Clinical Study Protocol

Safety and Efficacy of Panhematin™ for Prevention of Acute Attacks of Porphyria

Trial phase: II

Table of Contents

1. Introduction

The trial will be conducted in compliance with the protocol, Good Clinical Practice (GCP)[1] and applicable regulatory requirements. The single site for this study is the University of Texas Medical Branch (UTMB) in Galveston, Texas. Conducting the study at additional sites is not planned at this time.

1.1 Description of acute porphyrias and current treatment

The acute porphyrias are four types of porphyria that can present with attacks of identical neurological symptoms. Each is due to a deficiency of a different enzyme in the heme biosynthetic pathway [1]. Patients with the three most common of these disorders, namely acute intermittent porphyria (AIP), hereditary coproporphyria (HCP) and variegate porphyria (VP) will be eligible for inclusion in this study. AIP, HCP and VP are autosomal dominant genetic diseases that are classified as hepatic porphyrias and cause symptoms in adults and most commonly in women. The fourth acute porphyria, ALA-dehydratase porphyria (ADP), is extremely rare (only six well-documented cases described) [2,3]. In contrast to the other acute porphyrias, ADP is an autosomal recessive disorder, is perhaps more commonly symptomatic in males, and excess erythrocyte zinc protoporphyrin suggests a significant erythropoietic component. Given these possibly significant dissimilarities, patients with ADP will not be included in this protocol. Moreover, ADP is the rarest of the porphyrias, with only one known case in the U.S. [3], and it is unlikely that any patients would be available for inclusion. Therefore, in this and other study documents "acute porphyria" will refer to AIP, HCP and VP.

Molecular basis

AIP is the most common of the acute porphyrias in most countries, with an estimated prevalence of 5-10 gene carriers per 10,000 in western countries [1,4,5]. AIP results from a deficiency of the third enzyme of the heme biosynthetic pathway, porphobilinogen deaminase [PBGD – also known as hydroxymethylbilane synthase (HMBS)]. Both affected individuals and asymptomatic carriers, who are said to have latent AIP, are heterozygous for mutations of the PBGD gene. The disease is heterogeneous at the molecular level, with more than 400 mutations described in different families. Most known mutations cause the enzyme to be ~50% of normal in all tissues from birth, as

[1] Abbreviations used (in alphabetical order): AE, adverse event; ALA, 5-aminolevulinic acid; ADP, ALA-dehydratase porphyria; ALAS, ALA synthase; ALAS1, ubiquitous or housekeeping form of ALAS; ALAS2, erythroid form of ALAS; APF, American Porphyria Foundation; BMI, body mass index; CPOX, coproporphyrinogen oxidase; CRF, case report form; CYPs, cytochrome P450 enzymes; DMCC, Data Management and Coordinating Center; FDA, Food and Drug Administration; GCP, Good Clinical Practice; HIPAA, Health Insurance Portability and Accountability Act; HMBS, hydroxymethylbilane synthase; HPLC, high-performance liquid chromatography; ICTSA, Institutional Clinical and Translational Science Award; IRB, Institutional Review Board; NRS, numeric rating score, PBG, porphobilinogen; PBGD, porphobilinogen deaminase; PPOX, protoporphyrinogen oxidase; RDCRN, Rare Disease Clinical Research Network; SAE, significant adverse event; SIADH, syndrome of inappropriate antidiuretic hormone secretion; SPID, sum of pain intensity differences; UTMB, University of Texas Medical Branch.

most conveniently demonstrated in erythrocytes. However, mutations affecting exon 1 may reduce enzyme activity only in nonerythroid tissues, and in these families erythrocyte PBGD activity is normal [4].

HCP and VP are due to deficiencies of coproporphyrinogen oxidase (CPOX) and protoporphyrinogen oxidase (PPOX), the sixth and seventh enzymes in the heme biosynthetic pathway, respectively. Like AIP, HCP and VP are genetically heterogeneous. Fewer mutations have been identified in HCP and VP, perhaps reflected their lower prevalence in most countries [1,6-8]. VP is especially common in South Africans of Dutch ancestry, due to a founder effect, and the great majority of VP patients in that country share the same *PPOX* mutation [6].

Clinical presentation

AIP can be considered the prototypic acute porphyria. The majority of individuals who inherit PBGD mutations remain clinically unaffected throughout their lives, and most do not have elevations in porphobilinogen (PBG) and porphyrins. Clinical expression of AIP is more common in women, and is determined by additional factors, including certain drugs, nutritional alterations, endogenous or exogenous hormones, infections and other stressful illnesses, and probably unidentified modifying genes [1].

The most common presentation is an acute attack of neurological symptoms, including abdominal pain, vomiting, constipation, pain in the back, chest and extremities, muscle weakness and sensory loss. Peripheral neuropathy may progress to quadriplegia and respiratory paralysis, especially if diagnosis and treatment are delayed. Central nervous system manifestations may include mental symptoms, convulsions and hyponatremia from the syndrome of inappropriate antidiuretic hormone secretion (SIADH). Some patients develop frequently recurring attacks or chronic symptoms [1,9].

Blistering skin lesions on sun exposed areas of skin, which are identical to those found in porphyria cutanea tarda, are common in VP, much less common in HCP, and never occur in AIP (except rarely when there is concomitant end stage renal disease) [1,6,10].

Many patients do well after one or a few attacks. However, some develop frequently recurring attacks and more lasting symptoms, including depression and pain [11]. Acute porphyria patients, and especially those with high excretion of urinary ALA and PBG are at increased risk for developing hepatocellular carcinoma and renal disease, especially after 40-50 years of age [12,13].

Pathogenesis

These disorders are classified as hepatic porphyrias because the accumulation of pathway intermediates proximal to the deficient enzyme occurs initially in the liver, followed by excretion in urine or feces. Excretion of products derived from intermediates distal to the deficient enzyme is also increased, which suggests that excess intermediates can be metabolized further, perhaps in nonhepatic tissues. AIP, HCP and VP are readily differentiated by distinctive patterns of excess

porphyrin precursors and porphyrins in urine, plasma and feces. A diagnosis should be confirmed by DNA studies, which is now standard of care. The identified mutation can then be sought in relatives to detect those at risk for the disease [9].

In AIP, the accumulation of heme pathway intermediates, namely 5-aminolevulinic acid (ALA, also known as δ-aminolevulinic acid), PBG and porphyrins, results from the specific inherited enzyme deficiency as well as induction of hepatic ALA synthase, the first enzyme in the pathway [1]. PBGD is not genetically deficient in HCP and VP, but its normal activity may become rate-limiting when heme synthesis is stimulated. Moreover, coproporphyrinogen and protoporphyrinogen that accumulate in HCP and VP may inhibit hepatic PBGD [14]. Therefore, ALA and PBG are increased during attacks of HCP and VP, but the increases may be less than in AIP, and return to normal more quickly.

Heme synthesis in the liver is controlled by the ubiquitous form of ALA synthase, termed ALAS1, which is the initial and rate-controlling enzyme of the pathway in the liver. ALAS1 is inducible and subject to sensitive feedback repression by the end-product heme. A "free" pool of heme in hepatocytes down-regulates the synthesis of ALAS1. (The erythroid form of ALAS, termed ALAS2, is produced only in erythroid cells, and is regulated quite differently by heme.) Factors known to precipitate porphyric attacks include certain drugs and steroid hormones, alcohol, caloric or carbohydrate restriction, metabolic stress and infections. Many of these factors are inducers of hepatic ALAS1 [9].

The inherited partial deficiencies of PBGD, CPOX or PPOX in these acute porphyrias limit hepatic heme synthesis sufficiently to make ALAS1 more inducible. For this reason, gene carriers are susceptible to exacerbating factors that induce ALAS1 and heme synthesis in the liver. Because most heme made in the liver is used for synthesis of cytochrome P450 enzymes (CYPs), drugs, hormones and other substances that induce both CYPs and ALAS1 in the liver are potentially dangerous in these disorders [15]. Hepatic induction of ALAS1 and CYPs is controlled by similar nuclear receptor-mediated mechanisms [1,16,17].

The pathogenesis of the neurological symptoms and signs of the acute porphyrias is poorly understood [1,4,18]. A neurotoxic effect of ALA or one or more other intermediates or by-products of the pathway seems most likely. A role for PBG seems unlikely, especially after a recent study in which PBG was very effectively reduced by infusion of recombinant human PBGD demonstrated no clinical benefit (unpublished) [10]. Reports that liver transplantation cures AIP supports the view that the liver produces a neurotoxic effect in this disease [19-21]. Heme deficiency in the nervous system is also a possible cause of neurological damage, but is less supported in terms of evidence. Chronic blistering skin lesions in HCP and VP, as in other cutaneous porphyrias, are due to accumulation of porphyrins, which are known to be photosensitizing.

