A Randomized, Controlled, Double-Blind, Placebo-Controlled Study Evaluating the Efficacy of Intravenous Iron Sucrose in Adolescents with Non-anemic Iron Deficiency and Postural Orthostatic Tachycardia Syndrome (POTS)

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Study Product: Iron Sucrose marketed as Venofer®

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List of Abbreviations

LIST OF ABBREVIATIONS

AE	Adverse Event/Adverse Experience
CFR	Code of Federal Regulations
CRF	Case Report Form
DSMB	Data and Safety Monitoring Board
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
IND	Investigational New Drug Application
IRB	Institutional Review Board
PHI	Protected Health Information
PI	Principal Investigator
SAE	Serious Adverse Event/Serious Adverse Experience
SOP	Standard Operating Procedure

Study Summary

Title	A Randomized, Controlled, Double-Blind, Placebo-Controlled Study Evaluating the Efficacy of Intravenous Iron Sucrose in Adolescents with Non-anemic Iron Deficiency and Postural Orthostatic Tachycardia Syndrome (POTS)
Running Title	Iron Sucrose in Adolescents with Iron Deficiency and POTS
Protocol Number	12-009963
Phase	Phase II
Methodology	Randomized, controlled, double-blind, placebo-controlled
Overall Study Duration	Expected duration of 2 years
Subject Participation Duration	Six months
Single or Multi-Site	Single center
Objectives	1. To assess whether a single infusion of iron sucrose will improve orthostatic symptoms as measured by a validated survey instrument in adolescent subjects with POTS and non-anemic iron deficiency 2. To assess whether a single infusion of iron sucrose will improve cardiovascular indices, specifically a reduction in the intraval of measured heart rate change, during a ten minute head up tilt, in adolescent subjects with POTS and non-anemic iron deficiency 3. To determine if abnormalities in standing and supine catecholamine levels will improve following intravenous iron sucrose infusion in adolescent subjects with POTS and non-anemic iron deficiency
Number of Subjects	30
Diagnosis and Main Inclusion Criteria	Adolescent subjects 12-21 years of age who have a diagnosis of Postural Orthostatic Tachycardia Syndrome and non-anemic iron deficiency as defined by a serum ferritin level of less than or equal to 20 ug/L
Study Product, Dose, Route, Regimen	5 mg/kg of intravenous iron sucrose supplied as Venofer ® (maximum dose of 200 mg). Iron sucrose will be diluted to 1 mg of elemental iron in 1 mL of NaCl 0.9% (maximum volume of 210 mL).
Duration of Administration	Single dose administration
Reference therapy	Normal saline (NaCl 0.9%) 5 mL/kg (maximum volume 210 mL)

Statistical Methodology	Data will be visually inspected for normality. Group comparisons will include Fisher's exact test for categorical variables and Wilcoxon rank sums for continuous variables. Confidence intervals will be calculated at 95%. All P-values will be 2-sided with values less than 0.05 considered significant. All analyses will be performed on an "intention to treat" basis.

1 Introduction

This document is a clinical research 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

Postural orthostatic tachycardia syndrome (POTS) is characterized by an exaggerated heart rate response to the standing position in a patient with symptoms of orthostatic intolerance [1]. It is likely a heterogeneous disorder representing a common manifestation of a number of possible underlying derangements such as impaired sympathetically mediated vasoconstriction, excessive sympathetic drive, volume dysregulation and deconditioning [2]. POTS was first described in the literature by Low and Schondorf in 1993 and was recognized in adolescent by Stewart et al. in 1999 [3, 4] While the actual prevalence of POTS is unknown, it has been estimated to affect at least 500,000 individuals in the United States [5]. It predominately affects young individuals, and particularly women[6].

The prevalence of iron deficiency in adolescents with POTS has been reported to be greater than that expected in the general adolescent population [7, 8]. Iron is an essential element in both cellular and enzymatic function. Iron deficiency is one of the most common nutritional deficiencies noted worldwide [9]. Iron deficiency has been estimated to affect nearly 7.8 million adolescent girls and women of childbearing age in the United States [8]. Iron deficiency has been linked to numerous clinical findings in children and adolescents including decreased motor activity, social inattention, delayed cognitive development, neurally mediated syncope, restless legs syndrome and chronic fatigue [7, 9, 10]. Iron supplementation in non-anemic, iron-deficient adolescent girls has demonstrated improvements in cognitive functioning [11]. A double-blinded, placebo-controlled trial demonstrated a reduction of chronic fatigue symptoms following intravenous iron administration in iron deficient, non-anemic patients [12]. A similar study showed a reduction in symptoms of restless leg syndrome following iron infusion for iron-deficient patients [13].

Interestingly, elevations in urine and blood catecholamine levels have been described in non-anemic iron deficient animal models [14]. Similar urine catecholamine elevations were found in children with iron deficient anemia [15]. An adult study demonstrated elevated serum catecholamines levels in iron deficient patients with anemia, and found that anemia from other causes had no significant increase in plasma catecholamine levels [16]. While the pathophysiology of catecholamine synthesis, function and degradation is still not fully understood, it is possible that iron may be an integral component, given its ubiquitous role in enzyme function. Catecholamine derangements have been found in patients diagnosed with POTS, thus there may be a correlation between iron deficiency and POTS.

Adolescence is a complex time of physical, emotional, social, cognitive and moral development [17]. These changes are paralleled by transitions from parental to self-management for health care related issues. Although adolescents are increasingly responsible for their health care

decisions, there is an extensive literature highlighting poor medication adherence in this population [18]. Oral iron supplements, in particular, are commonly discontinued due to patient complaints of gastric irritation and lack of palatability [19]. In part given these limitations, intravenous iron supplementation has been used clinically for correction of iron deficiency in select adolescent patients who are otherwise healthy.

The use of intravenous iron sucrose has been shown to be a safe and effective therapy for correcting iron deficiency in children and adolescents [20, 21]. Iron sucrose administered at a dose of 7 mg Fe/kg, up to a maximum dose of 300 mg of iron sucrose, has been shown to be well-tolerated in children and adolescents [20]. Adult data have found similar safety and limited side effects with single intravenous doses less that 300 mg of iron sucrose [22].

