CLINICAL STUDY PROTOCOL

A Phase II Double-blind Randomized Controlled Trial of Intravenous Hydroxocobalamin in Septic Shock

Clinical Trials.gov Number: NCT03783091

Jayshil Patel, M.D.

Version 1.0, February 6, 2019

PROTOCOL SIGNATURE PAGE

Protocol No.: 2.0 **Version Date:** 06SEP2019

1. I agree to follow this protocol version as approved by the MCW Institutional Review Board (IRB) and Data Safety Monitoring Committee (DSMC).
2. I will conduct the study in accordance with applicable IRB requirements, federal regulations, and state and local laws to maintain the protection of the rights and welfare of study participants.
3. I certify that I, and the study staff, have received the requisite training to conduct this research protocol.
4. I have read and understand the information in the Investigator's Brochure (or Manufacturer's Brochure) regarding the risks and potential benefits. I agree to conduct the protocol in accordance with Good Clinical Practices (ICH-GCP), the applicable ethical principles, the Statement of Investigator (Form FDA 1572) and with local regulatory requirements. In accordance with the FDA Modernization Act, I will ensure the registration of the trial on the www.clinicaltrials.gov website.
5. I agree to maintain adequate and accurate records in accordance with IRB policies, Federal, state and local laws and regulations.

MCW Principal Investigator / Study Chair

Jayshil Patel, MD

Printed Name

Signature Date

Title: A Phase II Double-blind Randomized Controlled Trial of Intravenous Hydroxocobalamin in Septic Shock

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Investigational Agent(s): Hydroxocobalamin or Vitamin B12 or Cyanokit

REVISION HISTORY

Revision history is presented in reverse order so that the information pertaining to the most current version of the protocol is presented first in this section.

Version 2, Version Date 06SEP2019

Norepinephrine dose at enrollment changed from 0.2 mcg/kg/min to 0.1 mcg/kg/min.

Version 1, Version Date 06FEB2019

Initial submission of the protocol.

PROTOCOL SUMMARY

Title	A Phase II Double-blind Randomized Controlled Trial of Intravenous Hydroxocobalamin in Septic Shock
Protocol Number	PRO00032950
Principal Investigator	Jayshil Patel, M.D.
Study Sites	Medical College of Wisconsin & Froedtert Hospital
Clinical Trial Phase	II
Study Disease	Septic Shock
Main Eligibility Criteria	**Inclusion Criteria** 1. Adult patients at least 18 years of age 2. Admitted to an Intensive Care Unit (ICU) service at Froedtert Hospital 3. Current diagnosis of septic shock as defined by the sepsis-3 criteria[4], based on (a) hypotension despite IV fluid resuscitation of at least 30 mL/kg (b) vasopressor use (e.g., norepinephrine dose of at least 0.1 mcg/kg/min or equivalent), and/or lactic acidosis (lactic acid >2 mmol/L) 4. Ability to randomize within 48 hours of diagnosis of septic shock or admission to ICU, whichever occurs last 5. Patient or LAR able to understand a written informed consent document and willingness to sign it **Exclusion Criteria** A potential subject who meets any of the following exclusion criteria is ineligible to participate in the study. 1. Known hypersensitivity to any component of the hydroxocobalamin formulation 2. Patients who lack adequate IV access 3. Patients not anticipated to survive the next 24 hours as determined by the treating physician 4. Participation in another investigational trial within the last 30 days 5. Patients who require treatment with chloramphenicol 6. History of urinary calcium oxalate crystals

	7. Patients with an active bleed and/or hemolysis that require more than once daily hemoglobin monitoring 8. In the rare event a patient on vasopressor support is on intermittent hemodialysis, they will be excluded 9. Women who are pregnant or nursing as the effects of hydroxocobalamin are not known
Study Rationale	Short of early antibiotics and supportive care measures, there are few effective therapies for septic shock. High dose intravenous hydroxocobalamin, or vitamin B12, has shown promise in reversing post-operative vasoplegia. Septic shock is a vasodilatory state with a similar pathophysiology to post-operative vasodilatory shock. No human studies have evaluated tested hydroxocobalamin in septic shock. The primary aim of this study is to determine the feasibility of administering intravenous hydroxocobalamin and its impact on reducing vasopressor dose.
Primary Objectives	To measure the benefit of a single 5-gram IV dose of B12a in reducing vasopressor support in vasopressor-refractory septic shock
Secondary Objectives	1. To identify the pre and post intervention difference in blood H_2S concentration between those patients who received B12a to placebo. 2. To quantify the physiological effects of H2S and B12a on the heart, pulmonary vasculature, and end organs. 3. Examine the effect of cobalamin administration on cf-mtDNA. 4. Point of care (POC) spectrophotometric detection of plasma adducts of infused B12a (sulfocobalamin, nitrosocobalamin, hydroxocobalamin) as biomarkers for natural history of H_2S generation during septic shock, and for therapeutic titration of hydroxocobalamin infusion. 5. POC oximetry to quantify B12a effects on hydrogen sulfide-mediated intoxication of mitochondrial respiration in human peripheral blood mononuclear cells (PBMC).
Endpoints	1. The impact of hydroxocobalamin will be measured by the percent reduction in vasopressor dose 3 hours after administration. 2. The secondary endpoints will be the difference in serum H_2S after study drug administration and the rate of PODS at 28 days post ICU admission.
Study Design	Single-center prospective randomized phase II study comparing a single 5-gram IV dose of B12a as add-on to conventional care in ICU patients with vasopressor-refractory septic shock.

Study Agent/ Intervention Description	Hydroxocobalamin or Cyanokit
Number of Subjects	26-30
Duration of Follow up	30 Days
Estimated Time to Complete Enrollment:	24 months
Statistical Methodology:	The primary outcome will be compared between the groups using the Wilcoxon rank-sum test. In addition to the primary unadjusted analysis, regression-based approaches will be used to explore the effect of the prognostic covariates such as age, co-morbidities, APACHE scores, etc. on the outcomes of interest. Time-to-event outcomes, such as length of stay, time to 50% reduction in vasopressors, will be presented using cumulative incidence curves with in-hospital mortality as a competing risk. All analyses will be on an intention-to-treat basis, utilizing all patients randomized. Mechanistic outcomes include conditional differences in blood H_2S concentrations, cardiac function, pulmonary and systemic vascular resistance, and cellular respiration for individual subjects, by treatment group.
Efficacy Assessments	To measure the benefit of a single 5-gram IV dose of B12a in reducing vasopressor support in vasopressor-refractory septic shock
Unique Aspects of this Study	This is the first study to evaluate the safety and efficacy of hydroxocobalamin in addition to standard of care in patients with septic shock.

STUDY CALENDAR

The below study chart is described in detail in Section 5 Study Procedures.