Biochemical findings

During exacerbations of AIP, urinary excretion of PBG is typically in the range of 20~200 mg/day (normal range, 0~4 mg/day), and ALA excretion is approximately half that of PBG (normal range, 0~7 mg/day) [9]. Urinary porphyrins are also markedly elevated, usually with a predominance of uroporphyrin (derived in part from nonenzymatic polymerization of PBG and also from enzymatic formation of uroporphyrinogen III from accumulated PBG), which accounts for reddish urine. Excess PBG can also form porphobilin, a brownish degradation product.

Urinary ALA and PBG are often less elevated in HCP and VP than in AIP, and may decrease more rapidly to normal as the attack resolves. Porphyrin measurements in urine, plasma and feces are needed for diagnosis of HCP and VP, and especially to differentiate these disorders from AIP [6,9,22,23]. Urinary porphyrin levels generally remain substantially elevated in HCP and VP, even after ALA and PBG become normal, and are usually predominantly coproporphyrin III.

Plasma porphyrins are substantially increased in symptomatic VP, and in many cases of latent VP, with a distinctive fluorescence emission spectrum at neutral pH (maximum at ~626 nm) [24,25]. Plasma porphyrins are usually normal or slightly elevated in AIP and HCP, but are expected to be substantially elevated in the small number of HCP patients with cutaneous manifestations. Fecal porphyrins are substantially increased in HCP and VP, and are predominantly coproporphyrin III in HCP, and approximately equal amounts of coproporphyrin III and protoporphyrin IX in VP [6,9,22,23].

Diagnosis

A rapid, accurate diagnosis is paramount because delayed treatment of an attack can result in neurologic damage and even death. Acute porphyria should be considered in any patient with symptoms that are prominent in these conditions, particularly abdominal pain, when initial clinical evaluation does not support another cause [9]. No single sign or symptom is universal, and 5% to 10% of patients may not have the most common features, such as abdominal pain and tachycardia. The family history may be unrevealing because most carriers of the trait are asymptomatic.

Rapidly excluding acute porphyrias also avoids delay in establishing an alternative correct diagnosis. Misdiagnoses of porphyrias are common, so it cannot be assumed that a reported history of porphyria is accurate. It is important to obtain the original evidence for the diagnosis, and to repeat testing if that evidence is not convincing.

Biochemical diagnostic testing

A substantial increase in urinary PBG establishes the diagnosis of either AIP, HCP or VP. Because increases in PBG are so substantial during acute attacks of AIP, HCP and VP, measurement of PBG even on a spot urine sample is often diagnostic. Measuring creatinine allows evaluation for dilution. Further testing on

the same spot urine sample, and on plasma, feces and erythrocytes (obtained prior to initiating treatment) differentiates AIP, HCP and VP [9].

Initial rapid testing for increased urinary PBG is recommended for initial diagnosis of these acute porphyrias, especially at or near the time of symptoms. This will miss the diagnosis only in patients who have already received hemin (which can rapidly decrease PBG), in the very rare patient with ADP and in some cases of HCP and VP with more transient increases in ALA and PBG. Therefore, ALA and total porphyrins should also be measured, which will enable diagnosis of ADP, in which ALA and coproporphyrin are markedly elevated, and HCP and VP, in which porphyrins commonly remain increased even after ALA and PBG decrease to normal [9].

Most tests for PBG, a colorless pyrrole, rely on formation of a violet pigment with Ehrlich's reagent (*p*-dimethylaminobenzaldehyde). PBG must be separated from other urinary substances, principally urobilinogen, that also react with Ehrlich's aldehyde. The Mauzerall–Granick and closely related anion exchange methods are most reliable and are used for quantitative determination of ALA and PBG [26]. Mass spectrometry methods are also available. A kit for rapid detection of increased PBG levels in urine was recommended [9,27], but unfortunately is no longer available. Laboratories that measure urine PBG should be willing to expedite testing when requested.

Because excretion of these porphyrin precursors is so high when symptoms are present, differences in reference ranges between laboratories are of little consequence, and collection of urine for 24 hours, which delays diagnosis, is unnecessary for diagnosis. Urinary results expressed per gram of creatinine are readily compared with reference ranges for 24-hour excretion. Decreases occur with clinical improvement and are dramatic (but usually not long-lasting) after hemin therapy. After recovery from an attack of AIP, levels of ALA and PBG generally remain increased, except soon after hemin therapy or with prolonged latency. But in HCP and VP, ALA and PBG levels may be less markedly increased and may decrease more rapidly. Ideally, major medical facilities should provide for in-house determination of urinary PBG levels within hours of obtaining the sample, because life-threatening progression of the disease may occur with a delay of several days in testing. The single-void urine sample that is tested should then be refrigerated or frozen without additives and shielded from light for subsequent quantitative ALA, PBG, and total porphyrin determinations (which can detect ADP, and HCP or VP when ALA and PBG levels have already decreased to normal). In patients with substantial renal dysfunction, ALA and PBG levels can be measured in serum [9].

If PBG is increased in urine or serum, second-line testing will differentiate AIP, HCP and VP, although treatment (which is the same regardless of the type of acute porphyria) should not be delayed pending these results. Second-line tests include measurement of erythrocyte PBGD activity, as well as urine, plasma, and fecal porphyrin levels, measured in samples collected before beginning hemin

therapy. Marked increases in urinary and fecal total porphyrin levels and the relative, rather than absolute, amounts of the individual porphyrins [separated by high-performance liquid chromatography (HPLC)] are of greatest diagnostic importance. Therefore, spot urine and fecal samples are suitable for second-line testing. Total plasma porphyrin levels are best measured fluorometrically either by acidification and solvent extraction or in diluted plasma at neutral pH [9,25,28].

These second-line tests should not be relied upon for initial diagnosis of an acutely ill patient before treatment because they lack either sensitivity, specificity, or both. Urinary porphyrin levels, for example, can be increased in many nonporphyric conditions. Coproporphyrin is the predominant porphyrin in normal urine. But because coproporphyrin is also partially excreted in bile, even minor liver dysfunction may reduce biliary and thus increase urinary excretion of coproporphyrin [9,29].

Diagnosis of the acute attack

The diagnosis of an acute attack in a patient with documented AIP, HCP or VP is made on clinical grounds. While urinary ALA, PBG and porphyrins are higher during attacks than before or between attacks, there are no defined laboratory criteria for deciding that a patient is having an acute attack. Recurrent attacks are often similar over time and biochemical reconfirmation of the diagnosis of AIP, HCP or VP is not required for each attack. Treatment should be initiated immediately, after exclusion of other causes of symptoms (for example, pancreatitis and appendicitis) [9]. Criteria for diagnosis of an acute attack should be defined in clinical trials.

Enzymatic and DNA testing

Enzyme activity measurement and DNA testing help to confirm the type of acute porphyria and enable identification of asymptomatic but at-risk relatives. For example, half-normal activity of erythrocyte PBGD helps confirm a diagnosis of AIP in patients with increased PBG. This assay is also useful for screening family members once an index case has been identified. However, normal erythrocyte PBGD activity does not exclude AIP because 1) some mutations in the PBGD gene lead to a deficiency of the enzyme in the liver and other organs but not in erythrocytes [30,31]; 2) the normal range for erythrocyte PBGD activity is wide (up to 3-fold) and low-normal and high-carrier values overlap; and 3) the enzyme activity is much higher in younger than older erythrocytes and therefore enzyme activity in whole blood increases when erythropoiesis is stimulated [32]. A falsely low enzyme activity may be due to improper processing, storing, and shipping of blood samples. Assays of the enzymes deficient in HCP and VP are technically difficult, must be performed in extracts of cells with mitochondria, such as lymphocytes or cultured fibroblasts, and are not widely available [9].

Once biochemical studies have determined the type of acute porphyria, DNA studies can identify the disease-causing mutation in the defective gene. This further confirms the diagnosis, and permits rapid and accurate testing of asymptomatic at-risk family members by DNA studies. Patients with porphyria

should have genetic counseling and should be encouraged to inform family members about the disease and its genetics. Counseling enables family members to make informed decisions about lifestyle and to know the potential risks of certain drugs, preferably before the development of an acute illness [9].

Acute porphyria may be diagnosed prenatally with enzymatic and molecular studies, but this is seldom indicated because the outlook for most carriers is favorable [1].

Treatment of the acute attack

Precipitating factors, such as drugs, dietary restrictions, alcohol, metabolic stress, infection, and exogenous hormones should be identified and removed whenever possible. Treatment of symptoms such as pain, nausea, vomiting, agitation, etc. are important. Specific treatments include hemin, which must be administered intravenously, and carbohydrate loading, given by mouth (if tolerated) or intravenously. Glucose is often given in amounts of 200-400 g per day.