Anecdotal experience at our clinic has demonstrated an immediate reduction in self-reported orthostatic symptoms in several patients diagnosed with POTS following the administration of intravenous iron sucrose for iron deficiency.

1.2 Investigational Agent

The following text in this section 1.2 is taken directly from the Drug Summary available on MD Consult. This information was last updated September 28, 2012[23]:

Description: Iron sucrose is an intravenous iron formulation approved for the treatment of iron deficiency anemia in patients with chronic kidney disease. Iron sucrose is one of the oldest parenteral iron preparations available; it has been used clinically for more than 50 years. Parenteral iron is preferred over oral iron therapy when rapid repletion of iron-depleted patients is desired. Intravenous iron has been used to treat the anemia associated with dialysis and may reduce the need for erythropoietin dosage by about 40%[24]. Iron sucrose contains ferric hydroxide complexed with sucrose; this complex has a molecular weight of 34,000—60,000 daltons. This compound is free of ferrous ion and dextran polysaccharides. In contrast, iron dextran consists of a complex of ferric oxyhydroxide with dextrans. Parenteral iron sucrose (Venofer®) and iron gluconate (Ferrlecit® , see separate monograph) appear less likely to cause hypersensitivity reactions than iron dextran[25], and have been used in patients with a hypersensitivity to iron dextran[26-28]. However, hypersensitivity may occur with iron sucrose also; serious and fatal anaphylactic reactions have been reported. Intravenous iron sucrose rapidly increases serum iron concentrations, which can be reliably measured 48 hours after administration. Iron sucrose complex (Venofer) was originally approved by the FDA for administration by slow IV injection or IV infusion in November 2000 in adult patients with iron deficiency anemia and chronic kidney disease requiring hemodialysis who are receiving supplemental erythropoietin therapy. In June 2005, the FDA approved iron sucrose for the treatment of iron deficiency anemia in non-dialysis dependent adult patients with chronic kidney disease, and in October 2005, the FDA approved iron sucrose for the treatment of iron deficiency anemia in adult patients with chronic kidney disease on peritoneal dialysis who are receiving supplemental erythropoietin therapy. In September 2012, iron sucrose was approved for iron maintenance treatment in pediatric patients >= 2 years with iron deficiency anemia and chronic

kidney disease who require hemodialysis, peritoneal dialysis, or are non-dialysis dependent and are receiving supplemental erythropoietin therapy[28].
Mechanism of Action: Normal erythropoiesis depends on the concentration of iron and erythropoietin available in the plasma, both being decreased in renal failure. Exogenous administration of erythropoietin increases red blood cell production and iron utilization, contributing to iron deficiency in hemodialysis patients. Intravenous iron has been used to treat the anemia associated with hemodialysis and may reduce the need for erythropoietin dosage by about 40%. Following IV administration of iron sucrose, the complex of polynuclear iron (III)-hydroxide in sucrose is dissociated into iron and sucrose by the reticuloendothelial system. In addition, a competitive exchange of iron takes place from the iron sucrose complex to the iron-binding protein transferrin. A therapeutic response to treatment with iron therapy is dependent upon the patient's iron stores and the ability to use the iron. Use of iron is influenced by the cause of the deficiency as well as other illnesses that can affect normal erythropoiesis. Protein-energy malnutrition can prevent the incorporation of iron into the erythrocyte regardless of the quantity of iron stored. Only when lean body mass expands will iron be used. Iron therapy alone does not increase red blood cell production. Administration of iron only improves anemia which is associated with iron deficiency.

Iron-containing proteins and enzymes are important in oxidation-reduction reactions, especially those of the mitochondria. Iron is a component of myoglobin and several heme-enzymes, including the cytochromes, catalase, and peroxidase. Iron is an essential component of the metalloflavoprotein enzymes and the mitochondrial enzyme alpha-glycerophosphate oxidase. Iron-containing proteins and enzymes are important in oxidation-reduction reactions, especially those of the mitochondria. Furthermore, iron is a cofactor for enzymes such as aconitase and tryptophan pyrrolase. Iron deficiency not only causes anemia and decreased oxygen delivery, it also reduces the metabolism of muscle and decreases mitochondrial activity. Iron deficiency can also lead to defects in learning or thermoregulation. Thus iron is important to several metabolic functions which are independent of its importance to erythropoiesis.

Pharmacokinetics: Iron sucrose is administered intravenously. In healthy adults receiving IV doses of iron sucrose, its iron component appears to distribute mainly in blood and to some extent in extravascular fluid. A significant amount of the administered iron distributes in the liver, spleen and bone marrow. The bone marrow is considered an iron trapping compartment and not a reversible volume of distribution. Following IV administration, iron sucrose is dissociated into iron and sucrose by the reticuloendothelial system. The sucrose component is eliminated primarily by urinary excretion (75.4% in 24 h). Approximately 5% of the iron is excreted in the urine over 24 hours. In healthy adults treated with IV iron sucrose, the iron component exhibits linear kinetics with an elimination half-life of about 6 hours and a systemic clearance of 1.2 L/h.

Iron therapy dosage is individualized according to patient goals for serum iron levels, iron storage parameters (e.g., ferritin, transferrin saturation) and serum hemoglobin concentrations. Iron toxicity may occur with excessive or unnecessary iron therapy. Systemic iron is stored in compounds called ferritin and hemosiderin, which are used for future use in the production of hemoglobin. The absorption of iron depends upon the route of entry. The tissue that first clears parenterally administered iron from the bloodstream determines the bioavailability. If the

reticuloendothelial system clears iron, only small amounts will be available over time to the bone marrow. Transferrin accepts iron from the intestinal tract or from sites of storage or hemoglobin destruction. Iron is then transported in plasma bound to transferrin and distributed to the bone marrow for hemoglobin synthesis, to the reticuloendothelial system for storage, to all cells for enzymes containing iron and to placental cells if needed to meet fetal needs. Transferrin eventually becomes available for reuse. There is no destructive metabolism of iron because it takes place in a closed system. In normal adults, ninety percent of metabolized iron is conserved and reutilized repeatedly. Very little iron is eliminated. In normal, healthy adults, some daily loss of iron occurs through normal skin, hair, and nail loss, and GI losses. Menstruating women have an increased loss as do other persons with loss of blood.