Period/ Procedure	Screening	Day 1 Pre-Dose	Day 1 Post-Dose	Days 2-5				Hospital Discharge
Study Day/Visit Day	**0**	**1**	**1**	**2**	**3**	**4**	**5**	**≤30**
Eligibility Criteria	X							
Informed Consent	X							
Demographics		X						
Assessment of Adverse Events		X	X	X	X	X	X	X
Treatment/Drug Administration								
Study drug Infusion			X					
Clinical procedures								
Medical history	X	X						
Concomitant Medications	X	X	X	X	X	X	X	X
Vasopressor Usage & Fluid Resuscitation	X	X	X	X	X	X	X	X
Blood Products Given			X	X	X	X	X	
APACHE II and SOFA Scores	X	X	X	X	X	X	X	X
mNUTRIC Score & Calorie Counts			X	X	X	X	X	
Renal Replacement Therapy								X
Confirmation of negative pregnancy test	X							
Laboratory procedures								
Serum Lactic Acid	X	X						
Serum Leukocyte Count	X							
Serum Glucose	X							
Serum Creatinine	X							
Platelet Count	X							
INR	X							
Culture Results			X	X	X	X	X	X
Hydrogen Sulfide Test		X	X					
Outcome Measures								
Discharge Destination								X
Mortality								X

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LIST OF ABBREVIATIONS

AE	adverse event
CBC	complete blood cell (count)
CRC	clinical research coordinator
CTCAE	Common Terminology Criteria for Adverse Events
CTMS	Clinical Trial Management System
DSMB	Data and Safety Monitoring Board
FDA	Food and Drug Administration
GCP	Good Clinical Practice
ICH	International Conference on Harmonization
IND	investigational new drug application
IP	investigational product
IRB	Institutional Review Board
IV	intravenous
MedDRA	Medical Dictionary for Regulatory Activities
SAE	serious adverse event
SD	standard deviation
UP	unanticipated problem
UPIRSO	unanticipated problems involving risks to subjects or others
WBC	white blood cell (count)

1 BACKGROUND

Septic Shock

Sepsis is life-threatening organ dysfunction due to a dysregulated host response from infection, which affects millions of people worldwide and kills one in four.[1] Unfortunately, few effective treatments exist and early antibiotics and supportive care remain the cornerstone of therapies.[1] *Novel, non-toxic therapies are sorely needed*. High dose micronutrients have become promising forms of therapy in critically ill patients because they are non-toxic and exert pleiotropic effects to reduce inflammation.[2,3]

Septic shock, a subset of sepsis, is a form of vasodilatory shock, which increases the risk for multiple organ dysfunction and mortality rate can exceed 50%.[4-7] Increased and unregulated nitric oxide (NO) and hydrogen sulfide (H_2S) are key contributors to vasodilatation and hypotension and elevated levels have been associated with poor outcomes in septic shock.[5,8,9] Worse, NO increases H_2S production through increased gene expression, which sustains vasodilatation, circulatory dysfunction, myocardial dysfunction, and creates refractory hypotension.[7] Hydroxocobalamin scavenges, binds, and prevents NO and H_2S formation and has the potential to reduce capillary leak, promote capillary membrane stabilization, and accentuate recovery.[8 10-12] Case series of high dose intravenous (IV) hydroxocobalamin have been associated with reduced vasopressor support in post-operative vasodilatory states, which is also associated with increased NO and H_2S.[10,13-18]

There are no human studies evaluating the feasibility and impact of high dose IV hydroxocobalamin in septic shock. We propose a phase II double blind randomized controlled trial to evaluate the feasibility and impact of high dose vitamin B12 in reducing vasopressor support in septic shock.

Few therapies exist for septic shock. Micronutrients are increasingly used as a form of *therapy* to improve outcomes in septic shock.[2,3] Our study is the first to prospectively evaluate the effect of hydroxocobalamin in septic shock. Our study utilizes a modern design and contains an exploratory translational component to identify a mechanism (H_2S) by which nutrition (high dose hydroxocobalamin) improves clinical outcomes. Furthermore, sepsis requires time-sensitive management to mitigate downstream complications, such as PODS and its complications.

What is the problem to be addressed?

Sepsis is life-threatening organ dysfunction due to a dysregulated host response from infection.[21] In the United States, there are more than 750,000 annual cases of sepsis with a mortality rate up to 40%.[4,22,23] Septic shock is a continuum of sepsis and is recognized by hypotension despite intravenous (IV) fluid resuscitation and need for vasopressor support.[21] Septic shock is a form of vasodilatory shock, which often leads to multiple organ failure, which increases the risk for mortality, often exceeding 50%.[21] Unfortunately, few effective therapies beyond early antibiotics and supportive care exist, as evidenced by numerous negative trials.[24-29]

What is the pathogenesis of septic shock?

Sepsis is a dysregulated inflammatory and immune response secondary to an infection leading to organ dysfunction.[4] When the host response to an infection crosses the boundaries of the local environment, widespread malignant inflammation ensues, leading to an uncontrolled, unregulated, and self-sustaining inflammatory response.[30,31] Hypotension ensues because pro-inflammatory mediators that are usually contained in the interstitial space are now in the blood,

leading to widespread vasodilatation with circulatory and cellular abnormalities.[4,5,30] Components of the bacterial cell wall, such as lipopolysaccharide (LPS), endotoxin, peptidoglycan and bacterial products such as enterotoxins and exotoxins have been found in the blood of septic patients, suggesting they contribute to the pathogenesis of a dysregulated inflammatory and immune response, including excess pro-inflammatory mediators, complement activation, apoptosis, mitochondrial dysfunction, and eventual immunosuppression.[5] As a consequence, organ dysfunction ensues. Circulatory dysfunction manifests clinically as hypotension due to diffuse vasodilatation.

What is the role of nitric oxide in septic shock?

Nitric oxide (NO) plays a key role in the pathogenesis of vasodilatation.[8] The enzyme NO synthase is upregulated in sepsis. NO depresses metabolic autoregulation and all levels of circulation, which maintains vasodilatation and circulatory dysfunction.[32]

What is the role of hydrogen sulfide in septic shock?

Hydrogen sulfide (H_2S) has historically been known as a poisonous gas and is endogenously produced in various tissues. H_2S serves as a 'gasotransmitter' and depending on the clinical condition, H_2S has been found to be protective and detrimental.[7] In a mouse model, Zhang found *endogenous H_2S is time-dependent during sepsis* and overproduction was present in early stages.[9] Early H_2S level increases in septic shock is detrimental. In a subsequent mouse model of LPS induced septic shock, Zhang found increased H_2S gene expression was associated with organ dysfunction. One study suggests elevated H_2S are found in ventilated septic children, as compared to non-septic controls.[33] In nonsurgical adults with septic shock, Goslar found plasma H_2S levels were elevated and predicted survival.[34] Kosir confirmed H_2S was involved in the regulation of vascular tone and higher plasma levels inversely correlated with blood pressure and cardiac function.[7] Higher total plasma H_2S was associated with higher mortality, compared to lower levels.[7]

What is the interaction between NO and H_2S in septic shock?

NO plays a central role in vasodilatation and circulatory dysfunction in septic shock. NO induces gene expression for H_2S through a positive feedback loop.[35] Consequently, elevated H_2S and NO worsen vasodilatation, which sustains hypotension, potentially leading to refractory shock.

What is the proposed intervention?

As stated earlier, NO and H_2S contribute to widespread vasodilatation and circulatory dysfunction in septic shock, potentially leading to refractory hypotension, increasing the risk for mortality. Hydroxocobalamin, or vitamin B12, has been shown to scavenge, bind, and prevent the formation of NO and H_2S.[10] We propose a phase II randomized controlled trial to test the effect of high dose IV hydroxocobalamin, compared to placebo, on reducing vasopressor support in critically ill patients with septic shock.