Intravenous administration of heme (referred to generically as hemin) is regarded as the most effective treatment for acute attacks of porphyria [1,9]. After intravenous administration heme binds to hemopexin and albumin in plasma, and is then taken up primarily in hepatocytes, where it reconstitutes a “free” heme pool that regulates ALAS1. In patients with AIP, HCP and VP, heme promptly (within 24-48 hours) reduces excretion of ALA and PBG to normal or near-normal levels.

Human hemin (hemin for injection)[2] is approved in the U.S. as lyophilized hematin (Panhematin™, Recordati, the first drug approved under the U.S. Orphan Drug Act) and in Europe and South Africa as heme arginate (Normosang™, Orphan Europe). Approval of human hemin in these countries was based on biochemical efficacy and evident benefit in numerous individual cases and case series, rather than randomized, controlled studies [33-43]. One small blinded study of heme arginate, in which treatment was delayed for 2 days, showed biochemical but not clinical efficacy [44]. That study, which was clearly underpowered, showed trends suggesting efficacy, and is not considered as having demonstrated evidence against efficacy. This report contrasts with many case reports and series, including a large, uncontrolled case series that enrolled 22 patients who had 51 acute attacks, in which heme arginate was initiated within 24 h of admission in 37 attacks (73%). All patients responded, including two with paresis, and hospitalization was less than 7 days in 90% of cases [38].

[2] Human hemin and hemin for injection refer to heme that is derived from human blood as a biological product for administration to humans, and are generic names for all heme preparations used for intravenous administration, including hematin and heme arginate. Hemin is also a chemical term that refers to the oxidized (ferric) form of heme (iron protoporphyrin IX), and is usually isolated as hemin chloride. Hemin is insoluble at neutral pH, but in alkaline solution (pH 8 or higher), the chloride is replaced by the hydroxyl ion, forming hydroxyheme, or hematin, which can be prepared for intravenous infusion.

Human hemin has few side effects. Hematin is unstable in water, and degradation products are formed which, when infused intravenously, can cause phlebitis at the site of infusion and a transient anticoagulant effect [45-49]. Reconstitution with 25% human albumin, which has become common in clinical practice, stabilizes hematin and prevents formation of degradation products, such that coagulopathy and phlebitis are prevented [10,43,50,51]. This helps preserve peripheral venous access in patients who require repeated courses of hematin. Heme arginate is more stable in solution [52], but is also often reconstituted with albumin [53]. Less common reported side effects of hemin have included fever, aching, malaise, hemolysis, anaphylaxis, and circulatory collapse [54,55]. Excessive doses of hematin can cause acute renal tubular damage associated with excretion of heme in urine [56]. Clearance of drugs that are metabolized by hepatic CYPs is reduced in some patients with acute porphyrias [57] and rapidly restored after intravenous hemin [58-60].

In the past glucose was recommended as first line therapy and human hemin as second line therapy. Increasingly, hemin is used earlier, because it is considered more effective than glucose [9]. Moreover, clinical response to hemin may be delayed or incomplete when there is advanced neurologic damage, as may occur when treatment is started late [38]. Subacute or chronic symptoms, which may reflect persistent neurological damage after repeated or prolonged attacks, are unlikely to respond [35,61]. Therefore, it is important to reverse an attack before advanced neuronal damage has occurred. The standard regimen for hemin treatment of acute porphyric attacks is considered to be 3–4 mg/kg daily for 4 days (or sometimes longer for severe attacks with advanced neuropathy) [9,38,62], although product labeling for Panhematin™ recommends 1-4 mg/kg for up to 14 days. Doses lower than 3 mg/kg have less effect on porphyrin precursor excretion and probably less clinical benefit. Prophylactic regimens of weekly or biweekly single doses have sometimes been useful in preventing attacks in patients prone to frequent exacerbations, but have been little studied [63,64].

The clinical benefits of hemin treatment described above remain under discussion because randomized, controlled trials with adequate power were not conducted prior to regulatory approval. In a retrospective mortality study of AIP patients (referred to earlier), a reduction in mortality was noted after the introduction of treatment with human hemin in 1971, but the difference was not statistically significant [11]. Therefore, the level of evidence for efficacy of hemin treatment is not considered to be high, even though it is widely considered to be highly effective [65].

1.2 Description of the drug under study

Panhematin™ is a sterile, lyophilized powder suitable for intravenous administration after reconstitution. Each dispensing vial of Panhematin™ contains the equivalent of 350 mg hemin, 240 mg sodium carbonate and 355 mg of sorbitol. The pH may have been adjusted with hydrochloric acid; the product contains no preservatives.

When mixed as directed with sterile water for injection, USP, each 48 mL provides the equivalent of approximately 336 mg hematin (7 mg/mL). When reconstituted with 147 mL of 25% human serum albumin instead of sterile water, which is an off-label recommendation, the hemin concentration is 2.4 mg/mL. [50]

In this study Panhematin™ will be reconstituted with 25% human albumin, which has been found to enhance stability and reduce side effects such as infusion site phlebitis and transient coagulopathy [43,50,51]. Phlebitis and coagulopathy after reconstitution with sterile water result from degradation products that bind to vascular endothelial cells, platelets and circulating coagulation factors.

1.3 Rationale for this clinical trial

The quality of the evidence base for diagnosis and treatment is becoming increasingly important in clinical practice, even for uncommon disorders. Hemin treatment can be rated no higher than 1C based on current evidence-based evaluation [65]. The lack of strong evidence for efficacy makes it more difficult to convince practicing physicians that patients will benefit, and therefore limits availability of this treatment for patients with acute porphyrias. Experience has shown that some physicians regard this treatment as still “experimental.”

The low quality of the evidence applies to use of hemin for treatment of acute attacks of porphyria and also to its use for prevention of such attacks. A separate double blind placebo controlled study protocol aims to improve the quality of the evidence for use on Panhematin™ for treating acute attacks. This protocol addresses its use for prevention of frequently recurring attacks of porphyria. This study is important because there is evidence that Panhematin™ is used quite frequently to prevent recurrent attacks of porphyria in the U.S.[64] and there have been few studies to guide or justify its use for this indication [9,66]. Product labeling supports its use for prevention of recurrent attacks in women. This is based on a single case report [66], and it is generally believed that frequently recurring cyclic attacks are best treated with a hormonal intervention such as a GnRH analogue [67]. Therefore, the most promising and appropriate preventive use for hemin would likely be for frequently recurring noncyclic attacks.

This study, as well as the study in acute attacks, will not provide definitive evidence to support the changes in product labeling for Panhematin™ in the U.S., but will contribute significantly to the body of evidence to support current expert recommendations in the following areas. 1) Current expert opinion is that at least for acute attacks, hemin treatment should be started promptly, without an initial trial of glucose [9]. Product labeling recommends treatment with Panhematin™ only after a trial of glucose for several days is not successful. Glucose loading is sometimes effective for treating mild acute attacks [9], but is seldom effective for preventing recurrent attacks, and often leads to unwanted weight gain. This study will likely provide evidence to support initial treatment with Panhematin™ for prevention of frequently occurring attacks. 2) Although Panhematin™ labeling states that treatment is approved only for treatment of women with attacks of AIP related to the menstrual cycle, there is no evidence from previous cases series

that treatment response to hemin is different in men, in women when the attack is not related to the cycle or in HCP and VP. Therefore, this study will support the use of hemin for prevention of recurrent attacks of AIP, HCP or VP in men and in women with frequently recurring attacks unrelated to the menstrual cycle. 3) The study will provide evidence to support use of Panhematin™ reconstituted with 25% human albumin to enhance stability and reduce side effects [50] by demonstrating a low incidence of infusion site adverse effects. This method has become widely used in clinical practice, but published data supporting its use is limited. 4) The study will also focus on a dose of 4 mg/kg rather than the 1-4 mg/kg daily recommended in product labeling.

2. Objectives

Patients available for this trial will already be on a prophylactic hemin regimen, and as part of the study will be offered the usual number of periodic prophylactic hemin (or placebo) infusions, which for most patients will be a single weekly infusion. Patients will be offered blinded infusions that correspond to the number of infusions they usually receive within approximately one week for prophylactic treatment.

Primary Objectives:

- To evaluate in 20 patients who are on a Panhematin™ prophylactic regimen whether a blinded dose of Panhematin™ is more effective than placebo in preventing an attack within the next 1-4 weeks.
- To evaluate in these 20 patients whether hemin is as safe and well tolerated as placebo when administered in a blinded fashion. Safety parameters will include the frequency and severity of phlebitis, nausea, vomiting and coagulation abnormalities.

Secondary Objectives:

- To evaluate the biochemical effects of Panhematin™ in patients treated with Panhematin™ to prevent attacks of acute porphyria by measuring urinary 5-aminolevulinic acid and porphobilinogen and serum porphobilinogen. This will determine whether biochemical measurements are predictive of efficacy in preventing an attack.