•***Special Populations***

Pediatrics
Following a single-dose of iron sucrose 7 mg/kg (max = 200 mg) in patients 12-16 years (n = 11) with non-dialysis dependent chronic kidney disease, mean AUC was 31305 mcg x h/dl and mean C_{max} was 8545 mcg/dl, which were 1.67- and 1.42-fold higher than reported values in adults. The half-life of total serum iron was 8 hours[28].

Gender Differences
The effects of gender on the pharmacokinetics of iron sucrose have not been studied.

1.3 Clinical Data to Date

Several clinical studies were conducted by the manufacturer. One study was conducted in children. It was a randomized, open-label, dose-ranging study for iron maintenance treatment in pediatric patients with dialysis-dependent or non-dialysis-dependent CKD on stable erythropoietin therapy. The study randomized patients to one of three doses of Venofer (0.5 mg/kg, 1.0 mg/kg or 2.0 mg/kg). The mean age was 13 years (range 2 to 20 years). Over 70% of patients were 12 years or older in all three groups. There were 84 males and 61 females. About 60% of patients underwent hemodialysis and 25% underwent peritoneal dialysis in all three dose groups. At baseline, the mean hemoglobin was 12 g/dL, the mean TSAT was 33% and the mean ferritin was 300 ng/mL. Patients with HDD-CKD received Venofer once every other week for 6 doses. Patients with PDD-CKD or NDD-CKD received Venofer once every 4 weeks for 3 doses. Among 131 evaluable patients with stable erythropoietin dosing, the proportion of patients who maintained hemoglobin between 10.5 g/dL and 14.0 g/dL during the 12-week treatment period was 58.7%, 46.7%, and 45.0% in the Venofer 0.5 mg/kg, 1.0 mg/kg, and 2.0 mg/kg groups, respectively. A dose-response relationship was not demonstrated[28].

Since iron sucrose has been available for clinical use, it has been used in several research studies, two of these studies included children and adolescents with iron deficiency not related to underlying chronic renal failure [20, 21]. In one of these studies, 38 children were treated with parenteral iron sucrose for treatment of iron deficiency [20]. Five mild reactions were reported and included one patient with headache, two patients with abdominal pain, one patient with transient mild low blood pressure and one patient with fainting. In this study, one 15 year old

female had a serious allergic reaction that responded to treatment. This patient was treated with 500 mg of iron sucrose, which was above the recommended maximum dose of 300 mg. This study concluded that parenteral iron was a "safe and effective means to treat iron deficiency in children who cannot receive or do not respond to oral iron due to intolerance, poor adherence, or iron malabsorption" [20].

1.4 Dose Rationale and Risk/Benefits

Venofer®, an iron sucrose infusion preparation, has been approved by the U.S. Food and Drug Administration (FDA) for treatment of children and adolescents with iron-deficiency anemia and chronic kidney disease. The use of iron sucrose infusion is not FDA approved for the treatment of iron deficiency in adolescents with POTS.

In efficacy studies in non-dialysis dependent-chronic kidney disease patients (N=139), the most frequent adverse events (≥5%) whether or not related to Venofer® administration, were nausea (8.6%), taste disturbance (7.9%), peripheral edema (7.2%), diarrhea (7.2%), dizziness (6.5%), hypertension (6.5%), infusion site pain or burning (5.8%), dyspnea (5.8%), and vomiting (5.0%)[28]

In rare cases, serious hypersensitivity reactions, including anaphylactic-type reactions, some of which have been life-threatening and fatal, have been reported in patients receiving Venofer® (iron sucrose injection, USP). Patients may present with shock, clinically significant hypotension, loss of consciousness, and/or collapse[28].

Dexferrum®, an iron dextran infusion preparation, is FDA indicated for the treatment of children and adults with documented iron deficiency in whom oral administration is unsatisfactory or impossible. However, due to concerns for anaphylaxis reactions, iron dextran preparations have carried a FDA Black Box warning since 2009. Due to the improved safety profile of iron sucrose compared to iron dextran, iron sucrose has been selected as the iron infusion preparation for this study intervention. Iron sucrose does not carry a Black Box warning and does not require the pre-administration of medication test dose.

The dose selected for the study intervention is 5 mg/kg of Venofer® (iron sucrose) with a maximum dose of 200 mg. The dose will be diluted in normal saline 0.9% to a final concentration of 1 mg of elemental iron to 1 mL of normal saline 0.9%. In the study from Crary et al., doses up to 7 mg iron/kg up to a maximum dose of 300 mg were found to be safe and effective [20]. Five mild reactions were reported and included one patient with headache, two patients with abdominal pain, one patient with transient mild low blood pressure and one patient with fainting. One 15 year old female had a serious allergic reaction that responded to treatment. This patient was treated with 500 mg of iron sucrose, which was above the recommended maximum dose of 300 mg. In a similar study performed by Pinsk et al., doses of 5 mg iron/kg were used in pediatric patients and also found to be well tolerated with minimal adverse reactions [21].

In a single-dose pharmokinetics study of Venofer, patients with NDD-CDK ages 12 to 16 (N=11) received intravenous bolus doses of Venofer at 7 mg/kg (maximum 200 mg) administered over 5 minutes. Following single dose Venofer, the half-life of total serum iron was 8 hours[28].
.
In this pilot study, subjects will receive only one dose of iron sucrose.

Although adolescents are increasingly responsible for their health care decisions, there is an extensive literature highlighting poor prescription medication adherence in this population [18]. Oral iron supplements, in particular, are commonly discontinued due to patient complaints of gastric irritation and lack of palatability [19]. Nausea is also a common complaint of patients diagnosed with POTS, further complicating oral iron supplementation compliance. Given these limitations, intravenous iron supplementation has been used clinically for correction of iron deficiency in select adolescent patients.