Which preliminary data justifies a phase II randomized controlled trial?

In an LPS mouse model of septic shock, Zhang demonstrated H_2S to be a proinflammatory mediator that exacerbates the systemic inflammatory response.[36] When H_2S was inhibited, lung inflammation was blocked.[37] Greater H_2S level was identified in nonsurgical adults with shock, with survivors having lower total plasma sulfide concentrations as compared to non-survivors, and H_2S levels correlated with norepinephrine dose, severity of illness, and survival.[34] Animal models have suggested benefit when NO and H_2S are blocked by hydroxocobalamin.[9,11,12,36] Elevated NO and H_2S have been implicated in post-operative vasodilation leading to refractory shock. Numerous case series have demonstrated high dose IV hydroxocobalamin was associated with reduced vasopressor dose in post-operative vasoplegia (table 1). There are no human

studies evaluating high dose IV hydroxocobalamin in septic shock. The pathophysiologic insult in septic shock mirrors that observed in post-operative vasodilation; however, inferences drawn from observational data are weak, at best. To eliminate the effect of bias, a phase II randomized controlled trial to test feasibility and the impact of IV hydroxocobalamin on vasopressor dose is warranted.

Authors	Year	Patient	Surgery	Pre-HC NE dose	HC dose	Duration
An[13]	2018	66 year old male	Liver transplant	20 mcg/kg*^	5 grams	Multiple hours
Boettcher[17]	2017	44 year old male	Liver transplant	1.2 mcg/kg*&	500 mg/h!	Unclear
Boettcher[17]	2017	69 year old male	Liver transplant	0.1 mcg/kg/min*	250 mg/h!	Unclear
Zundel[10]	2017	28 year old male	TV replacement	0.2 mcg/kg/min	5 grams	15 minutes
Zundel[10]	2017	44 year old male	AA repair	0.45 mcg/kg/min*	5 grams	15 minutes
Burnes[16]	2017	69 year old male	AV and MVR	0.1 mcg/kg/min#	5 grams	15 minutes
Woehlck[18]	2016	63 year old female	Liver transplant	0.2 mcg/kg/min	5 grams	Multiple hours

Table 1: Observational (case series) of post-operative vasodilatory shock. AA, aortic aneurysm; AVR, aortic valve replacement; HC, hydroxocobalamin; N/A, not-applicable; NE, norepinephrine; MVR, mitral valve replacement; TV, tricuspid valve. *Also receiving vasopressin; ^weight not reported to identify weight-based NE dose; #Also receiving milrinone infusion; &Also receiving epinephrine; !Total dose unknown

2 HYPOTHESIS AND OBJECTIVES

2.1 Primary Objectives

The primary objective of this study is to measure the impact of high dose vitamin B12 in reducing vasopressor support in critically ill patients with septic shock.

2.2 Secondary Objectives

The secondary objectives of this clinical trial are to first identify the pre and post intervention differences in H_2S concentration between those patients who received hydroxocobalamin and placebo and secondly, to identify persistent organ dysfunction syndrome (PODS)[18] at 28 days in both groups.

Additional objectives of this clinical trial are to first identify the pre and post intervention differences in H_2S blood concentration between those patients who received hydroxocobalamin and placebo. Secondly, to identify persistent organ dysfunction syndrome (PODS)[18] at 28 days in both groups. Third, to examine the effect of cobalamin administration on cf-mtDNA.Fourth, to examine point of care (POC) spectrophotometric detection of plasma adducts of infused B12a (sulfocobalamin, nitrosocobalamin, hydroxocobalamin) as biomarkers for natural history of H_2S generation during septic shock, and for therapeutic titration of hydroxocobalamin infusion. Last, to use POC oximetry to quantify B12a effects on hydrogen sulfide-mediated intoxication of mitochondrial respiration in human peripheral blood mononuclear cells (PBMC).

3 STUDY DESIGN

3.1 General Description

We will conduct a phase II double-blind randomized controlled trial in a single center cohort of adult intensive care unit patients with septic shock to compare a single 5-gram intravenous dose of hydroxocobalamin to placebo on the primary outcome of reducing vasopressor dose by 15% at 3 hours. We will enroll 13 patients in each arm of this pilot study. There will be no co-interventions. We will measure pre- and post-intervention blood hydrogen sulfide levels and collect demographic, clinical, outcomes, and biochemical variables. We will employ concealed randomization, members of the study team, care team, and patients will be blinded; and all analyses will be conducted under intention-to-treat.

Number of Subjects

Up to 30 patients may be consented into this trial. Twenty-six patients are needed to meet the objectives of this clinical trial.

3.1.1 Primary Endpoint(s)

The impact of hydroxocobalamin will be measured by the percent reduction in vasopressor dose 3 hours after administration.

3.2 Secondary Endpoint(s)

The secondary endpoints will be the difference in serum H_2S after study drug administration and the rate of PODS at 28 days post ICU admission.

3.3 Study Timeline

See Study Calendar.

4 PATIENT SELECTION

4.1 Eligibility Criteria

Patients must have baseline evaluations performed prior to the first study drug dose and must meet all inclusion and exclusion criteria. In addition, the patient or LAR must be thoroughly informed about all study aspects, including the study visit schedule and required evaluations and all regulatory requirements for informed consent. The written informed consent must be obtained from the patient or LAR prior to enrollment. The following criteria apply to all patients enrolled onto the study unless otherwise specified.

4.2 Inclusion Criteria

1. Adult patients at least 18 years of age

2. Admitted to the Intensive Care Unit (ICU) service at Froedtert Hospital

3. Current diagnosis of septic shock as defined by the sepsis-3 criteria[4], based on (a) hypotension despite IV fluid resuscitation of at least 30 mL/kg (b) vasopressor use (e.g., norepinephrine dose of at least 0.1 mcg/kg/min or equivalent), and/or lactic acidosis (lactic acid >2 mmol/L)

4. Ability to randomize within 48 hours of diagnosis of septic shock or admission to ICU, whichever occurs last

5. Patient or LAR able to understand a written informed consent document and willingness to sign it

4.3 Exclusion Criteria

A potential subject who meets any of the following exclusion criteria is ineligible to participate in the study.

1. Known hypersensitivity to any component of the hydroxocobalamin formulation

2. Patients who lack adequate IV access

3. Patients not anticipated to survive the next 24 hours as determined by the treating physician

4. Participation in another investigational trial within the last 30 days

5. Patients who require treatment with chloramphenicol

6. History of urinary calcium oxalate crystals

7. Patients with an active bleed and/or hemolysis that require more than once daily hemoglobin monitoring

8. In the rare event a patient on vasopressor support is on intermittent hemodialysis, they will be excluded

9. Women who are pregnant or nursing as the effects of hydroxocobalamin are not known

5 STUDY ENTRY AND WITHDRAWAL; STUDY PROCEDURES

5.1 Study Entry Procedures

5.1.1 Required Screening

The study-specific assessments are detailed in this section and outlined in the Study Calendar.

