Withdrawn subjects may be replaced during the accrual period. Pre-specified reasons for discontinuing include, but are not limited to, the following:

- Patient Request: Patient decided that he/she did not want to continue (for any reason)
- Adverse Event: Patient experienced a related or unrelated event that would interfere with the study objectives/evaluation
- Treatment Failure: If in the Principal Investigator and/or Investigators' judgment, the patient's condition required another form of treatment
- Inclusion/Exclusion Discrepancy/Violation: Patient should not have been enrolled
- Noncompliance: Patient is not complying with the protocol requirements (i.e. visit schedule, dosing, regimen, etc.); a patient is to be withdrawn if he/she misses two consecutive visits
- Other: Any other reason

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

If a Participant withdraws from the study, no additional attempts will be made to contact the Participant.

5 Study Drug

5.1 Description

Hydroxocobalamin, the active ingredient in Cyanokit™, is cobinamide dihydroxide dihydrogen phosphate (ester), mono (inner salt), 3'-ester with 5,6-dimethyl-1-□-D-ribofuranosyl-1H benzimidazole. The drug substance is the hydroxylated active form of vitamin B12 and is a large molecule in which a trivalent cobalt ion is coordinated in four positions by a tetrapyrol (or corrin) ring. It is a hygroscopic, odorless, dark red, crystalline powder that is freely soluble in water and ethanol, and practically insoluble in acetone and diethyl ether.
Hydroxocobalamin direct binds nitric oxide and has a direct inhibitory effects on nitric oxide synthase and soluble guanylate cyclase.
After reconstitution, the vial contains hydroxocobalamin for injection, 25 mg/mL. One 5 g vial is a complete starting dose.

Methylene Blue (PROVAYBLUE™) is an oxidation-reduction agent. Methylene Blue is a sterile solution intended for intravenous administration. Each Methylene Blue 10 mL ampule contains 50 mg Proveblue® methylene blue and water for injection. Each mL of solution contains 5 mg methylene blue and water for injection. It has been demonstrated that methylene blue is capable of binding nitric oxide (a reactive oxygen species), inhibiting both constitutive and inducible nitric oxide synthase, and inhibiting soluble guanylate cyclase.
Each 10 mL ampule of PROVAYBLUE™ contains 50 mg methylene blue. PROVAYBLUE™ is hypotonic and may be diluted before use in a solution of 50 mL 5% Dextrose in Water (D5W) in order to avoid local pain, particularly in the pediatric population. Use the diluted solution immediately after preparation. Do not mix with sodium chloride 9 mg/mL (0.9%) solution for injection, because it has been demonstrated that chloride reduces the solubility of methylene blue. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit.

5.2 Treatment Regimen

10 study participants will receive 5g of hydroxocobalamin intravenously intraoperatively
10 study participants will receive 2mg/kg of Methylene Blue intravenously intraoperative.

5.3 Method for Assigning Subjects to Treatment Groups

This is an open-label pilot investigation and all study participants are assigned to either of the two active treatments. There is no placebo arm in this study.

The randomization will be performed utilizing REDCap and assigned anesthesia clinical care team will be informed of patients' assigned to guide them with the selection of the assigned treatment option.

5.4 Preparation and Administration of Study Drug

The study drug will be obtained as per standard clinical anesthesia practice from pharmacy's dispensing system Pyxes. Assigned clinical anesthesia team member will prepare and administer either treatment option to the patient as per standard practice.

5.5 Prior and Concomitant Therapy

Concomitant administration of selective serotonin reuptake inhibitors (SSRIs), serotonin and norepinephrine reuptake inhibitors (SNRIs), and monoamine oxidase inhibitors (MAOIs) is not permitted.

5.6 Packaging

Hydroxocobalamin and Methylene Blue are currently part of Mayo Clinic Florida pharmacy formulary and their storage will follow standard pharmacy practice.

5.7 Masking/Blinding of Study

This is an open-label pilot investigation. Masking and blinding procedures are not applicable.

6 Study Procedures

6.1 Visit 1a (Screening – placement on transplant waiting list and up to the day of surgery)

- Review of medical record
- Initial review of Informed Consent

6.2 Visit 1 b (Enrollment – day of surgery)

- Capacity to consent assessment
- Informed consent
- Review of medical record
- Concomitant medications

6.3 Visit 2 (Randomization and Treatment – day of surgery)

- Liver Transplantation surgery (as per standard of care)
- Administration of Hydroxocobalamin or Methylene Blue

6.4 Visit 3 (Off Study)

- Review of medical record will continue until study participants are discharged from the hospital

	Schedule of Events			
Study Activity	Visit 1a	Visit 1b	Visit 2	Visit 3
Hydroxocobalamine or Methylene Blue			X	
Initial review of informed consent	X			
Capacity to consent		X		
Informed consent		X		
Review of Medical Record	X	X		X
Concomitant Medications		X	X	
Adverse event evaluation			X	X

7 Statistical Plan

7.1 Sample Size Determination

Per the biostatistician from the Mayo Division of Biomedical Statistics and Informatics, the existing information on the changes of SVR, blood pressure, and amount of administered vasopressors altered by either medication are not sufficient for sample size calculation. The primary aim of this pilot study will be to determine feasibility and to collect descriptive data that can be used in future to obtain statistical power.