Exploratory Objectives:

- To evaluate effects of clinical features, such as sex, age and the factors that precipitate attacks of porphyria on response to preventive administration of Panhematin™.
- To evaluate effects of genetic features, including the nature or the *PBGD*, *CPOX* or *PPOX* mutation on response to preventive Panhematin™
- To evaluate the use of Panhematin™ reconstituted with 25% human albumin in patients treated to prevent acute attacks of porphyria

2.1 Endpoints.

The primary efficacy endpoint is whether a Panhematin™ is more successful than placebo in preventing a porphyria attack within the next 1-4 weeks when given as the patient's usual periodic prophylactic regimen given within approximately one week. Symptoms during past acute attacks should have included severe abdominal pain requiring a narcotic analgesic. An acute porphyric attack is defined by the presence of abdominal pain and one or more other characteristic manifestations such as tachycardia (heart rate 100 per minute or greater), nausea, vomiting, constipation, extremity pain, acute hypertension, low-grade fever, objective evidence of peripheral neuropathy, ileus, dehydration, mild leukocytosis, or hyponatremia. The manifestations of the recurrent attacks should have been similar to each other in nature (although not necessarily in degree or severity). The presence or absence of all such symptoms will be recorded on the CRFs by study personnel who are blinded to the treatment that was given.

The primary safety parameters will include the frequency and severity of phlebitis, nausea, vomiting and coagulation abnormalities. An additional series of safety measures will include other symptoms, physical findings and laboratory measurements.

Secondary efficacy endpoints will include urinary 5-aminolevulinic acid and porphobilinogen and serum porphobilinogen and comparisons of changes in these levels with prophylactic treatment with hemin and placebo.

Exploratory objectives. We will explore the effects of clinical features, such as sex, age, the factors that precipitate attacks of porphyria, and the nature of the *PBGD*, *CPOX* or *PPOX* mutation on response to Panhematin™ prophylaxis. We will also gain experience on the use of Panhematin™ reconstituted with 25% human albumin in patients treated to prevent acute attacks of porphyria.

3. Trial Design

3.1 Type of Trial

The trial is a double-blind, randomized, placebo-controlled, parallel group trial investigating the efficacy and safety of Panhematin™ for preventing acute attacks in at least 20 patients with well-documented acute porphyria (AIP, HCP or VP). These patients will (1.) have had frequent attacks in the past, with symptoms such as abdominal, back and/or limb pain and diagnosed after exclusion of other causes, and (2.) be on hemin prophylaxis for prevention of frequent attacks. Although clear guidelines have not been developed, it is expected that patients will have had 6 or more attacks in one year before starting hemin prophylaxis. This would be considered justification for a preventive regimen of hemin on clinical grounds. Most patients on hemin prophylaxis are given a single dose once weekly. However, other prophylactic regimens are effective in some patients, such as twice weekly infusions or a series of 3-4 infusions at monthly intervals. Giving only one blinded infusion may not be meaningful for a patient for whom more than a single infusion is needed to prevent attacks. Therefore, patients will

be offered the number of blinded infusions they usually are given within approximately one week for prophylaxis. All infusions for each patient will be either Panhematin™ or placebo. An interim analysis will be carried out after completion of ~10 patients to assess progress and possibly adjust the sample size. The trial consists of the following:

- A screening visit to determine eligibility and obtain informed consent
- A treatment visit for administration of one or more double blind prophylactic doses of Panhematin™ or placebo, corresponding to each patient's usual prophylactic regimen. Depending on the regimen, this visit may be completed in one day or after the number of days corresponding to their usual prophylactic regimen over approximately one week.
- Follow up visit at 1, 2, 3, and 4 weeks to assess response to the infusion of Panhematin™ or placebo. These visits will be in person or by telephone.
- Additional visits may be scheduled if needed, for example for treatment of symptoms.
- Follow-up visits 3 and 6 months after the end of treatment either in person or by telephone

Patients will have laboratory documentation of one of the acute porphyrias. Molecular documentation is also expected, although rarely a causative mutation cannot be detected. Upon entry into the study they will be given in a blinded fashion one or more preventive dose of either Panhematin™ (4 mg/kg) or placebo. The number of infusions will correspond to the number of prophylactic doses of hemin they customarily receive over approximately one week. A recurrent attack within the next 1, 2, 3 and 4 weeks will represent treatment failures. Because at study entry most patients are expected to be on weekly prophylactic hemin treatment, and hemin is a short-acting drug, emphasis in the analysis will be on attacks occurring within 1 week after study treatment.

Any attacks that occur during the study will be treated according to standard of care, which may include Panhematin™, either at the study site or at the patient's usual treatment location.

It is intended that 20 patients will complete treatment with a single blinded dose and at least 4 weeks of follow up. A completed patient is one who meets all entrance criteria, has no exclusion criteria and completes the single dosing and at least one week of follow up, or is withdrawn because of an adverse event.

The site for this study is the University of Texas Medical Branch (UTMB) at Galveston. The UTMB Porphyria Laboratory will carry out laboratory determinations related to porphyria as needed for this study. DNA studies to identify the familial mutation are now standard of care and will not be done as part of the study.

3.2 Rationale for the trial design

A randomized, double-blind, placebo controlled study design is important for evaluation of clinical efficacy of a treatment or preventive regimen for acute porphyrias because the symptoms of these disorders (e.g. pain) are highly variable and subjective. Physical signs other than pulse and blood pressure are also at least somewhat subjective. A single dose of blinded treatment, or the number of doses corresponding to patients' usual prophylactic treatment over approximately one week, was adopted because a blinded study of an extended course of preventive treatment would require repeated treatment visits to the study site, which is difficult unless a patient lives close to the site. Also, most patients on hemin prophylaxis have been on a preventive regimen for an extended period, and are not available to be studied when prophylactic Panhematin™ is initiated. On the other hand, many more patients are available who can travel even long distances to UTMB for a study that focusses on prophylactic dosing for less than approximately one week.

A single dose or multiple doses of study drug within approximately one week does not fully test the effectiveness of a continued regimen of hemin prophylaxis. However, if Panhematin™ prophylaxis is effective in preventing attacks, a difference should be seen between the Panhematin™ and placebo groups in the effectiveness in preventing an attack over the next 4 weeks. However, some patients may not remain constantly susceptible to attacks, and at times might not have an attack after a single dose of placebo. Therefore, efficacy data will be collected for 4 weeks or until the next time the patient is treated with Panhematin™ (either for an acute attack or for prophylaxis), whichever is longer. Given the uncertainty of the follow up interval that will be most informative, and other related uncertainties, an interim analysis will be done after the first ~10 patients to see if an adjustment in sample size or other protocol modification may be needed.

Both groups will receive either a single dose, which can presently be considered the standard dose of Panhematin™ for a prophylactic regimen, or the individualized prophylactic regimen that has been judged clinically to be effective in an individual patient when given during approximately one week. Any recurrent acute attacks that occur during the study will be treated as part of the study (i.e. with glucose or Panhematin™). Observations made during treatment of individual patients will be made available to their doctors at the end of the study and may contribute to their future management.

Potential study participants will be identified through the Porphyrias Consortium and its Longitudinal Study or referred by the American Porphyria Foundation as well as physicians countrywide.

3.3 Treatment of Subjects and Rationale for Treatment

Panhematin™ 4 mg/kg will be reconstituted with 25% human albumin [50] and infused over a 1 hour period. The amount of albumin is based on achieving a 1:1 molecular ratio for hemin and albumin [43,50]. Product labeling suggests that

Panhematin™, after reconstitution with sterile water, be infused within 15 minutes. After reconstitution with albumin, an infusion time of 1 hour is based on guidelines for infusion of the amount of human albumin used for reconstitution. The longer infusion time of 1 hour is acceptable given the enhanced stability of hemin in the presence of albumin, and based on experience of investigators in the Porphyrias Consortium [9].

Glucose infusions will be permitted during the study for treatment of acute attacks. Its use will be recorded but will not be specified by the study. For treatment of acute attacks, Panhematin™ product labeling suggests an initial trial of glucose at a dose of 400 grams daily, whereas a dose of 300 grams daily as 10% glucose is generally accepted [9,68-70] and a higher dose given as 10% glucose may increase the risk of fluid overload and hyponatremia. Smaller amounts (e.g. 200 grams as 2L 5% glucose in saline) have been recommended for meeting fluid, electrolyte and caloric needs, but not as an alternative to hemin therapy [71]. There are no guidelines or published experience for glucose loading for prevention of attacks, but based on expert experience the impression is that it is not very effective and may lead to unwanted weight gain.

Symptomatic treatment, including opioids, needed to control pain and other symptoms during an attack that occurs during the study will be recorded. Use of medications needed to control chronic symptoms, including pain, will also be recorded.

4. Trial Population

4.1 Number of patient and sites

Patients to be randomized: 20

Patients to be evaluated and screened: 40

Approximately 2 patients will need to be evaluated for every patient found suitable for study and randomization. Patients who do not complete the study will need to be replaced.