To make the most out of the 48-hour eligibility window, patients will be identified using a pre-screening algorithm in the electronic medical record. This algorithm will be designed to notify designated members of the study team when a patient 18 years of age or older is admitted to the ICU service and requires vasopressor support. Once received, study staff with review the patient against the IRB approved criteria to ensure eligibility. If appropriate, they will pursue an informed consent discussion with the patient and/or their legally authorized representative (LAR). All patients reviewed will be documented in a screening log and reason for exclusion for reporting purposes.

A signed ICF copy will be given to the subject and a copy will be filed in the medical record. The original will be kept on file with the study records.

All patients who are consented will be registered and randomized in OnCore®, the MCW Clinical Trial Management System. The system is password protected and meets HIPAA requirements.

Screening Assessments

- Confirmation of Eligibility Criteria listed in Section 4.2 and 4.3
- Confirmation of negative pregnancy test
- Signed written informed consent form by either the patient or their LAR
- Collection of medical history
- Review of current medications
- Documentation of vasopressor use and fluid resuscitation
- APACHE II and SOFA Scores
- Serum laboratory tests including lactic acid, leukocyte count, glucose, creatinine, platelet count, and INR

5.1.2 Study Procedures, Day 1 Pre-Dose

- Collection of demographic information
- Evaluation of adverse events
- Concomitant medications
- Documentation of vasopressor use and fluid resuscitation
- APACHE II and SOFA Scores
- Serum lactic acid
- Hydrogen Sulfide Test

5.1.3 Study Procedures Day 1 Post-Dose

- Hydrogen Sulfide Test
- Evaluation of adverse events
- Concomitant medications
- Documentation of vasopressor use and fluid resuscitation

- Documentation of blood products given
- APACHE II and SOFA Scores
- Nutric Score and Calorie Counts
- Culture Results

5.2 Study Procedures Days 2-5

- Evaluation of adverse events
- Concomitant medications
- Documentation of vasopressor use and fluid resuscitation
- Documentation of blood products given
- APACHE II and SOFA Scores
- mNUTRIC Score and Calorie Counts
- Culture Results

5.2.1 Hospital Discharge or Day 30

- Evaluation of adverse events
- Concomitant medications
- Documentation of vasopressor use and fluid resuscitation
- APACHE II and SOFA Scores
- Use of Renal Replacement Therapy
- Culture Results
- Discharge Destination
- Mortality

5.3 Study Withdrawal Procedures

5.3.1 Duration of Therapy

In the absence of treatment delays due to adverse events, treatment may continue for or until:

- Disease progression
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the investigator's judgment.
- Inter-current illness that prevents further treatment administration
- Patient decides to withdraw from the study
- Unacceptable adverse event(s)

5.3.2 Patient-Initiated Withdrawal: A patient may decide to withdraw from the study at any time.

5.3.3 Investigator-Initiated Withdrawal: The Investigator will withdraw a patient whenever continued participation is no longer in the patient's best interests. Reasons for withdrawing a

patient include, but are not limited to, disease progression, the occurrence of an adverse event or a concurrent illness, a patient's request to end participation, a patient's noncompliance or simply significant uncertainty on the part of the Investigator that continued participation is prudent.

5.3.4 Withdrawal Documentation Procedure: The reason for study withdrawal and the date the patient was removed from the study must be documented in OnCore.

6 TREATMENT PLAN

6.1 Hydroxocobalamin

Patients will receive a single IV infusion to be administered by their nurse over a 10-15-minute period. The infusions will be prepared by a pharmacist. Opaque or dark bag and tubing covers will be utilized in order to protect the blind.

6.1.1 Usage of Concurrent/Concomitant Medications

Study staff will document the concomitant medications each patient has while participating in this clinical trial. Currently, the only documented drug interaction is with chloramphenicol as it may diminish the therapeutic effect of Vitamin B12. It is not anticipated that any patients participating in this trial would receive treatment with this antibiotic.

6.1.2 Usage of Hemodialysis

Patients on continuous veno-venous hemodialysis (CVVH), which is the form of dialysis patients on vasopressor support would receive, will be included. The Medical College of Wisconsin utilizes a NxStage CVVH machine. Vitamin B12 does not interfere with its use nor is calibration required. It is not anticipated that any patient will be on intermittent hemodialysis at the time of B12 infusion (since these patients are on vasopressor support); however, in the rare event they are, we will exclude them.

6.2 Follow-Up Period

Patients will be followed for 30 days after randomization or until death, whichever occurs first.

Patients removed from the study treatment for unacceptable SAEs will be followed until resolution or stabilization of the adverse event.

7 ADVERSE EVENTS: DEFINITIONS AND REPORTING REQUIREMENTS

7.1 Definitions

7.1.1 Adverse Event (AE) and Serious Adverse Events (SAE)

Patients requiring treatment for septic shock are recognized to be critically ill. It is expected in this patient population to have worsening of disease, symptoms, and in some cases death. Patients disease and symptoms will be monitored while in the study but will not be considered 'reportable' adverse events unless an investigator has reasonable doubt regarding the relatedness of the event to the investigational product. In the case where an event may be related the following will apply:

The investigator and his or her team will follow the Medical College of Wisconsin policies related to adverse event reporting. This information may be found on the Human Research Protection Program website.

Serious AE (SAE) means any untoward medical occurrence that at any dose:

- **Death.** Results in death.
- **Life threatening**. Is life threatening (refers to an AE in which the patient was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe).
- **Hospitalization**. Requires inpatient hospitalization or prolongation of an existing hospitalization (see clarification in the paragraph below on planned hospitalizations).
- **Disability/incapacity**. Results in persistent or significant disability or incapacity. (Disability is defined as a substantial disruption of a person's ability to conduct normal life functions).
- **Medically important event**. This refers to an AE that may not result in death, be immediately life threatening, or require hospitalization, but may be considered serious when, based on appropriate medical judgment, may jeopardize the patient, require medical or surgical intervention to prevent 1 of the outcomes listed above, or involves suspected transmission via a medicinal product of an infectious agent. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse; any organism, virus, or infectious particle (e.g., prion protein transmitting Transmissible Spongiform Encephalopathy), pathogenic or nonpathogenic, is considered an infectious agent.

Clarification should be made between a serious AE (SAE) and an AE that is considered severe in intensity (Grade 3 or 4), because the terms serious and severe are NOT synonymous. The general term severe is often used to describe the intensity (severity) of a specific event; the event itself, however, may be of relatively minor medical significance (such as a Grade 3 headache). This is NOT the same as serious, which is based on patient/event outcome or action

criteria described above, and is usually associated with events that pose a threat to a patient's life or ability to function. A severe AE (Grade 3 or 4) does not necessarily need to be considered serious. For example, a white blood cell count of 1000/mm3 to less than 2000 is considered Grade 3 (severe) but may not be considered serious. Seriousness (not intensity) serves as a guide for defining regulatory reporting obligations

7.1.2 Unanticipated Problem Involving Risk to Subject or Other (UPIRSO)

The investigator and his or her team will follow the Medical College of Wisconsin policies related to unanticipated problems involving risks to subjects or others. This information may be found on the Human Research Protection Program website.

7.1.3 AE Attribution and Grading

Adverse Event Grading

Grade	Description
0	No AE (or within normal limits).
1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
2	Moderate; minimal, local or noninvasive intervention (e.g., packing cautery) indicated; limiting age-appropriate instrumental activities of daily living (ADL).
3	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.
4	Life-threatening consequences; urgent intervention indicated.
5	Death related to AE

Adverse Event Attribution

Attribution is an assessment of the relationship between the AE and the medical intervention.