7.2 Statistical Methods

Descriptive Statistics

Continuous variables will be summarized using the sample mean, median, standard deviation, interquartile range, and range. Categorical variables will be summarized using number and percentage of patients

Handling of Missing Data

This is a prospective study and therefore we do not anticipate any missing data. In the event of any unexpected missing data, no attempt to impute this missing data will be made; missing data will simply be treated as missing in the statistical analysis.

7.3 Subject Population(s) for Analysis

Each participant who received the study drug will be included in the primary analysis regardless of study withdrawal for any reason. In the event of any study withdrawals, in secondary analysis we will examine the sensitivity of our results to the exclusion of patients who withdrew.

8 Safety and Adverse Events

8.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others (UPIRTSO)
Any unanticipated problem or adverse event that meets the following three criteria:

- Serious: Serious problems or events that results in significant harm, (which may be physical, psychological, financial, social, economic, or legal) or increased risk for the subject or others (including individuals who are not research subjects). These include: (1) death; (2) life threatening adverse experience; (3) hospitalization - inpatient, new, or prolonged; (4) disability/incapacity - persistent or significant; (5) birth defect/anomaly; (6) breach of confidentiality and (7) other problems, events, or new information (i.e. publications, DSMB reports, interim findings, product labeling change) that in the opinion of the local investigator may adversely affect the rights, safety, or welfare of the subjects or others, or substantially compromise the research data, **AND**
- Unanticipated: (i.e. unexpected) problems or events are those that are not already described as potential risks in the protocol, consent document, not listed in the Investigator's Brochure, or not part of an underlying disease. A problem or event is "unanticipated" when it was unforeseeable at the time of its occurrence. A problem or event is "unanticipated" when it occurs at an increased frequency or at an increased severity than expected, AND
- Related: A problem or event is "related" if it is possibly related to the research procedures.

Adverse Event
An untoward or undesirable experience associated with the use of a medical product (i.e. drug, device, biologic) in a patient or research subject.

Serious Adverse Event
Adverse events are classified as serious or non-serious. Serious problems/events can be well defined and include;

- death
- life threatening adverse experience
- hospitalization
- inpatient, new, or prolonged; disability/incapacity
- persistent or significant disability or incapacity
- birth defect/anomaly

and/or per protocol may be problems/events that in the opinion of the sponsor-investigator may have adversely affected the rights, safety, or welfare of the subjects or others, or substantially compromised the research data.

All adverse events that do not meet any of the criteria for serious, should be regarded as **non-serious adverse events**.

Adverse Event Reporting Period

For this study, the study treatment follow-up period is defined as 90 minutes following the last administration of study treatment.

Preexisting Condition
A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings
At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event
All unresolved adverse events should be followed by the sponsor-investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the sponsor-investigator should instruct each subject to report, to the sponsor-investigator, any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for an adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

8.2 Recording of Adverse Events

At each contact with the subject, the study team must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse

event section of the electronic case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic, laboratory or procedure results should recorded in the source document.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been ultimately determined that the study treatment or participation is not the probable cause. Serious adverse events that are still ongoing at the end of the study period must be followed up, to determine the final outcome. Any serious adverse event that occurs during the Adverse Event Reporting Period and is considered to be at least possibly related to the study treatment or study participation should be recorded and reported immediately.

8.3 Reporting of Serious Adverse Events and Unanticipated Problems

When an adverse event has been identified, the study team will take appropriated action necessary to protect the study participant and then complete the Study Adverse Event Worksheet and log. The sponsor-investigator will evaluate the event and determine the necessary follow-up and reporting required.

8.3.1 Sponsor-Investigator reporting: notifying the Mayo IRB

The sponsor-investigator will report to the Mayo IRB any UPIRTSOs and Non-UPIRTSOs according to the Mayo IRB Policy and Procedures.

Any serious adverse event (SAE) which the Principal Investigator has determined to be a UPIRTSO will be reported to the Mayo IRB as soon as possible but no later than 5 working days after the investigator first learns of the problem/event.

The following information will be collected on the adverse event worksheet (and entered in the research database):

- Study ID
- Disease
- The date the adverse event occurred
- Description of the adverse event
- Relationship of the adverse event to the research (drug, procedure, or intervention*)
- Determination if the adverse event was expected
- The severity of the adverse event (severity scale described below**)
- If any intervention was necessary
- Resolution (was the incident resolved spontaneously, or after discontinuing treatment)
- Date of Resolution

The sponsor-investigator will review all adverse event reports to determine if specific reports need to be made to the IRB and FDA. The sponsor-investigator will sign and date the adverse event report when it is reviewed. For this protocol, only directly related SAEs/UPIRTSOs will be reported to the IRB.