This study will be conducted at UTMB. Although it will not be a project of the Porphyrias Consortium, which has been funded in part by a U54 grant from the National Institutes of Health (NIH) beginning in 2009, the other sites in the Consortium are aware of this protocol and will refer patients that seem appropriate for evaluation. The Porphyrias Consortium is one of 23 NIH-funded consortia that comprise the Rare Disease Clinical Research Network (RDCRN). Funds for the Porphyrias Consortium U54 grant is provided by the National Institute for Diabetes, Digestive and Kidney Diseases (NIDDK) and the NIH Office of Rare Diseases Research. At present, the Consortium consists of 6 sites funded by the NIH grant, and 2 satellite sites that receive some support from the American Porphyria Foundation.

The American Porphyria Foundation (APF) is an active patient support group and is a supporting partner in the Porphyrias Consortium. The U54 grant supports

infrastructure for clinical research on porphyrias but not the costs of major clinical trials. Funding by the FDA Office of Orphan Product Development beginning in September 2014 supports costs for this study and an acute treatment study, which is also a Porphyrias Consortium single-site study conducted at UTMB. Recordati Rare Diseases, the manufacturer of Panhematin™, provides some additional grant support for both studies and supplies study drug at no cost.

4.2 Recruitment of Subjects

The investigators will contact patients known to them who are likely to be eligible for the trial by phone. Many of these patients will already be in contact with the Porphyrias Consortium. Additional patients will be referred by the American Porphyria Foundation (APF), which has been an important referral source for previous porphyria studies. Those patients not previously known to the study team at UTMB, the APF and the Porphyrias Consortium will be contacted through their primary treating physician. Patients newly referred will also be considered and enrolled for screening to determine if they meet the entry criteria. Written material and transcripts of planned verbal descriptions of the study will be approved in advance by the Institutional Review Board (IRB) at UTMB. A Screening Log will be kept of all subjects who are contacted by phone.

4.3 Inclusion Criteria

1. Male or female aged ≥18 years
2. Willing to provide written informed consent
3. A diagnosis of AIP, HCP or VP confirmed by the following criteria, which are based on the criteria for enrollment in the Longitudinal Study of the Porphyrias Consortium. For each type of porphyria, the inclusion criteria are based on 1) clinical features, 2) biochemical findings, as documented by laboratory reports (or copies) of porphyria-specific testing, and 3) molecular studies to identify a mutation in a porphyria-related gene. Equivocal biochemical measurements may require confirmatory testing. DNA testing is now considered standard of care and will not need to be done as part of this study. DNA testing for acute porphyrias is available through participation in the Porphyrias Consortium's Longitudinal Study (IRB#10-183), and is done by the CAP-approved laboratory at Mt. Sinai, which is the molecular laboratory resource for the Consortium. Such testing is also offered by major referral laboratories such as Mayo and GeneDx, and is currently offered at no cost by inVitae as part of a porphyria testing program supported by Alnylam Pharmaceuticals. An identified mutation is not essential for enrollment, since it is known that a mutation cannot be found in a small fraction (<5%) of biochemically proven cases of porphyria.

   ***Diagnostic inclusion criteria*:**

   Acute intermittent porphyria (AIP):

   1. Clinical features – a history of consistent clinical features such as acute attacks of abdominal, back and/or limb pain

2. Biochemical findings:

a. A marked increase in urinary or serum PBG before treatment with Panhematin™.

i. Urinary PBG >8 mg/24 hours or g of creatinine, or >2 fold increase (relative to upper limit of normal (ULN) of 4 mg/24 hours or mg/g creatinine)

ii. Serum PBG >0.2 ug/dL, or >2 fold increase (relative to ULN of 0.1 ug/dL)

iii. Marked increases are expected at initial diagnosis and before treatment with Panhematin™. But increases may be less marked in patients already on prophylactic treatment with Panhematin™.

b. Normal or only slight increases in plasma and fecal porphyrins.

i. Plasma porphyrins <4.5 ug/dL, or <5-fold increase (relative to ULN of 0.9 ug/dL); fluorescence scanning at neutral pH should show no peak or a small peak at ~620 nm. *Note*: AIP patients with severe renal disease are an exception and may have substantial increases in plasma porphyrins.

ii. Total fecal porphyrins <400 ug/g dry weight or <2-fold increase (relative to ULN of 200 ug/d dry weight

c. Erythrocyte HMBS/PBGD activity - deficient in ~90% of cases

i. Activity should be below the lower limit of normal, or <60% of the normal mean for the laboratory; this enzyme activity may be falsely low due to improper sample handling or physiologically increased when erythrocyte turnover is increased.

3. Molecular findings known:

a. Disease-causing mutation in *HMBS* (also known as *PBGD*) in >95% of cases.

b. Biochemical criteria will suffice if no mutation can be identified.

4. Previous acute attacks of porphyria, consisting of symptoms such as abdominal, back and/or limb pain, diagnosed by the investigator as caused by porphyria after exclusion of other causes, and currently on a prophylactic regimen of Panhematin™.

<u>Hereditary coproporphyria (HCP)</u>:

1. Clinical features – a history of consistent clinical features such as acute attacks of abdominal, back and/or limb pain

2. Biochemical findings:
    a. A marked increase in urinary or serum PBG and/or in urinary coproporphyrin III before treatment with Panhematin™.
        i. For urinary and serum PBG, same as for AIP (see above)
        ii. For urinary total porphyrins: >450 nmol/24 hours or g of creatinine, or more than 1.5-fold increase (relative to ULN of 300 nmol/24 hours or g of creatinine
    b. Normal or only slight increases in plasma porphyrins.
        i. Plasma porphyrins <5 ug/dL, or <5-fold increase (relative to ULN of 0.9 ug/dL); fluorescence scanning at neutral pH should show no peak or a small peak at ~620 nm. *Note:* this criterion applies to HCP patients without skin lesions. Skin lesions are rare in this disease, but if present are often accompanied by substantial increases in plasma porphyrins. An exception will be made for this criterion in such cases.
    c. A substantial increase in fecal porphyrins, with a predominance of coproporphyrin III.
        i. Total fecal porphyrins >400 ug/g dry weight or >2-fold increase (relative to ULN of 200 ug/g dry weight, with a predominance of coproporphyrin III and a coproporphyrin III/I ratio >1.5
3. Molecular findings known:
    a. Disease-causing mutation in *CPOX* in >95% of cases.
    b. Biochemical criteria will suffice if no mutation can be identified.

4. Previous acute attacks of porphyria, consisting of symptoms such as abdominal, back and/or limb pain, diagnosed by the investigator as caused by porphyria after exclusion of other causes, and currently on a prophylactic regimen of Panhematin™.

<u>Variegate porphyria (VP)</u>:

1. Clinical features – a history of consistent clinical features such as acute attacks of abdominal, back and/or limb pain

2. Biochemical findings:
    a. A marked increase in urinary or serum PBG and/or in urinary coproporphyrin III (same criteria as for HCP) before treatment with Panhematin™.

b. Increases in plasma porphyrins and a fluorescence emission peak at ~626 nm.

i. Plasma porphyrins >2.7 ug/dL, or >3-fold increase (relative to ULN of 0.9 ug/dL)

ii. Fluorescence scanning at neutral pH showing a peak at ~626 nm.

c. Substantial increase in fecal porphyrins, with a predominance of coproporphyrin III and protoporphyrin IX.

i. Total fecal porphyrins >400 ug/g dry weight or >2-fold increase (relative to ULN of 200 ug/d dry weight, with a predominance of coproporphyrin III and protoporphyrin

3. Molecular findings known:

a. Disease-causing mutation in *PPOX* in >95% of cases.

b. Biochemical criteria will suffice if no mutation can be identified.

5. Previous acute attacks of porphyria, consisting of symptoms such as abdominal, back and/or limb pain, diagnosed by the investigator as caused by porphyria after exclusion of other causes, and currently on a prophylactic regimen of Panhematin™.

4.4 Exclusion Criteria

1. Symptoms such as abdominal, back or limb pain are explained by another condition, as judged by the investigator
2. Known or suspected allergy to Panhematin™ or related products
3. A known or suspected allergy to human albumin
4. Any disease or condition that the investigator judges would lead to an unacceptable risk to the patient or interfere with the successful collection of data for the trial
5. Previous randomization in this trial

All inclusion and exclusion criteria must be satisfied for inclusion of a patient in the efficacy analysis.

4.5 Withdrawal Criteria

The subject may be withdrawn from the trial if judged non-compliant with the study procedures or if there is a safety concern, at the discretion of the investigator.

The subject may withdraw from the study at any time.

For subjects withdrawn prematurely, assessments should be completed up to the time of withdrawal.

Patients who require treatment of acute attacks with open label Panhematin™ are not withdrawn from the study.

Patients withdrawn will be offered standard of care treatment, which may include Panhematin™, at the study site or through their own physician.