Relationship	Attribution	Description
Unrelated to investigational agent/intervention	Unrelated	The AE *is clearly* ***NOT*** *related* to the intervention
	Unlikely	The AE *is* ***doubtfully related*** to the intervention
Related to investigational agent/intervention	Possible	The AE ***may be related*** to the intervention

	Probable	The AE ***is likely related*** to the intervention
	Definite	The AE ***is clearly related*** to the intervention

Relationship Assessment: In-Depth Definitions

For all collected AEs, the clinician who examines and evaluates the subject will determine the adverse event's causality based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below:

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 drug administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the drug (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 sequence to administration of the drug, 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.

Possibly 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, the influence of other factors may have contributed to the event (e.g., the subject's clinical condition, other concomitant events). Although an adverse drug event 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", as appropriate.

Unlikely: A clinical event, including an abnormal laboratory test result, whose temporal relationship to drug administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the trial medication) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the subject's clinical condition, other concomitant treatments).

Unrelated: The AE is completely independent of study drug administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

7.2 Known AEs List

Most common adverse reactions reported during clinical trials (>10% of patients) are:

- Central nervous system: Headache (6% to 33%)

- Dermatologic: Erythema (94% to 100%; may last up to 2 weeks), skin rash (20% to 44%; predominantly acneiform eruption; can appear 7 to 28 days after administration and usually resolves within a few weeks)
- Gastrointestinal: Nausea (6% to 11%)
- Genitourinary: Urine discoloration (100%; may last up to 5 weeks after administration)
- Hematologic & oncologic: Lymphocytopenia (8% to 17%)
- Local: Infusion site reaction (6% to 39%)

7.3 Monitoring and Recording an Adverse Event

Definition. Any clinically relevant deterioration in laboratory assessments or other clinical finding is considered an AE.

Reporting source. AEs may be spontaneously reported by the patient and/or in response to an open question from study personnel or revealed by observation, physical examination or other diagnostic procedures.

Prior to the trial. Planned hospital admissions or surgical procedures for an illness or disease that existed before the patient was enrolled in the trial are not to be considered AEs unless the condition deteriorated in an unexpected manner during the trial (e.g., surgery was performed earlier or later than planned).

Pretreatment events following signed informed consent. For serious pretreatment events, the investigator must determine both the intensity of the event and the relationship of the event to study procedures.

Treatment events. For serious AEs, the investigator must determine both the intensity of the event and the relationship of the event to study drug administration.

Not serious AEs. For non-serious AEs, the investigator must determine both the intensity of the event and the relationship of the event to study drug administration.

Follow-up of Adverse Events

All adverse events will be followed with appropriate medical management 30 days following the last dose of the study drug or treatment or until they are resolved, if they are related to the study treatment.

7.3.1 Procedure for Reporting Drug Exposure during Pregnancy and Birth Events

If a woman becomes pregnant, or suspects that she is pregnant, while participating in this study, she must inform the investigator immediately and permanently discontinue the study drug. The sponsor-investigator must notify the DSMC by email. The pregnancy must be followed for the final pregnancy outcome.

If a female partner of a male patient becomes pregnant during the male patient's participation in this study, the sponsor-investigator must also immediately notify the DSMC by email. Every effort should be made to follow the pregnancy for the final pregnancy outcome.

7.3.2 Subject Complaints

If a complaint is received by anyone on the study staff, it will be discussed with the study staff and will be addressed on a case-by-case basis. The PI will be notified of any complaints. Complaints will be reported to the IRB if indicated.

If the subject has questions about his or her rights as a study subject, wants to report any problems or complaints, obtain information about the study or offer input, the subject can call the Medical College of Wisconsin/Froedtert Hospital research subject advocate at 414-955-8844. This information is provided to the subject in their consent.

A product complaint is a verbal, written or electronic expression that implies dissatisfaction regarding the identity, strength, purity, quality or stability of a drug product. Individuals who identify a potential product complaint situation should immediately contact the sponsor and report the event. Whenever possible, the associated product should be maintained in accordance with the label instructions pending further guidance from a sponsor representative. Product complaints in and of themselves are not Reportable Events. If a product complaint results in an SAE, an SAE form should be completed.

7.3.3 Routine Reporting Procedures for AEs

Expedited Reporting Procedures for SAEs, SARs, UPIRSOs and DLTs.

Since this is an investigator-initiated study, the principal investigator, also referred to as the sponsor-investigator, is responsible for reporting serious adverse events (SAEs) to any regulatory agency and to the sponsor- investigator's IRB. All applicable SAEs must be reported to the DSMB as soon as possible, but no later than five calendar days of the sponsor-investigator's observation or awareness of the event.

Signs or symptoms reported as adverse events will be graded and recorded by the investigator, according to the CTCAE. When possible, signs and symptoms indicating a common underlying pathology should be noted as one comprehensive event.

The investigator will assess all adverse events and determine reporting requirements to the Data and Safety Monitoring Board (DSMB) and MCW's Institutional Review Board, and, when the study is conducted under an Investigational New Drug Application (IND), to the Food and Drug Administration (FDA), if it meets the FDA reporting criteria. The investigator will report SAEs to any regulatory agency and to the sponsor- investigator's IRB.

Reporting to the Data and Safety Monitoring Board

All applicable SAEs must also be reported to the DSMB as soon as possible, but no later than five calendar days of the sponsor-investigator's observation or awareness of the event.

Report Method: The investigator will use email to report SAEs to the DSMB. The SAE report must include event term(s), serious criteria, and the sponsor-investigator's or sub-investigator's determination of both the intensity of the event(s) and the relationship of the event(s) to study drug administration. Intensity for each SAE, including any lab abnormalities, will be determined by using the NCI CTCAE as a guideline whenever possible.

The criteria are available online at http://ctep.cancer.gov/reporting/ctc.html.

Reporting to MCW Committee Institutional Review Board

The principal investigator must report events to the MCW IRB within five business days of his/her awareness of the event.

Please see the below table that outlines reporting information.

Event Type	Report Recipients				
	PI/Study Chair/ Coordinating Center	**Institutional Review Board**	**DSMB**	**FDA**	**Other**
Related Serious Adverse Event	ASAP	5 days (or annual CPR)[1]	5 days	7 or 15 days[2]	
Unanticipated Problems Involving Risks to Subjects of Others	ASAP	5 days (or annual CPR)[1]	5 days	7 or 15 days[2]	
Evidence of Causal Relationship between Drug and AE	ASAP	5 days (or annual CPR)[1]	5 days	7 or 15 days[2]	

Contacts					
Role	**Name**	**Entity/Department**	**Institution**	**Telephone**	**Email**
Sponsor-Investigator	Jayshil Patel, MD	Medicine	MCW	414-955-7040	Jpatel2@mcw.edu
Research Coordinator	Stephanie Jones, MS, CCRC	Medicine	MCW	414-955-7085	stejones@mcw.edu
IRB Representative	TBD		MCW	414-955-8844	
DSMB	Todd Rice, MD	Medicine	Vanderbilt University		
FDA			FDA	1-800-FDA-1088	www.fda.gov/medwatch

Footnotes

[1] Consult MCW IRB Policies (contact your regulatory representative)

[2] FDA guidelines: Suspected adverse reaction, Unexpected and Serious = 7 Days; If not = 15 days

8 PHARMACEUTICAL INFORMATION

8.1 Hydroxocobalamin (Cyanokit)

8.1.1 Product Description

Hydroxocobalamin, the active ingredient in Cyanokit, is cobinamide dihydroxide dihydrogen phosphate (ester), mono (inner salt), 3'-ester with 5,6-dimethyl-1-α-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.