2 Study Objectives

1. To assess whether a single infusion of iron sucrose will improve orthostatic symptoms as measured on a validated survey instrument in adolescent subjects with POTS and non-anemic iron deficiency
2. To assess whether a single infusion of iron sucrose will improve cardiovascular indices, specifically a reduction in heart rate change during a ten minute head up tilt, in adolescent subjects with POTS and non-anemic iron deficiency.
3. To determine if abnormalities in standing and supine catecholamine levels will improve following intravenous iron sucrose infusion in adolescent subjects with POTS and non-anemic iron deficiency

3 Study Design

3.1 General Design

This study is a randomized, double blind, placebo-controlled study to assess the efficacy of a single iron sucrose infusion in adolescents with non-anemic iron deficiency and POTS. Subjects will be screened at outpatient clinic visit appointments. Interested qualified subjects will be consented and offered participation in this trial. Once consent and assent (for patients less than 18 years of age) has been obtained, subjects will be randomized to either treatment or placebo group. Subjects will participate in two on site study visits. During the first visit subjects will complete baseline laboratory studies, tilt table test and study questionnaires. Subjects will then receive the intervention study drug or placebo. The second study visit will occur 7 days $\pm$ 2 days from the first study visit. During the second study visit subjects will repeat laboratory studies, tilt table test and study questionnaires. Follow up questionnaires will be sent to all subjects six months following the initial study visit.

3.2 Primary Study Endpoints

The primary endpoint for this study is to determine the effects of iron sucrose on 1) self-reported symptoms of autonomic dysfunction assessed by a validated questionnaire and 2) cardiovascular indices measured by tilt table testing.

3.3 Primary Safety Endpoints

Subjects will be continuously monitored during iron sucrose infusion and for no less than thirty minutes following the conclusion of the infusion. During this time, subjects will have continuous cardiopulmonary monitoring of heart rate and oxygen saturation, with blood pressure measurement no less than every fifteen minutes. Subjects will also be clinically assessed by medical staff from the clinical research unit for side effect symptoms. Signs and symptoms of side effects will be recorded in the subject's study record. The primary investigator will review all side effects and correlation to dose amount and infusion rate will be carefully monitored. All serious side effects will be reported promptly to the IRB. Evidence that serious side effects occur at a rate beyond that reported in manufacture labeling will be reported promptly to the IRB and will be potential cause for study termination.

All subjects will have ferritin, iron, and total iron binding capacity measured prior to iron infusion as well as one week following infusion. Evidence of iron overload at the screening visit will preclude subject participation in the study. Evidence of iron overload following the infusion will result in medical management recommendations from the primary investigator. Data will be monitored for evidence of dose-dependent iron overload. If a subject is found to develop clinically significant iron overload, the primary investigator will promptly report to the IRB and this will be potential cause for study termination.

4 Subject Selection Enrollment and Withdrawal

4.1 Inclusion Criteria

- Adolescent patients (age 12 to 21 years) with chronic (>3 months) symptoms of orthostatic intolerance, including but not limited to lightheadedness, syncope, headache, fatigue, weakness, sweating, nausea and heart palpitations.

- Symptomatic orthostatic heart rate increment ≥40 bpm during a 10 minute 70 degree head up tilt study
- Presence of non-anemic iron deficiency, defined as serum ferritin levels ≤20 ug/L with hemoglobin no less than 1 gm/dL below the normal reference range as defined for age and gender
- Consent obtained from responsible guardian AND from subjects, 12-17 years of age
- Consent obtained for subjects 18 years of age and older

4.2 Exclusion Criteria

- Orthostatic hypotension (decrease of systolic BP>30 mmHg and/or diastolic BP>15mmHg within 3 minutes of 70 degree head up tilt study)
- Pregnant or lactating females
- The presence of failure of other organ systems or systemic illness that can affect autonomic function
- Concomitant therapy with anticholinergic, alpha-adrenergic antagonists, beta-adrenergic antagonists or other medications which could interfere with autonomic testing. Patients may participate if the potentially interfering medication is held for five half-lives prior to the study
- Laboratory evidence of anemia or iron overload
- Personal history of hematochromatosis or first degree relative with hematochromatosis
- Known sensitivity to Venofer® (iron sucrose injection, USP) or other intravenous iron preparation

4.3 Subject Recruitment, Enrollment and Screening

Subjects will be recruited for this study from patients seen in the Department of Pediatric and Adolescent Medicine (DPAM) on the Mayo Clinic Rochester campus. The study will be advertised to medical providers within DPAM, particularly those practicing in the divisions of General Pediatrics and Adolescent Medicine (GPAM), Pediatric Neurology, Pediatric Cardiology, Pediatric Gastroenterology and Community Pediatrics and Adolescent Medicine (CPAM). Study information will be shared with these providers through verbal communication, printed materials and e-mail. The information will include inclusion and exclusion criteria, contact information for the Primary Investigator and study coordinator and brief study overview.

Patients will be screened by a study investigator or the study coordinator for review of study entry requirements.

Study enrollment and screening:

1. Will take place between 21 days and the day of first study visit
2. Will take place either at patient point of care or the Clinical Research Unit
3. Will review and document the following:
    a. Consent and assent forms based on age of subject

b. Subject demographics
c. Physical exam by Mayo Clinic medical provider within 12 weeks of study enrollment
d. Lab data (CBC, ferritin, total serum iron, percent iron saturation) within 12 weeks of study enrollment that meets inclusion and exclusion parameters
e. Current medication review and compliance with exclusion parameters
f. Randomization procedure to treatment or placebo group

Patients meeting the criteria will be offered participation in the study. A screening log will be kept to document reasons for ineligibility and for non-participation of eligible candidates. Those choosing to enroll will meet with a research study team member to complete the consent process. A research record will be generated for each study subjects. Documentation that the subject satisfied inclusion and exclusion criteria will be made.