4.6 Subject Replacement

Subjects who are enrolled initially and then found not to meet inclusion criterion will be replaced in order to provide 20 patients eligible for randomization and trial completion.

5. Study materials

5.1 Study drug reconstitution and administration

Panhematin™ (human hemin), a lyophilized preparation of hematin (hydroxyheme or heme hydroxide), is provided by Recordati Rare Diseases in a vial containing either 350 mg of product, and reconstituted with 147 mL of 25% human serum albumin [43,50]. Standard directions for dose preparation using either vial are in place in the UTMB Pharmacy and are reflected here.

The patient's body weight is provided to the Pharmacy, which prepares each Panhematin™ dose of 4 mg/kg body weight. Representative calculated dosages are shown in the **Table**. No more than 313 mg should be used for each administration, i.e. the dose is 4 mg/kg body weight, not to exceed a total of 313 mg. After the first dose, the same calculated dosage is used for subsequent doses.

The dose should be calculated and venous access obtained before reconstituting the study drug.

Procedure for reconstitution of Panhematin™ [50].

The following materials are needed:

1. One 350-mg vial of Panhematin™.
2. One 150-mL or larger sterile empty glass bottle for infusion; a second bottle is needed for processing the 350-mg vial of Panhematin.
3. Three 50-mL vials of 25% albumin (only 147 mL will be used)
4. One 5-micron filter needle
5. One vent needle.

Table. Volumes of heme–albumin solution needed for each dose based on representative body weights

Body Weight (kg)	Hemin dosage (4 mg/kg)	Heme–Albumin Mixture (mL)
50	200	83
60	240	100
70	280	117
80	313*	132

* No more than 313 mg Panhematin™ should be used per single dose.

To prepare Panhematin™ for infusion. (Note that the 350-mg vial requires 147 mL of 25% albumin. Because only 132 mL of 25% albumin will fit in the vial, this amount is used initially, followed by the additional 15 ml of 25% albumin):

1. Reconstitute the 350-mg vial of Panhematin™ initially with 132 mL of 25% albumin. Because this volume will almost completely fill the vial, the albumin must be injected into the vial slowly and the vial must be vented. Use a vented needle or make a vent with a separate needle to release the air pressure.
2. *Do not shake the mixture*. Swirl the vial 15 to 20 times to ensure that it is thoroughly mixed (it will be difficult to see if the materials are blended because of the dark color of hemin).
3. Withdraw the full contents of the vial into a syringe.
4. Inject the full contents (350 mg hemin reconstituted with 132 mL 25% albumin) into a 150-mL empty sterile bottle.
5. After the full contents is transferred to a sterile glass bottle (as described in step 4 above) add an additional 15 mL of 25% albumin to the original vial, swirl as in step 2 and transfer this using a syringe to the same 150-mL bottle, which will now contain 350 mg hemin reconstituted with 147 mL of 25% albumin.
6. After reconstitution of the entire contents of the vial (350 mg hemin) with 147 mL of 25% albumin, the concentration of hemin in the glass bottle is 2.4 mg/mL. The volume required to deliver the desired dose (usually 4 mg/kg of body weight) should be calculated based on the patient's body weight, but not to exceed 313 mg. See **Table** for example volumes for some example body weights.
7. Withdraw the calculated required dose for the patient into a syringe by using a 5-micron filter needle.
8. Inject the dose into a 150-mL empty sterile bottle.
9. Label the bottle.
10. Place the bottle in an amber bag to protect the mixture from light. Also place a vented spike adapter in the bag. To preserve blinding, do not label the amber bag. (Customarily, a yellow Medication Administrations Recording blood products label (for both albumin and Panhematin™) would be attached to the amber bag.) Then place the amber bag inside a STAT-labeled bag.
11. Hand-deliver the bag to the clinical unit immediately. The infusion should be started within 1 hour or less of preparation. The infusion is administered by an unblinded nurse and other blinding procedures are followed (as described in sections **5.2 and 5.5**). The heme–albumin complexes may be

stable for much longer, but the solution does not contain bacteriostatic agents and therefore should be infused promptly.

Procedure for administration of Panhematin™.

1. Access a large peripheral vein using an indwelling intravenous catheter. Based on clinical indications, such as a need for frequent intravenous infusions or poor venous access, a peripherally inserted central line or a central line or port may be used.
2. Piggyback the Panhematin™-albumin dose to an intravenous line that is infusing 0.9% sodium chloride at a moderate rate (at least 100 mL/hr). The piggyback site should be as close as possible to the venous access site.
3. Infuse the dose over a period of 60-90 minutes or at a rate that should not exceed 1 mL/min, which corresponds to the recommendation for infusing 25% human albumin [43,50]. A somewhat shorter infusion time may be acceptable but may entail some risks from intravascular volume expansion. Some patients have experienced headaches shortly after infusions of heme–albumin, perhaps related to transient expansion of intravascular volume.
4. After the heme–albumin is infused, continue the infusion of 0.9% saline for at least 10 minutes at a rate of at least 100 mL/hr to clear the line, catheter (or port) and vein of the drug.

The study drug is administered at a dose of 4 mg/kg body weight. The infusion is given over 60-90 minutes [50], after which the IV set and other materials are removed and discarded.

5.2 Placebo preparation and administration

It is not feasible to design a placebo for intravenous administration with the same appearance as Panhematin™ (human hemin), which is administered as a black solution. The placebo for this study will be 117 mL of 0.9% sterile saline in the same 150 mL sterile glass bottle used for the active drug, and labeled and delivered from the Pharmacy in the same manner as the active drug. The placebo will be infused in the same manner as the active drug by a research nurse who is unblinded. Other study personnel will remain blinded. To maintain blinding in this study, study drug (reconstituted Panhematin™ or placebo) is delivered from the Pharmacy in a non-transparent container, so is not visible to study personnel or the patient. One research nurse will have responsibility for the infusion and will not be blinded. This nurse will interact minimally with the patient and other study personnel and will drape the administration set and the IV site in a manner that will maintain blinding. The drug or placebo will be administered through an already established intravenous access. The unblinded nurse will remain for the entire infusion time and assist in any manner necessary to maintain blinding of other study personnel and the patient, which may include adjusting drapes and the infusion set-up. The unblinded nurse will not otherwise be involved in the patient's care or in collecting data. At completion of the one-hour infusion, the unblinded

nurse will remove the IV set and other materials to another location for disposal. The patient will also be blind-folded before the study drug arrives and until the IV set and other materials are removed from the unit. Other research nurses who are blinded will carry out other study procedures that do not involve the infusion.

5.3 Timing of administration of Panhematin™ or placebo

Treatment with Panhematin™ or placebo should be given within ±2 days of the time a prophylactic dose or series of doses would have been administered. Panhematin™ is FDA approved at the dosage level used in this study.

It is recommended in product labelling that Panhematin™ be given once or twice daily. Therefore, in this study a dose of Panhematin™ will not be given within 12 hours of a previous preventive dose of active study drug. If an attack occurs within 12 hours of the study dose of Panhematin™ or placebo, open label treatment with Panhematin™ will be delayed until 12 hours after the last study dose of Panhematin™ or placebo. If it considered clinically necessary to start open label Panhematin™ for treatment of the attack in less than 12 hours, the first therapeutic dose for the attack will be blinded, and will be Panhematin™ if the patient was randomized to placebo, and will be placebo if the patient was randomized to Panhematin™. Whether active drug or placebo is given in this instance will be determined by the Pharmacy, where 1-2 individuals will not be blinded.

5.4 Glucose administration

Glucose loading will be permitted for treatment of acute attacks during the study at the discretion of the patient's physician. No particular amount or regimen will be required during participation in this study, although a recommendation may be made to treat intravenously with glucose 300 grams daily, which is considered the standard dosage for glucose loading [9,10,68-70,72]. This amount is usually delivered as 3 liters of 10% glucose infused daily for 4 days or longer. Although larger daily amounts are sometimes recommended, this results in larger volumes of intravenous fluid and increased risk for fluid overload and hyponatremia.

5.5 Blinding

Blinding of treatment with a darkly colored, intravenous drug poses significant challenges, but is feasible because the drug is administered only once, and personnel directly involved in drug reconstitution and administration will be different from those who establish intravenous access and are otherwise involved in the study patient's care. The PI, other physicians, study coordinators and nurses involved in patient care will remain blinded. As described above (Section 5.2), pharmacy personnel (1-2 individuals) and one study nurse who will administer the drug will not be blinded. Blinded staff will not be in the room during study drug administration. Every effort will be made to avoid compromise in blinding if, for example, there are problems with the infusion after it is started. Problems with the infusion will be handled by the unblinded nurse without compromising blinding of other personnel, if possible. Any difficulties that might compromise blinding will be recorded.