Classification: Vitamin

Mechanism of Action: The mechanisms by which IV hydroxocobalamin mitigates catecholamine resistant vasodilatory shock are not firmly established, but hydroxocobalamin has been shown to scavenge, bind, and prevent the formation of NO and H_2S.[10, 16,18, 44] Due to this effect, IV hydroxocobalamin has been used in catecholamine refractory shock.[14] Specifically, NO oxidizes the cobalt (Co) atom in hydroxocobalamin.[45] The Co-NO complex could transfer its NO to hemoglobin, reducing the circulating NO level.[46] Less circulating NO and H_2S may stabilize the capillary membrane and restore vascular tone.[10] In effect, hydroxocobalamin serves as a sink to dispose of excess circulating gasotransmitters.

Metabolism: Cobalamins are excreted in the urine with the majority of urinary excretion occurring during the first 24 hours following administration.

Contraindications:

There are no contraindications listed in the manufacturer's labeling.

Side Effects: Complete and updated adverse event information is available in the product package insert.

8.1.2 Solution Preparation

The 5 g vial of hydroxocobalamin for injection is to be reconstituted with 200 mL of diluent (not provided with Cyanokit) using the supplied sterile transfer spike. The recommended diluent is 0.9% Sodium Chloride injection (0.9% NaCl). Lactated Ringers injection and 5% Dextrose injection (D5W) have also been found to be compatible with hydroxocobalamin and may be used if 0.9% NaCl is not readily available. The line on the vial label represents 200 mL volume of diluent. Following the addition of diluent to the lyophilized powder, the vial should be repeatedly inverted or rocked, not shaken, for at least 60 seconds prior to infusion. Hydroxocobalamin solutions should be visually inspected for particulate matter and color prior to administration. If the reconstituted solution is not dark red or if particulate matter is seen after the solution has been appropriately mixed, the solution should be discarded.

8.1.3 Storage Requirements

Lyophilized form: Store at 25°C (77°F); excursions permitted to 15-30°C (59 to 86°F) [see USP Controlled Room Temperature].

Cyanokit may be exposed during short periods to the temperature variations of usual transport (15 days submitted to temperatures ranging from 5 to 40°C (41 to 104°F), transport in the desert (4 days submitted to temperatures ranging from 5 to 60°C (41 to 140°F)) and freezing/defrosting cycles (15 days submitted to temperatures ranging from -20 to 40°C (-4 to 104°F)).

Reconstituted solution: Store up to 6 hours at a temperature not exceeding 40ºC (104°F). Do not freeze. Discard any unused portion after 6 hours.

8.1.4 Route of Administration

Intravenous infusion over a 15-minute time period via a dedicated line.

8.1.5 Agent Accountability

The Investigational Pharmacist will manage drug accountability records.

8.1.6 Agent Destruction and Return

At the conclusion of the study, any unused agent will be destroyed according to institutional policies. The destruction will be recorded on the *Drug Accountability Record Form*.

9 STATISTICAL CONSIDERATIONS

9.1 Study Design

This is a single site, prospective, randomized, phase II study.

9.2 Randomization

Patients will be randomized utilizing OnCore by Forte Clinical Trial Management Software. Patients will be randomized with a 1:1 ratio with a block scheme randomization.

9.3 Sample Size and Power Estimate

Using the variability values from ATHOS-3 trial,[42] if we assume a standard deviation of 15% in percent change in blood pressure from baseline to 3 hours, then with 13 patients per group the study would have 80% power to detect a 15 percentage point difference in the percent change of norepinephrine at 3 hours between the two groups at a one-sided 5% significance level.

9.4 Replacement Policy

Patients will only be replaced if they were consented into the trial but failed to receive study drug for any reason. Patients who began treatment but were unable to complete the infusion will be included in the analysis with intent to treat.

9.5 Interim Analyses and Stopping Rules

No interim analysis is planned at this time.

After each 25% increment in completed enrollment, the study will be reviewed by the DSMB. The DSMB will provide a recommendation to either continue the study or stop early.

9.6 Analyses Plans

Appropriate descriptive statistics such as median, mean, standard deviation, range, and proportions reported with 95% confidence interval will be used to summarize the demographic and clinical characteristics of our patient population. Time-to-event outcomes, such as length of stay, time to 50% reduction in vasopressors, will be presented using cumulative incidence curves with in-hospital mortality as a competing risk. The primary outcome, the reduction in norepinephrine dose at 3 hours, will be compared between the groups using the

Wilcoxon rank-sum test. In addition to the primary unadjusted analysis, regression-based approaches will be used to explore the effect of the prognostic covariates such as age, co-morbidities, APACHE scores, etc. on the outcomes of interest. All analyses will be on an intention-to-treat basis, utilizing all patients randomized.

Statistical support will be provided by Aniko Szabo, PhD. She is an Associate Professor of Biostatistics and Director of Biostatistics Consultation at the Medical College of Wisconsin in Milwaukee, WI.

10 DATA AND SAFETY MONITORING PLAN (DSMP)

Please refer to the DSMB Charter.

Data and Safety Management Overview

The Data Safety Monitoring Board (DSMB) and the MCW Institutional Review Board (IRB) will approve protocol-specific DSM plans. A local, investigator-initiated trial will be required to be continuously monitored by the principal investigator of the study with annual safety and progress reports submitted to the DSMC.

10.1 Study Team

The study team minimally consists of the principal investigator, the clinical research coordinator, and the study biostatistician. While subjects are on treatment, the principal investigator will meet regularly with the research coordinator and the study biostatistician to review study status. This review will include but not be limited to reportable SAEs and UPIRSOs and an update of the ongoing study summary that describes study progress in terms of the study schema. The appropriateness of further subject enrollment and the specific intervention for a next subject enrollment is addressed.

10.2 Quality Assurance

The MCW Office of Research provides ongoing quality assurance audits when requested.

10.3 DSMB

The Medical College of Wisconsin places the highest priority on ensuring the safety of patients participating in clinical trials.

This study will be reviewed by the Data and Safety Monitoring Board (DSMB). A summary of the DSMB activities are as follows:

- Review the clinical trial for data integrity and safety
- Review all unexpected grade 3, and all grade 4, and 5 adverse events, as well as any others requiring expedited reporting as defined in this protocol. (Grades 4 & 5 events must be reported to the DSMC within 5 calendar days of study staff's knowledge.)
- Review all DSM reports
- Submit a summary of any recommendations related to study conduct
- Terminate the study if deemed unsafe for patients

A copy of the Data and Safety Monitoring Plan and membership roster will be maintained in the study research file and updated as membership changes. The committee will review reports from the study PI annually (or more frequently if needed) and provide recommendations on trial continuation, suspension or termination as necessary.