For subjects less than 18 years of age, the parent or legal guardian will be required to participate in the consent process along with the subject. The consent form will be reviewed by the study team member. An opportunity will be given for questions. Subjects will be asked to reflect the purpose and interventions of the study back to the research study team member. A waiting period for contemplation will be offered to the potential subjects. Subjects aged 12-17 years old will sign giving assent AND the parent or legal guardian will sign the consent form. Subjects 18 years of age will sign the consent form. All signatures will be witnessed and co-signed by a study team member. The original form will be stored in the subject's research record.

Subjects will be provided with a copy of the consent form and will be provided a copy of subject responsibility during the study.

4.4 Early Withdrawal of Subjects

4.4.1 When and How to Withdraw Subjects

Subjects may stop their participation in the study at any time. Withdrawal of consent or assent will terminate the subject's participation in the study. The Principal Investigator will follow up with subjects to determine if ongoing medical management that may be indicated for patient safety as a result of study discontinuation. If the subject was in the treatment arm of the study, this may include follow up laboratory studies to assess laboratory response to therapy. Medical recommendations will be made by the Principal Investigator based on these results. The Principal Investigator will document reasons for subject dropout in the research record.

If a subject is withdrawn from the study, an additional subject may be enrolled to maintain the statistical power of the study.

In addition, the Principal Investigator or Mayo Clinic may stop the study at any time if there are severe adverse events or if adverse events are reported at a frequency higher than expected.

Subjects may be withdrawn from the study prior to completing all of the study if there is failure to adhere to the subject responsibility parameters.

Subject responsibility during study:

1. Subjects will be asked to stop taking medications that can potentially interact with tilt table test results at least 5 half-lives prior to study entry. This includes, but is not limited to anticholinergic, alpha-adrenergic antagonists and beta-adrenergic antagonists
2. Subjects will be asked to avoid caffeine and nicotine for at least 24 hours before each study site visit
3. Upon enrollment, subjects will be asked to discontinue iron supplements and/or vitamins containing iron until after the second study visit is complete
4. Subjects of child-bearing potential must have a negative urine beta-HCG (pregnancy test) prior to study entry
5. Subjects who are sexually active and able to become pregnant must agree to use one of the birth control methods listed below until after the last study site visit:
    a. Hormonal methods, such as birth control pills, patches, injections, vaginal ring or implants
    b. Barrier methods (such as a condom or diaphragm) used with a spermicide (a foam, cream, or gel that kills sperm)
    c. Intrauterine device (IUD)
    d. Abstinence (no sex)

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

Even though a subject has withdrawn from the study, it may be important to collect some follow-up data on such subjects throughout the protocol defined follow-up period. Such data is important to the integrity of the final study analysis since early withdrawal could be related to the safety profile of the study drug. If a subject withdraws consent to participate in the study, for subject safety reasons, attempts will be made to obtain permission to collect follow up information whenever possible.

5 Study Drug

5.1 Description

Iron sucrose solution supplied as Venofer® in a liquid form available in vials of 100 mg/5 mL and 200 mg/10 mL.

5.2 Treatment Regimen

One treatment dose will be administered during this study and will take place during the First Study Visit. The treatment dose will be 5 mg/kg of intravenous iron sucrose (maximum dose of 200 mg). Iron sucrose will be diluted to a concentration of 1 mg of elemental iron in 1 mL of NaCl 0.9% (maximum volume of 210 mL). The infusion will be infused at 3.5 mL/minute. A flush of 10 mL of NaCl 0.9% will be given after the infusion.

5.3 Method for Assigning Subjects to Treatment Groups

Subjects entering the study will be randomized through the clinical trials research pharmacy using a 1:1 randomization process. The Primary Investigator will be blinded to the randomization process. The randomization key will be readily available in the event of a medical emergency associated with the study intervention.

5.4 Preparation and Administration of Study Drug

The Clinical Research Unit's Research Pharmacy will obtain Venofer® supplied in vials of 100 mg/5 mL, from standard hospital supply at Mayo Clinic Hospital Rochester, MN. The preparation of the study drug will take place in the Clinical Research Unit Research Pharmacy and will include the dilution of the drug to the recommended concentration of 1 mg/mL. The drug will be maintained within recommended temperature ranges of 20°C to 25°C with excursions permitted to 15°C to 30°C[28]. Venofer® does have a brown color in concentrated form. To ensure adequate blinding of study team members, the IV solution bag and tubing will be wrapped by the Research Pharmacy to conceal color.

The study drug will have an accessory label reading "**CAUTION: NEW DRUG LIMITED BY FEDERAL LAW TO INVESTIGATIONAL USE**" as required by Federal Law. It will also have a finished product label from the Research Pharmacy. Below is a sample template :

IRB – Iron Sucrose/Placebo Dose: 1 each
Sodium Chloride 0.9% Dose: 100 mL
Route: IV Frequency: Once
Rate: _____ mL/hour
Start Date/Time: _____
Comment: IRB# 12-009963; Iron Sucrose _____ mg vs placebo

Clinical Research Pharmacy Director:
Christine M. Formea, Pharm.D., R.Ph.
Domitilla Building
Floor 05, Room 248
Saint Mary's Hospital
1216 Second Street SW
Rochester, MN 55905

Research Pharmacist assigned to protocol:
Jill Randolph, Pharm.D., R.Ph.
507-255-5712

5.5 Subject Compliance Monitoring

Subjects will receive only one dose during the study. No additional medication adherence will be required.

5.6 Prior and Concomitant Therapy

Subjects will be required to discontinue therapy with anticholinergic, alpha-adrenergic antagonists, beta-adrenergic antagonists and other medications which could interfere with autonomic testing. Subjects may participate if the potentially interfering medication is held for five half-lives prior to the study. Enrolled subjects may not receive iron supplementation outside of study administration until after the Second Site Study Visit has been completed.