5.6 Treatment of acute attacks during the study

Attacks during the study that occur at the study site will be treated at the study site with the usual hemin regimen of 4 mg/kg body weight daily for 4 days, or longer if clinically indicated, as part of the study. Attacks that occur after the patient leaves the study site and returns home will be treated in the manner considered optimal by the patient's physicians. Such treatment will be recorded but not prescribed by the protocol. Study site physicians will be available to provide advice on management, as appropriate. This will not be considered formally as rescue treatment since it is not prescribed in the study protocol. It will be recommended that Panhematin™ be reconstitution with human albumin, as described above, since this is now considered optimal [9]. Blinding of the study treatment will be maintained during unblinded treatment of any acute attacks, unless doing so would compromise patient safety.

Administration of two Panhematin™ doses of 4/mg/kg doses daily was common in the past, although a single daily dose for 4 days is now more commonly recommended [9]. When two doses are given, an interval of at least 6-8 hours is customary. During this study, a need to give two doses of Panhematin™ within a period of 12 hours will occur only if an acute attack develops and needs to be treated within 12 hours of the previous study dose of Panhematin™ or placebo.

As noted above, if open label treatment with Panhematin™ is required less than 12 hours after a blinded infusion, the first infusion for treating the acute attack will also be blinded, and the first infusion will be Panhematin™ if the patient was randomized to placebo, and will be placebo if the patient was randomized to Panhematin™. This will avoid unblinding of the randomly assigned study treatment.

Symptomatic treatment for pain, nausea and vomiting will be given as needed to control these symptoms of the porphyric attack, which can be severe. These are regarded as expected treatments rather than rescue treatments and are not a prescribed part of this study.

5.7 Randomization

Treatment in this double-blind, symmetrically randomized, parallel group trial study will be assigned by the study statistician, who will inform the Pharmacy of the assignment. Randomization will be in blocks of 4, and occur after the inclusion and exclusion criteria are satisfied. Labels showing the study randomization number will be generated to label all study samples and materials.

The randomization code for a particular subject can be broken if knowing the identity of the treatment allocation is felt to be necessary for optimal management of the patient and the treating physician concludes that breaking the code is in the best interest of the patient. Whenever a code is broken, the person breaking the code must record the time, date and reasons. It must also be recorded who is unblinded as a result of breaking the code, i.e. specific study personnel and/or the patient.

6. Methods and Assessments

This study will be carried out in an outpatient setting, with an option for inpatient visits if needed, and will consist of screening and enrollment visits (designated Visit 1), followed by a treatment visit for blinded hemin or placebo administration (Visit 2), and post-treatment visits: weekly for the first month, then at3 and at 6 months. Visits 1 and 2 may be combined to occur on the same day.

6.1 Visit Procedures

Visits are designated as shown below to facilitate scheduling of procedures and recording of study-related data. All study visits will be numbered as a single consecutive series. Variations outside specific windows will be recorded as a protocol deviations.

Visit 1	Screening and enrollment. (If screening and enrollment require more than one visit, they will be designated 1a, b, etc.)
Visit 2	Administration of Panhematin™ or placebo. (This visit might be on the same day as Visit 1.) If additional days are needed at the study site, such as for additional doses of study drug for prevention, or for treatment of an attack of porphyria, these will be designated 2a, b, etc.
Visit 3,4,5,6, 7 & 8	These will occur in person or by phone at weekly intervals for the first month to determine efficacy, and 3 and 6 months after completion of blinded treatment to record long terms effects of the study treatment and subsequent course and clinical treatment of the disease

6.1.1 Visit 1 – Screening and Enrollment

Patients will be fully informed about the purposes and procedures of the study, orally and in writing, and asked to sign an informed consent form approved by the IRB, which describes study procedures and the risks and potential benefits of the study. Patients will be screened for eligibility and enrolled at Visit 1. Any additional studies needed to determine eligibility will be done at that time.

Procedures:

After enrollment each subject will be allocated a unique study enrollment number. If the inclusion and exclusion criteria (see below) are satisfied, the patient will be randomized during the screening visit or before and assigned a unique study randomization number. The following will be performed and recorded in the CRF:

1. Checks of Inclusion and Exclusion Criteria.
2. Demographic Information, to include:
   - Date of Birth
   - Sex
   - Race and ethnicity

3. History, to include:
   - Year of first porphyria attack
   - Number of attacks during the past 6 months
   - Attacks related to the menstrual cycle or not
   - Attacks related to other precipitating factors (harmful drugs, nutritional alterations, etc. or not
   - Time of onset of the most recent attack
   - Previous treatment with Panhematin™
4. Concomitant illnesses
5. History of allergy
6. Concomitant medications
7. Use of opioid agonists during the previous 6 months
8. Physical Examination, including:
   a. Body height and weight
   b. Vital Signs
9. Electrocardiogram, if clinically indicated
10. Recording of porphyria-related signs and symptoms
11. Blood samples will be drawn for testing, to include erythrocyte PBGD activity, serum or plasma PBG and porphyrins, complete blood counts, and metabolic and hepatic panels
12. Urine sample will be collected for assessment of urine ALA, PBG, and porphyrins.
13. Fecal sample will be collected for porphyrins.
14. Blood sample for DNA isolation and mutation analysis (unless done previously). This is now considered standard of care diagnostic testing for porphyrias, and is widely available. A separate consent form for this testing will be provided only if required by the testing laboratory.
15. Urine pregnancy tests (for females of childbearing potential only).

6.1.2 Visit 2:

Procedures:

The following will be performed and recorded in the CRF (at time of treatment):

1. Concomitant medications, and any changes not recorded previously
2. Vital Signs
3. Recording of porphyria-related signs and symptoms
4. Urine, blood and fecal samples for ALA, PBG and porphyrins (pretreatment)
5. Recording of adverse events

6. Administration of a dose of study treatment (Panhematin™ or placebo)

6.1.3 Visit 3, 4, etc.

Procedures:

Recording of clinical information and obtaining samples, as appropriate clinically.

Observation and treatment of any attacks or symptoms of porphyria.

6.2 Assessments for Efficacy

6.2.1 Clinical improvement

Attack prevention will be assessed especially over the 4 weeks following study drug administration. The expectation is that efficacy in preventing an attack is most likely within the first week, or within the time interval between usual prophylactic dosing.

6.2.1.1 Number of attacks and porphyria-related signs and symptoms

At the post-treatment visits the patients will be queried about symptoms attributable to porphyria, treatment that was given for such symptoms and any doctor for hospital visits or admissions since the previous visit. Symptoms recorded will include pain, nausea, vomiting, constipation and specified neurological and psychiatric symptoms. This information will be recorded on the CRFs.

6.2.1.2 Other clinical information

Use of Panhematin™, glucose loading and potent opioid agonists since the last visit including the generic and trade names, dose, route of administration and time of each dose will be recorded.

6.2.2 Biochemical measures of improvement

Biochemical measures, to include urinary ALA, PBG and porphyrins, plasma PBG and porphyrins and fecal porphyrins will be measured at each visit, but will be optional for the follow up visits at 1, 2, 3, and 4 weeks and 3 and 6 months. Analyses will be performed at the Porphyria Laboratory of the University of Texas Medical Branch.

6.3 Safety Assessment

6.3.1 Symptoms

Any new symptoms not related to porphyria will be recorded during the treatment period.

6.3.2 Physical Examination

Physical examination, to include vital signs, weight, body mass index (BMI) and evaluation of the major systems will be recorded at each visit, but will be optional for the follow up visits. Height will be recorded only at Visit 1.

6.3.3 Blood counts and chemistries

Blood samples will be drawn for complete blood counts and metabolic, hepatic and coagulation panels at each visit, but will be optional for follow up visits. Analyses will be performed either by the hospital laboratory. The site investigator will review the report, sign and date it, and comment on any laboratory abnormality that is judged to be clinically relevant. A clinically relevant abnormality is defined as one that suggests a disease or organ toxicity and is of a severity that requires active management (e.g. change in treatment, more frequent follow-up or diagnostic investigation).

6.3.4 Urinalysis

A standard urinalysis will be performed at UTMB, using a urine strip, at Visit 1.

6.3.5 Pregnancy test

For women of childbearing potential a pregnancy test (urine hCG) will be performed at UTMB at Visit 1. Since pregnancy is not a reason for avoiding treatment with Panhematin™, a positive pregnancy test will not exclude a patient from entering or continuing the study. Pregnancy will be recorded as a concomitant condition.

6.3.6 Adverse Events

Adverse Events will be recorded at each visit.

7. Adverse Events

7.1 Definitions (ICH)

Adverse Event (AE):

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. The following should not be recorded as AEs, if recorded at screening:

- Pre-planned procedure, unless the condition for which the procedure was planned has worsened from the first trial related activity after the subject has signed the informed consent.
- Pre-existing conditions found as a result of a screening procedure.