Any available DSMB letters will be submitted to the IRB of record as required.

11 REGULATORY COMPLIANCE, ETHICS AND STUDY MANAGEMENT

11.1 Ethical Standard

This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki as stated in 21 CFR §312.120(c)(4); consistent with GCP and all applicable regulatory requirements.

11.2 Regulatory Compliance

This study will be conducted in compliance with:

- The protocol
- Federal regulations, as applicable, including: 21 CFR 50 (Protection of Human Subjects/Informed Consent); 21 CFR 56 (Institutional Review Boards) and §312 (Investigational New Drug Application; and 45 CFR 46 Subparts A (Common Rule), B (Pregnant Women, Human Fetuses and Neonates), C (Prisoners), and D (Children), GCP/ICH guidelines, and all applicable regulatory requirements. The IRB must comply with the regulations in 21 CFR §56 and applicable regulatory requirements.

11.3 Pre-study Documentation

Prior to implementing this protocol at MCW, the protocol, informed consent form, HIPAA authorization and any other information pertaining to participants must be approved by the MCW IRB.

The clinical investigation will not begin until either FDA has determined that the study under the Investigational Drug Application (IND) is allowed to proceed or the Investigator has received a letter from FDA stating that the study is exempt from IND requirements.

11.4 Institutional Review Board

The protocol, the proposed informed consent form and all forms of participant information related to the study (e.g. advertisements used to recruit participants) will be reviewed and approved by the MCW Institutional Review Board. The initial protocol and all protocol amendments must be approved by the IRB prior to implementation.

Informed Consent Process

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Extensive discussion of risks and possible benefits of this therapy will be provided to the subjects and their families. Consent forms describing in detail the study interventions/products, study procedures, and risks are given to the subject and written documentation of informed consent is required prior to starting intervention/administering study product.

Potential subjects will be told, and a statement will be included that this study is designed to determine both safety and effectiveness. The consent forms will include the approved MCW IRB template language.

Consent forms will be IRB-approved and the subject (and Legally Authorized Representative, if necessary) will be asked to review the document. Upon reviewing the document, the investigator will explain the research study to the subject and answer any questions that may arise. In accordance with 46 CR 46.111, the subject will sign and date the informed consent document prior to any procedures being done specifically for the study.

A witness should only sign when required, per FH/MCW IRB policy. If a witness signs the document when not required, the study staff should document in the legal medical record (or note to file) the relationship to the patient and why a witness signed. (i.e., “Although not required, the subject’s spouse was present during the consenting process and signed as the witness.” Or “Although not required, hospital staff was present for consenting process and signed as a witness.”)

The subjects (or their LAR) will have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The subjects may withdraw consent at any time throughout the course of the trial.

Decisional ability to provide informed consent is determined by the treating team. The treating team utilizes the teach back method to determine a patient's decisional ability i.e. asking if the patient understands the risks and benefits of whatever interventions may be/have been presented to them for their care, confirm that they patient understands the current state of their health etc. If the treating team indicates the subject has decisional ability, the study team will approach them to potentially consent them for the project. During the consent process, study staff are trained to utilize the teach back method in order to gauge patient understanding. If study staff do not feel the patient is understanding a consent discussion, they will consult the treating team to contact the appropriate person (LAR).

Study staff work with the treating team to contact the appropriate person (LAR). Study team will consult and work with the treating team/social work if a POA/LAR has not been identified in EPIC. A copy of the informed consent document will be given to the subjects (or their LAR) for their records. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely impacted if they decline to participate in this study. Study subjects will not be reconsented for continuing reviews.

If a patient is awake, but non-decisional, the study team will ask for assent through nodding, etc. in the presence of the LAR and/or witness. This will be documented in the patient’s medical record with the rest of the consent documentation. If a LAR is providing consent, the patient is likely sedated/unconscious; that being the case, assent will not be any more possible than the patient providing consent directly.

Decisional ability is determined by the treating team, including the attending physician. Study staff will consult with the treating team to determine when a patient has been deemed decisional. Formal re-consenting will not occur. The interventional portion of the study will be completed over the course of about 3 hours. All interventions will be completed before the subject would regain decisionality if the LAR were to have consented on the subject’s behalf. There are no formal visits or follow-up procedures after the treatment day. The subject will only be followed in EPIC for up to 30 days after randomization or death, whichever comes first. Formal re-consent could potentially cause much confusion since the intervention is complete. Study staff will be available if LAR requests further explanation for the subject once the subject has been deemed decisional by the treating team. This conversation will be documented in EPIC that the study was informally explained to the subject and all questions were addressed.

After the subject’s visit in which the consent is signed, it is documented in the medical chart that the consent has been signed and that all questions have been answered to the subject’s satisfaction after adequate time for review of the consent. It is also documented that a copy of the consent is given to the subject. The original consent is kept with the subject’s study file, and a copy of the consent is sent to the OCRICC office, which will then submit to HIM a copy of the signed consent to be scanned into EPIC, the legal medical record.

11.5 Subject Confidentiality and Access to Source Documents/Data

Subject confidentiality is strictly held in trust by the sponsor-investigator, participating investigators, and any staff. This confidentiality includes the clinical information relating to participating subjects.

The study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the principal investigator.

The conditions for maintaining confidentiality of the subjects' records are required for the life of the data. These rules apply equally to any and all MCW projects.

One risk of taking part in a research study is that more people will handle the personal health information collected for this study. The study team will make every effort to protect the information and keep it confidential, but it is possible that an unauthorized person might see it. Depending on the kind of information being collected, it might be used in a way that could embarrass the subject or affect his/her ability to get insurance.

While data are being collected and after all data have been collected but are still in the process of being analyzed, the subject's data/PHI are stored in the locked Clinical Research offices in the PI's Division. Databases in which the study subject information is stored and accessed are password protected, allowing for limited access by authorized personnel only. Data/PHI kept in the Case Report Forms contain the study identifiers, subject initials, date of birth and date of service.

Personal identifiers, such as name and medical record number, will be removed from accompanying lab reports and test results. Any data/PHI that are not stored for the purposes of the study are shredded.

After all study queries and analyses are completed, the data/PHI will not be destroyed but will be archived in a secure long-term storage site in order to keep an accurate record of screened and enrolled subjects for the sponsor and potential audit purposes only specific for this study. Data/PHI would not be destroyed until permission is granted by the sponsor to destroy the records.

The principal investigator will allow access to all source data and documents for the purposes of monitoring, audits, IRB review, and regulatory inspections.

The study monitor or other authorized representatives of the investigator may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the subjects in this study. The clinical study site will permit access to such records.

11.6 Protection of Human Subjects

11.6.1 Protection from Unnecessary Harm

Each clinical site is responsible for protecting all subjects involved in human experimentation. This is accomplished through the IRB mechanism and the informed consent process. The IRB reviews all proposed studies involving human experimentation and ensures that the subject's rights and welfare are protected and that the potential benefits and/or the importance of the knowledge to be gained outweigh the risks to the individual. The IRB also reviews the informed consent document associated with each study in order to ensure that the consent document accurately and clearly communicates the nature of the research to be done and its associated risks and benefits.