5.7 Packaging

The following is taken directly from the manufacture drug information:
Venofer® is supplied sterile in 10 mL, 5 mL, and 2.5 mL single-use vials. Each 10 mL vial contains 200 mg elemental iron, each 5 mL vial contains 100 mg elemental iron, and each 2.5 mL vial contains 50 mg elemental iron (20 mg/mL):
NDC-0517-2310-05 200 mg/10 mL Single-Use Vial Packages of 5
NDC-0517-2310-10 200 mg/10 mL Single-Use Vial Packages of 10
NDC-0517-2340-01 100 mg/5 mL Single-Use Vial Individually Boxed
NDC-0517-2340-10 100 mg/5 mL Single-Use Vial Packages of 10
NDC-0517-2340-25 100 mg/5 mL Single-Use Vial Packages of 25
NDC-0517-2325-10 50 mg/2.5 mL Single-Use Vial Packages of 10
NDC-0517-2325-25 50 mg/2.5 mL Single-Use Vial Packages of 25

5.8 Blinding of Study

This study will be double-blinded such that the subject and Primary Investigator will be blinded to the randomization of the subject.

5.9 Receiving, Storage, Dispensing and Return

5.9.1 Receipt of Drug Supplies

Venofer® is available in the United States market and is maintained as routine medication stock supply in the Mayo Clinic Hospital Pharmacy. The Clinic Research Pharmacy will obtain the drug used in this study from the stock supply in the Mayo Clinic Hospital Pharmacy.

5.9.2 Storage

All manufacture recommendations as listed in the drug monograph[28] will be followed:

1. Store in original carton at 20°C to 25°C (68° F to 77° F); excursions permitted to 15° to 30°C (59° to 86°F). [See USP Controlled Room Temperature].
2. Do not freeze.

3. *IV Admixture Stability:* Venofer®, when added to IV infusion bags (PVC or non-PVC) containing 0.9% NaCl at concentrations ranging from 1 mg to 2 mg of elemental iron per mL, has been found to be physically and chemically stable for 7 days at controlled room temperature (25°C ± 2°C).
4. Do not dilute to concentrations below 1 mg/mL.
5. Do not mix Venofer® with other medications or add to parenteral nutrition solutions for intravenous infusion.
6. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to infusion.

5.9.3 Dispensing of Study Drug

Regular study drug reconciliation will be performed to document drug assigned, drug dispensed, drug returns, and drug remaining. This reconciliation will be logged on the drug reconciliation form, and signed and dated by the study team.

5.9.4 Return or Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of drug dispensed, drug returns, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be documented and investigated, prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files.

6 Study Procedures

Assessment	Screening/Enrollment Visit (day -21 to day 0)	Study Visit 1 (day 0)	Study Visit 2 (day 7 ±2 days)	Follow up by mail (6 months from day 0)
Informed consent/assent form	X			
Demographics	X			
Vital signs	X	X	X	
General physical exam	X			
Current medications	X	X	X	X
Inclusion/Exclusion Criteria	X	X	X	
Randomization	X			
Urine Beta-HCG for female subjects		X		
Questionnaires (COMPASS-31 and PedsQL)		X	X	X
IV/Venipuncture (CBC, ferritin, iron, TIBC, percent of iron saturation, upright and supine		X	X	

catecholamine)				
Tilt table test		X	X	
Orthostatic Symptom Scale (during tilt table)		X	X	
Intervention (study drug or placebo)		X		

6.1 Visit 1

1. Study visit ONE will be conducted in the Clinical Research Unit. All Study ONE visits will be scheduled such that the tilt table tests will be started during the window of 12 pm-4 pm. Study ONE visit will include:
    a. Review of inclusion and exclusion criteria
    b. Vital sign screen
    c. For all female subjects:
        i. Documentation of last menstrual period date
        ii. Review of current birth control method and adherence
        iii. Urine Beta-HCG obtained and documented as negative
    d. Questionnaire completion
        i. COMPASS-31 Instrument to assess autonomic symptoms [29]
        ii. Pediatric Quality of Life Inventory (PedsQL) Version 4.0 [30]
    e. IV/Venipuncture
        i. Complete blood count
        ii. Serum ferritin
        iii. Total serum iron, total iron binding capacity and percent iron saturation
        iv. Supine and standing catecholamine plasma levels
        v. Estimated blood volume to be drawn is less than 15 mL
    f. Ten minute, 70 degree head-up tilt table test which includes measurement of cardiovagal responses via heart rate variation to deep breathing (HRVDB), cardiac and vasomotor adrenergic responses via beat-to-beat heart rate, and blood pressure response with tilt and Valsalva maneuver.
    g. Orthostatic symptom scale assessing orthostatic symptoms during tilt on a visual analog severity scale from 0-10
    h. Administration of intervention (study drug OR placebo) followed by 10 mL NaCl 0.9% flush. Patients will have continuous cardiopulmonary monitoring (heart rate, respiratory rate and pulse ox) with blood pressure documented no less than every minutes during the infusion
    i. Following the intervention, subjects will be monitored for signs and symptoms of intervention hypersensitivity for an additional thirty minutes. Patients will have continuous cardiopulmonary monitoring (heart rate, respiratory rate and pulse ox) with blood pressure documented no less than every fifteen minutes.
2. Time estimates for Study Visit One (4-4.5 hours)
    a. Review of patient information (30 minutes)
    b. Questionnaire completion (1 hour)
    c. IV/Venipuncture (20 minutes)

d. Tilt table test (1 hour)
e. Intervention (1-1.5 hours)
f. Subject observation (30 minutes)

6.2 Visit 2

3. Study visit TWO will take place 7 days ±2 days following study visit ONE. Study visit TWO will be completed in the Clinical Research Center. The visit will be scheduled such that the tilt table test will be started during the window of 12 pm-4pm. Study visit TWO will include:
    a. Vital sign screen
    b. Questionnaire completion
        i. COMPASS-31 Instrument to assess autonomic symptoms
        ii. Pediatric Quality of Life Inventory (PedsQL) Version 4.0
    c. IV/Venipuncture
        i. Complete blood count
        ii. Serum ferritin
        iii. Total serum iron, total iron binding capacity and percent iron saturation
        iv. Supine and standing catecholamine plasma levels
        v. Estimated blood volume to be drawn is less than 15 mL
    d. Ten minute, 70 degree head-up tilt table test which includes measurement of cardiovagal responses via heart rate variation to deep breathing (HRVDB), cardiac and vasomotor adrenergic responses via beat-to-beat heart rate, and blood pressure response with tilt and Valsalva maneuver
    e. Orthostatic symptom scale assessing orthostatic symptoms during tilt on a visual analog severity scale from 0-10
4. Time estimates for Study visit TWO (3-3.5 hours)
    a. Review of patient information (30 minutes)
    b. Questionnaire completion (1 hour)
    c. IV/Venipuncture (20 minutes)
    d. Tilt table test (1 hour)