*** Relationship Index**

The relationship of an AE to the Investigational Drug is a clinical decision by the sponsor-investigator (PI) based on all available information at the time of the completion of the eCRF and is graded as follows:

1. Not related: a reaction for which sufficient information exists to indicate that the etiology is unrelated to the study drug; the subject did not receive the study medication or the temporal sequence of the AE onset relative to administration of the study medication is not reasonable or the event is c1early related to other factors such as the subject' s clinical state, therapeutic intervention or concomitant therapy.

2. Unlikely: a clinical event, including laboratory test abnormality, with a temporal relationship to drug administration which makes a causal relationship improbable and in which other drugs, chemicals, or underlying disease provide plausible explanations.

3. Possible: a clinical event, including laboratory test abnormality, with a reasonable time sequence to administration of the drug but which could also be explained by concurrent disease or other drugs or chemicals; information on drug withdrawals may be lacking are unclear.

4. Probable: a clinical event including laboratory test abnormality, with a reasonable time sequence to administration of the drug, unlikely to be attributed to concurrent disease or other drugs or chemicals and which follows a clinically reasonable response on withdrawal (de-challenge): re-challenge information is not required to fulfi1 this definition.

5. Definite: a reaction that follows a reasonable temporal sequence from administration of the drug, or in which the drug level has been established in body fluids or tissues, that follows a known or expected response pattern to the suspected drug, and that is confirmed by improvement on stopping or reducing the dosage of the drug, and reappearance of the reaction on repeated exposure (re-challenge).

**** Severity Scale**

The maximum intensity of an AE during a day should be graded according to the definitions below and recorded in details as indicated on the CRF. If the intensity of an AE changes over a number of days, then separate entries should be made having distinct onset dates.

1. Mild: AEs are usually transient, requiring no special treatment, and do not interfere with patient’s daily activities.

2. Moderate: AEs typically introduce a low level of inconvenience or concern to the patient and may interfere with daily activities, but are usually ameliorated by simple therapeutic measures.

3. Severe: AEs interrupt a patient's usual daily activity and traditionally require systemic drug therapy or other treatment.

8.4 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 10 "Study Monitoring, Auditing, and Inspecting"). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

9 Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (long term survival status that the subject is alive) at the end of their scheduled study period.

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, pharmacy records, recorded data from automated instruments, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3 Case Report Forms

All data necessary for this study will be obtained from EHR and recorded on the electronic Case Report Forms (CRFs) created in REDCap. All missing data will be explained.

Data Management

Study data to be collected and managed using EHR and transcribed into electronic CRFs in REDCap, electronic data capture software, hosted by CTSA at Mayo Clinic. REDCap (Research

Electronic Data Capture) is a secure, web-based application designed to support data capture for research studies, providing 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources.

Data Processing

All study date will be stored and analyzed at Mayo Clinic in Florida using the REDCap electronic data capture tool.

Data Security and Confidentiality

All source documents including clinical findings, observations or other activities will be stored in a REDCap database that will be designed by an Investigator. Access to the REDCap database will be limited to the Principal Investigator, Investigators, Study Team members, and Statistician.

Data Quality Assurance

Once the study is completed the Principal Investigator will randomly select 3 participants and compare the data documented in the EHR with what is entered into the REDCap database. If there is any discrepancy, the Principal Investigator and/or Investigators will cross-reference all 20 patients to ensure accuracy.

Data Clarification Process

For any data query the Principal Investigator and Investigators will meet to clarify the data queried and make corrections based on consensus.

9.4 Records Retention

The sponsor-investigator will maintain records and essential documents related to the conduct of the study. These will include subject case histories and regulatory documents.

The sponsor-investigator will retain the specified records and reports for;

1. Up to 2 years after the marketing application is approved for the drug; or, if a marketing application is not submitted or approved for the drug, until 2 years after shipment and delivery of the drug for investigational use is discontinued and the FDA has been so notified. OR
2. As outlined in the Mayo Clinic Research Policy Manual –"Retention of and Access to Research Data Policy" [redacted]

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the compliance or quality assurance reviewer is given access to all the study-related documents and study related facilities (e.g. pharmacy, etc.), and has adequate space to conduct the visit.

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, and government regulatory agencies, of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable compliance offices.

11 Ethical Considerations

This study is to be conducted according to United States government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted local Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study. The decision of the IRB concerning the conduct of the study will be made in writing to the sponsor-investigator before commencement of this study.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of a subject, using the Approved IRB consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject and the individual obtaining the informed consent.

12 Study Finances

12.1 Funding Source

This investigator initiated study is funded by a research grant Research Accelerator Program provided by Mayo Clinic Florida [redacted] to cover study coordinator time to consent and statistic data analysis.

12.2 Conflict of Interest

Any study team member who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor-investigator prior to participation in this study.

No financial conflicts of interested are anticipated or have been identified for this study.

12.3 Subject Stipends or Payments

No payment is given to study participants.

13 Publication Plan

The primary responsibility for publication of the study results is with the Primary Investigator. After the complication of study and prior to publication, the study results will be shared with all Investigators. The study will be registered at ClinicalTrials.gov prior to subject recruitment along with the posting of the results within 12 months of final data collection for the primary outcome measure.

14 References

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