11.6.2 Protection of Privacy

As noted, patients will be informed of the extent to which their confidential health information generated from this study may be used for research purposes. Following this discussion, they will be asked to sign informed consent documents. The original signed document will become part of the patient's medical records, and each patient will receive a copy of the signed document.

11.7 Changes in the Protocol

Once the protocol has been approved by the MCW IRB, any changes to the protocol must be documented in

the form of an amendment. The amendment must be signed by the investigator and approved by IRB prior to implementation.

If it becomes necessary to alter the protocol to eliminate an immediate hazard to patients, an amendment may be implemented prior to IRB approval. In this circumstance, however, the Investigator must then notify the IRB in writing within five working days after implementation.

The IRB may provide, if applicable regulatory authority(ies) permit, expedited review and approval/favorable opinion for minor change(s) in ongoing studies that have the approval /favorable opinion of the IRB. The investigator will submit all protocol modifications to the sponsor and the regulatory authority(ies) in accordance with the governing regulations.

Any departures from the protocol must be fully documented in the source documents.

11.8 Investigator Compliance

The investigator will conduct the study in compliance with the protocol given approval/favorable opinion by the IRB and the appropriate regulatory authority(ies).

Onsite Audits

Auditing is essential to ensure that research conducted at the Medical College of Wisconsin (MCW) is of the highest quality and meets MCW and regulatory agency standards.

Regulatory authorities, the IRB and/or sponsor may request access to all source documents, data capture records and other study documentation for on-site audit or inspection. Direct access to these documents must be guaranteed by the investigator, who must provide support at all times for these activities.

12 DATA HANDLING AND RECORD KEEPING

All subjects enrolled into the trial will be assigned a unique study ID number in order to maintain patient privacy and confidentiality. All data collected for the purpose of research will be entered in a secure electronic data capture system on secured servers. Information recorded for research purposes will be captured directly from the patient's electronic medical record or recorded on CRFs (or eCRFs). The patient's medical record, study CRFs, and any notes or print outs made by the study staff will be considered source documents and will be made available for inspection or review in the event of an inspection or audit by a regulatory official. All images that require Principal Investigator review will be sent de-identified using the provided de-identifying software.

All study staff will undergo appropriate training to ensure accuracy in data capture. The Principal Investigator remains responsible for the accuracy and integrity of the data collected under his or her supervision.

All study records will be retained for a minimum of 10 years per MCW policy or until two years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region.

12.1 Overview

Every effort is made to uphold the integrity of the project, the research, the institution, and the researchers involved. Data collection guidelines and methodologies are carefully developed before the research begins. Investigators focus on the following to ensure data integrity: well-trained data collectors/recorders to ensure consistency and quality, well-designed data collection protocols and ongoing monitoring. In this way, study rigor and validity are maintained. Data is protected from physical damage as well as from tampering, loss or

theft. This project's data management is a multidisciplinary activity that includes investigators, research coordinators and nurses, data mangers, support personnel, biostatisticians and database programmers. Quality control will be applied to each stage of data handling to ensure that all data are reliable and have been processed correctly.

12.2 Data Management Responsibilities

12.2.1 Principal Investigator
The principal investigator oversees the management of patient records/case report forms and ensures that a) complete and accurate data will be obtained; b) patient records are maintained to include history, prescribed medication and investigational product(s), measurements, exams, evaluations and adverse events; c) corrections are applied to clinical research data according to principles of good research practice (i.e., single-line delete, date and initial). He or she will ensure that there is correlation between the case report forms and the source documents**.**

12.2.2 Research Coordinator
A research coordinator creates, collects and organizes clinical trial documentation. He or she ensures that source documentation and data abstraction and entry are being done at protocol specified time points.

12.2.3 Biostatistician
The biostatistician may assist in CRF development (content and design), dataset specifications (annotation of CRFs and record layout) and validation.

12.3 Handling and Documentation of Clinical Supplies

The MCW Principal Investigator will maintain complete records showing the receipt, dispensation, return, or other disposition of all investigational drugs. The date, quantity and batch or code number of the drug, and the identification of patients to whom study drug has been dispensed by patient number and initials will be included. The sponsor-investigator will maintain written records of any disposition of the study drug.

The principal investigator shall not make the investigational drug available to any individuals other than to qualified study patients. Furthermore, the principal investigator will not allow the investigational drug to be used in any manner other than that specified in this protocol.

12.4 Source Documents

Source documents for clinical information (patient history, diagnosis, clinical and diagnostic test reports, etc.) are maintained in the patient's clinical file.

All source documents will be written following ALCOA standards:

ALCOA Attribute	Definition
Attributable	Clear who has documented the data.
Legible	Readable and signatures identifiable.
Contemporaneous	Documented in the correct time frame along with the flow of events. If a clinical observation cannot be entered when made, chronology should be recorded. Acceptable amount of delay should be defined and justified.
Original	Original, if not original should be exact copy; the first record made by the appropriate person. The investigator should have the original source document.
Accurate	Accurate, consistent and real representation of facts.
Enduring	Long-lasting and durable.

Available and accessible	Easily available for review by treating physicians and during audits/inspections. The documents should be retrievable in reasonable time.
Complete	Complete until that point in time.
Consistent	Demonstrate the required attributes consistently.
Credible	Based on real and reliable facts.
Corroborated	Data should be backed up by evidence.

12.5 Study Record Retention

The principal investigator is required to maintain adequate records of the disposition of the drug, including dates, quantity and use by subjects, as well as written records of the disposition of the drug when the study ends.

The principal investigator is required to prepare and maintain adequate and accurate case histories that record all observations and other data pertinent to the investigation on each individual administered the investigational drug or employed as a control in the investigation. Case histories include the case report forms and supporting data including, for example, signed and dated consent forms and medical records including, for example, progress notes of the physician, the individual's hospital chart(s), and the nurses' notes. The case history for each individual shall document that informed consent was obtained prior to participation in the study.

Study documentation includes all CRFs, data correction forms or queries, source documents, sponsor-investigator correspondence, monitoring logs/letters, and regulatory documents (e.g., protocol and amendments, IRB correspondence and approval, signed patient consent forms).

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary for the evaluation and reconstruction of the clinical research study.

In accordance with FDA regulations, the investigator shall retain records for a period of two years following the date a marketing application is approved for the drug for the indication for which it is being investigated; or, if no application is to be filed or if the application is not approved for such indication, until two years after the investigation is discontinued and FDA is notified.

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APPENDIX 1. *SPECIMEN COLLECTION AND PROCESSING*

Sampling and information

- Draw blood into three plastic BD vacutainer tubes containing lithium heparin
- Place immediately on ice and transfer to Medical Education Building
- Samples will be centrifuged at 1,500g for four minutes to separate plasma
- In a hypoxic chamber, 50µL of sample will be transferred into 3 vacutainer tubes and mixed with the appropriate phosphate buffer for measurement of acid-labile sulfide, bound sulfane sulfur, and free sulfide
- A 10mM MBB solution will be added to the samples and incubated for 30min at room temperature
- The reaction will be stopped by the addition of 200mM 5-sulfosalicylic acid
- Samples will be stored at 4°C until they are analyzed by HPLC
- Approximately 10µL of each sample will be injected into the HPLC using the fluorescence detector setting of 390nm/475nm for excitation and emission respectively