6.3 Mail follow-up

5. Follow up by MAIL will take place 6 months after study visit ONE and will include:
    a. Questionnaire completion
        i. COMPASS-31 Instrument to assess autonomic symptoms
        ii. Pediatric Quality of Life Inventory (PedsQL) Version 4.0
    b. Self-reported current medication list
    c. Self-reported use of iron treatments (oral or intravenous) from study completion until 6 months post-study completion
    d. Subjects will be provided a self-addressed, pre-paid envelope to return the materials to the primary investigator
6. Time estimate for Mail Follow-Up
    a. Questionnaire completion (1 hour)

7 Statistical Plan

7.1 Sample Size Determination

The two primary outcome variables in this study will be change in score on the COMPASS-31 survey and change in postural heart rate increase. For an anticipated change in the COMPASS-31 survey of 10 points, a sample size of 15 subjects per group (30 total) setting alpha at 0.05 and beta at 0.80 would be an adequate sample size to reject or accept the null hypothesis (power estimate >80%). For an anticipated change in postural tachycardia from 50 beats per minute to 30 beats per minute, a sample size of 15 subjects per group (30 total) setting alpha at 0.05 and beta at 0.80 would be adequate an adequate sample size to reject or accept the null hypothesis (power estimate >80%).

7.2 Statistical Methods

Descriptive Statistics

Univariate descriptive statistics and frequency distributions will be calculated, as appropriate for all variables. Baseline values for demographic, clinical, and outcome variables (primary and secondary) will be tabulated for the treatment groups. These analyses will help identify potential confounding variables to be used as covariates in sensitivity analyses. Distributions across subgroups used in randomization will be compared to assess whether the randomization was successful in equalizing distributions of these prognostic variables across treatment groups. Putative prognostic variables that will be investigated through these descriptive analyses include subjective symptoms of autonomic dysfunction and cardiovascular indices.

Handling of Missing Data

Continuous data monitoring will be done by both study coordinator and the Primary Investigator using computer based systems. Scheduled review of study documentation by study coordinator and Primary Investigator will take place at a frequency of no less than every four to six weeks to assure that all reports are on file, accurate and complete. Prior to data analysis, trends and outliers will be reviewed to consider for potential missing data.

Primary Hypothesis: Treatment of iron deficiency with intravenous iron sucrose in an adolescent with Postural Orthostatic Tachycardia Syndrome will reduce their score on the COMPASS-31 survey by 10 points.

Data will be visually inspected for normality. Group comparisons will include Fisher's exact test for categorical variables and Wilcoxon rank sums for continuous variables. Confidence intervals will be calculated at 95%. All P-values will be 2-sided with values less than 0.05 considered significant. All analyses will be performed on an "intention to treat" basis

Secondary Hypothesis 1: Treatment of iron deficiency with intravenous iron sucrose in an adolescent with Postural Orthostatic Tachycardia Syndrome will reduce heart rate change during tilt table testing from 50 beats per minute to less than 30 beats per minute.

Data will be visually inspected for normality. Group comparisons will include Fisher's exact test for categorical variables and Wilcoxon rank sums for continuous variables. Confidence intervals will be calculated at 95%. All P-values will be 2-sided with values less than 0.05 considered significant. All analyses will be performed on an "intention to treat" basis

7.3 Subject Population(s) for Analysis

Protocol-compliant population: Any subject who was randomized and received the protocol required study drug exposure and required protocol processing

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 birth defect/anomaly

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

Risk associated with use of Venofer®
Information taken directly from the manufacturer[28]:
In efficacy studies in non-dialysis dependent-chronic kidney disease patients (N=139), the most frequent adverse events (≥5%) whether or not related to Venofer® administration, were nausea (8.6%), taste disturbance (7.9%), peripheral edema (7.2%), diarrhea (7.2%), dizziness (6.5%), hypertension (6.5%), infusion site pain or burning (5.8%), dyspnea (5.8%), and vomiting (5.0%)

In a randomized, open-label, dose-ranging trial for iron maintenance treatment with Venofer in pediatric patients with CKD on stable erythropoietin therapy at least one treatment-emergent adverse reaction was experienced by 57% (27/47) of the patients receiving Venofer 0.5 mg/kg, 53% (25/47) of the patients receiving Venofer 1.0 mg/kg, and 55% (26/47) of the patients receiving Venofer 2.0 mg/kg. A total of 5 (11%) subjects in the Venofer 0.5 mg/kg group, 10 (21%) patients in the Venofer 1.0 mg/kg group, and 10 (21%) patients in the Venofer 2.0 mg/kg group experienced at least 1 serious adverse reaction during the study. The most common treatment-emergent adverse reactions (> 2% of patients) in all patients were headache (6%), respiratory tract viral infection (4%), peritonitis (4%), vomiting (4%), pyrexia (4%), dizziness (4%), cough (4%), renal transplant (4%), nausea (3%), arteriovenous fistula thrombosis (2%), hypotension (2%), and hypertension (2.1%).

In rare cases, serious hypersensitivity reactions, including anaphylactic-type reactions, some of which have been life-threatening and fatal, have been reported in patients receiving Venofer® (iron sucrose injection, USP). Patients may present with shock, clinically significant hypotension, loss of consciousness, and/or collapse[28].

- **Observations to be considered as adverse reactions will include but may not be limited to the following:**
  Nausea, taste disturbance, peripheral edema, diarrhea, vomiting, dizziness, hypertension, hypotension, infusion site pain or burning, dyspnea, cough, headache, myalgia, arthralgia or pyrexia[28].

- **Observations to be considered SERIOUS adverse reactions will include but may not be limited to the following:**
  Anaphylactic-type reaction, shock, clinically significant hypotension or hypertension or other symptoms leading to hospitalization, surgery, disability, incapacity, birth defect/anomaly or death[28]

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

Other potential risks associated with study participation, but not related to Venofer® use include the following:

1. Subjects may have worsening of orthostatic symptoms upon discontinuation of medications as indicated in exclusion criteria. These may include but are not limited to lightheadedness, fainting, headache and nausea

2. IV and venipuncture may cause pain, bruising, lightheadedness, and/or fainting, or rarely, infection or infiltration of fluids at the site of the needle stick

3. Valsalva maneuver can increase pressure in the chest by 40 mm Hg. There is a potential risk for detached retina and retinal hemorrhage

4. Ten minute, 70 degree, head up tilt table test may lead to exacerbation of underlying orthostatic intolerance symptoms, including but not limited to lightheadedness, fainting, headache and nausea

5. Confidentiality risk includes the possibility for inappropriate breach of confidentiality

Adverse Event Reporting Period

For this study, the study treatment follow-up period is defined as six months 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 will 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 will also be recorded and documented as an adverse event.

Post-study Adverse Event
All unresolved adverse events will be followed by the 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 investigator will instruct each subject to report, to the investigator, any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study.

Abnormal Laboratory Values
A clinical laboratory abnormality will be documented as an adverse event if iron overload is detected in the post-treatment study visit (Study Visit 2). Subjects found to have iron overload following treatment with the study drug will have medical recommendations made by the

Primary Investigator. These recommendations may include follow up laboratory and clinical monitoring. With the subject's approval, the Primary Investigator will communicate with the subject's primary care provider for coordination of medical management.

Additional medical recommendations may also be made by the Principal Investigator for the subject if post-treatment laboratory data suggests that the subject remains iron deficient (serum ferritin ≤12ug/L) or iron insufficient (serum ferritin ≤25ug/L).

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization will be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery will 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 diagnostic or elective surgical procedures for a preexisting condition. Surgery should **not** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- 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 will be recorded immediately in the source document, and also in the appropriate adverse event section of the subject's research record. All clearly related signs, symptoms, and abnormal diagnostic, laboratory or procedure results will be recorded in this source document.

All adverse events occurring during the study period will be recorded. The clinical course of each event will 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 will be followed up, to determine the final outcome. Any serious adverse event that occurs after the study period and will be considered to be at least possibly related to the study treatment or study participation will 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 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.

Information will be collected on an adverse event worksheet (*and entered in the research database)*:

- Subject's name
- Medical record number
- Disease diagnoses
- The date the adverse event occurred
- Description of the adverse event
- Relationship of the adverse event to the research (drug, procedure, or intervention)
- If the adverse event was expected
- The severity of the adverse event (severity table with scale 1-5)
- If any intervention was necessary
- Resolution (was the incident resolved spontaneously, or after discontinuing treatment)
- Date of Resolution

The investigator will review all adverse event reports to determine if specific reports need to be made to the IRB. The 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.

8.4 Un-blinding Procedures

Un-blinding will occur in the event that a subject's safety is at risk. This will be reported with any required study adverse event reporting to the IRB.

Un-blinding for other, unanticipated reasons will also be reported as indicated to the IRB.

8.5 Stopping Rules

Evidence that serious side effects occur at a rate beyond that reported in manufacture labeling will be reported promptly to the IRB and will be potential cause for study hold or termination. If study is placed on hold due to serious side effects, approval by the IRB will be required before study continuation.

8.6 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. Medical monitoring will include a regular assessment of the number and type of serious adverse events.

8.6.1 Internal Data and Safety Monitoring Board

A Data and Safety Monitoring Plan (DSMP) will be filed with the Mayo Clinic IRB.

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 will 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, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, 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

Case report form (CRF) will be used for data collection for the study. All data requested on the CRF will be recorded in a computer-based program. All missing data will be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, "N/D" will be noted. If the item is not applicable to the individual case, "N/A" will be noted. All such changes of data entry will be noted, initialled and dated.

Data Management

Data will be entered into the subject's research record by study team members. The data will be maintained on a computer based system through the Clinical Trials Research Unit.

Data Security and Confidentiality

All data forms and study specific information will be kept on password protected computers and locked file cabinets. Data access will be limited to Principal Investigator and study coordinator.

Data Quality Assurance

The Primary Investigator will be responsible for monitoring study documentation made by study team members. Review will occur at least every four to six weeks to document that all reports are on file, accurate and complete. These documents will be made available to the IRB upon request.

Data Clarification Process

Any missing data or other data queries will be reviewed by the study coordinator and Primary Investigator. An account of the data in need of clarification will be created. Log entries will explain the issue and actions take for resolution. Data and study team members involved in the clarification process will also be documented.

9.4 Records Retention

The investigator will maintain records and essential documents related to the conduct of the study as outlined in the Mayo Clinic Research Policy Manual –"Access to and Retention of Research Data Policy" http://mayocontent.mayo.edu/research-policy/MSS_669717 IRB guidelines. These will include subject case histories and regulatory documents.

Any report, publication or other disclosure of clinical study outcomes will be done without subject identifiers.

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 monitor or other compliance or quality assurance reviewer is given access to all the study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring 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 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 or the subject's legally authorized representative, and the individual obtaining the informed consent. For subjects under the age of 18 an additional assent form will be used. The assent form will be submitted with the protocol for review and approval by the IRB for the study.

12 Study Finances

12.1 Funding Source

Applications for funding will be submitted to the Mayo Clinic Center for Translational Science Activities (CTSA), Mayo Clinic Department of Pediatrics and Adolescent Medicine and Mayo Clinic Institutional Funds.

12.2 Conflict of Interest

Any study team member who has a conflict of interest with this study 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.

12.3 Subject Stipends or Payments

Subjects will receive $100.00 if the study is completed. If the study is not completed the subjects will receive $25.00 for each of the two on-site study visits.

13 Publication Plan

The Primary Investigator holds the primary responsibility for publication of the study results.

14 References

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