Clinical Laboratory Adverse Event:

A clinical laboratory AE is any clinical laboratory abnormality that suggests a disease and/or organ toxicity and that is of a severity that requires active management (i.e. change of dose, discontinuation of drug, more frequent follow-up or diagnostic investigation). Clinical laboratory abnormalities that are found at screening and that fall under the above description should be recorded as a concomitant illness.

Serious Adverse Event (SAE):

A SAE is defined and distinguished from a non-serious AE as follows:

Serious/Non-Serious Adverse Event Definitions:	
Serious Adverse Event (SAE)	An SAE is any untoward medical occurrence that at any dose: - results in death, - is life-threatening*, - requires inpatient hospitalization or prolongation of existing hospitalization, - results in persistent or significant disability/incapacity, or - is a congenital anomaly/birth defect - is an important medical event that may not result in death, be life-threatening*, or require hospitalization when, based upon appropriate medical judgment, it may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.
Non-Serious Adverse Event	A non-serious adverse event is any AE which does not fulfill the definition of an SAE
* The term life-threatening in the definition of serious adverse event refers to an event in which the subject 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 was more severe.	

8. Case Report Forms

Case Report Forms (CRFs) will be prepared at UTMB for this study.

8.1 Rules for Completing CRFs

CRFs may be completed by investigators, coordinators and study nurses. They will print legibly using a black ballpoint pen, and ensure that all relevant questions are answered and that no data entry spaces are left empty.

Any assessment or test data that was not done and will not be available is indicated by writing “N/D” (Not Done) in the answer field. If the question is irrelevant or not applicable, this is indicated by writing “N/A” (Not Applicable) in the field.

The investigator and site study team must ensure that all information derived from source documentation is consistent with the source information. By signing the Affirmation Statement, the Investigator confirms that the information in the CRF is complete and correct.

8.2 Corrections to CRFs

Corrections to the data on the CRFs must only be made by drawing a straight line through the incorrect data and then writing the correct value next to data that has been crossed out. Each correction must have initials of the individual who made the correction and the date of the correction. An explanation for the correction should also be written next to the correction, if necessary for clarity. If corrections are made after the date of the Investigator's signature on the Affirmation Statement, the Statement must be signed and dated again by the Investigator.

8.3 CRF Review and Data Entry

The original CRFs are reviewed by the investigator and study coordinator during the study and after completion of each subject. CRFs are finalized after no further corrections or amendments to the content are expected. After CRFs are verified and finalized, data will be compiled for analysis by the study biostatistician. CRFs will be archived at a secure archiving location at UTMB.

9. Monitoring Procedures

Safety Monitoring Plan

The risk level for this study is judged to be low, since treatment with Panhematin™ is already part of clinical practice and treatment of enrolled patients, and no investigational products are involved. Although use of albumin for reconstituting Panhematin™ is an off-label method, there is strong evidence and considerable experience to suggest that this increases safety. Potential risks of the study are described in detail under Assessment of Risks (See Section **13.1** below). Because this is not a Phase III clinical trial, a Data and Safety Monitoring Board (DSMB) is not required.

Recruitment, enrollment, retention, adverse events, and study procedures will be monitored carefully by the PI and study coordinator(s). They will review individual subjects' study records to ensure that appropriate safety procedures are being followed, that protocol requirements are being adhered to, and that data is accurate, complete, and secure. Study records include consent forms, case report forms, flow charts, data forms, laboratory specimen records, inclusion and exclusion criteria forms, adverse event logs, and medical charts. Investigators will review available data at study meetings that usually occur monthly, and discuss any instances of adverse events or unexpected problems encountered regarding patient safety or data collection.

Plan for Adverse Event (AE) Reporting. See Section **7**. The investigators will be notified immediately if an AE occurs, and a medical member of the team will evaluate the patient and enter a note in the CRFs. The investigators will be responsible for notifications to the IRB and others, as appropriate. In particular, all unanticipated, serious, fatal and/or life-threatening adverse events will be reported to the IRB, and others as required, within 24 h of occurrence or notification. The investigators, and the IRB are primarily responsible for determining whether modifications to the protocol and consent form are required. If a determination is

made that participants are found to be exposed to excessive risks in relation to anticipated benefits, the study may be immediately suspended, unless corrective changes can be made quickly. Studies will continue after modifications are made that are deemed to result in an acceptable risk/benefit ratio. Aggregate reports of SAEs will be prepared on an annual basis and forwarded to the IRB and others as required.

Plan for Safety Review. Every effort will be undertaken to monitor and minimize the risks to subjects. Prior to obtaining informed consent, subjects will be encouraged to thoroughly read the informed consent form and ask questions regarding the outlined procedures and risks, and be informed of all tests involved in the screening process.

Data Monitoring Plan

To ensure data quality and study integrity, all study data will be collected by the research team, recorded on data flow sheets or case report forms, and stored in locked file cabinets or secure electronic databases. The PI will be responsible for ongoing monitoring of data integrity and patient safety.

This is an investigator-initiated single site clinical study that is supported by a grant from the FDA Office of Orphan Product Development. Some additional grant support as well as study drug are provided by Recordati Rare Diseases. External monitoring will not be required. Data monitoring will be done, as described above, by the investigators and study coordinator. The following items must be verifiable in source documentation:

- Existence of subject (initials, date of birth)
- Confirmation of participation in trial (subject ID, trial ID and signed and dated research consent form)
- Diagnosis/indication under investigation
- Visit dates
- Adverse events or signs and symptoms (description and duration)
- Relevant medical history and/or concomitant illness(es)
- Concomitant medications
- Blood pressure, pulse, weight and height
- Reason for exclusion or withdrawal

The data recorded in the CRF are considered as source data.

10. Data Management

The PI and research staff will be responsible for management of data as recorded on the CRFs or secure electronic databases. Data downloaded from the database for analysis will identify subjects by study number, without personal identifiers. The identity of subjects will be excluded from all presentations and publications.

11. Evaluability of Subjects for Analysis

The data analysis for efficacy will include all randomized subjects who were randomized and exposed to the study drug or placebo, fulfilled inclusion and exclusion criteria, and for whom there were no protocol violations or deviations that affect assessments of efficacy. All subjects exposed to active drug or placebo will be included in the safety analysis.

The decision to exclude any subject or observation will be recorded, and the reasons for their exclusion will be documented and signed by those responsible for the exclusion. This documentation will be stored with other trial documentation.

12. Statistical Analyses

Statistical analyses will be conducted in the Office of Biostatistics at UTMB by Kristofer Jennings, PhD, Assistant Professor in the Division of Epidemiology and Biostatistics of the Department of Preventive Medicine and Community Health at UTMB.

12.1 Purposes

The main purpose of the statistical analyses is to test the null hypothesis that observed differences between the two treatment groups could have been produced solely by chance – the alternative being that differences were due to the difference in treatment (i.e. Panhematin™ vs. placebo) – and to estimate the true differences between measurements for the two treatment groups. The emphasis in the efficacy analysis will be the effects of treatment with Panhematin™ vs. placebo in preventing a subsequent attack in the week following a single infusion. Attacks occurring at weeks 2, 3 and 4 will also be subject to analysis, since recurrent attacks do not always occur at precisely predictable intervals.

Biochemical parameters, such as urine and serum levels of PBG, will be analyzed also, but will not be primary efficacy measures.

12.2 Variables for statistical analyses

12.2.1 Efficacy variables

The primary efficacy endpoint is the prevention of an acute attack during the first week following a blinded infusion of either Panhematin™ or placebo, and at other weekly intervals up to one month.

Secondary efficacy and exploratory endpoints include changes in biochemical parameters during treatment, and the effects of genetic, clinical and demographic features on treatment response.

The following biochemical endpoints will be analyzed:

- Urine (or plasma/serum) ALA, PBG
- Urinary total porphyrins, including fractionation of individual porphyrins by HPLC

- Plasma porphyrins, including fractionation of individual porphyrins by HPLC (if elevated initially)
- Fecal porphyrins, including fractionation of individual porphyrins by HPLC (if elevated initially)

Data related to these secondary endpoints will be subjected to descriptive analyses and differences between the treatment groups analyzed for significance as described below.

12.2.2 Safety variables

As noted earlier, safety endpoints will include:

- Symptoms
- Findings on physical examination including vital signs
- Routine clinical testing daily including
    - Complete blood counts
    - Metabolic and liver panels
    - Coagulation panel (platelets, prothrombin time and partial thromboplastin time)
- Unexpected adverse events

These will be subjected to descriptive analyses and differences between the treatment groups analyzed for significance as described below.

12.3 Statistical Methods

All tests for significance will be two sided at the 5% significance level and accordingly 95% confidence intervals will be determined.

The main clinical efficacy variable, the occurrence or nonoccurrence of an acute attack each of the 4 weeks following test infusion, will be analyzed by logistic regression with treatment as a factor and drug dose as a covariate. Data for any post randomization exclusions will be listed and the possible impact assessed.

Use of Panhematin™ and opioids for treatment of porphyria symptoms during the 4 weeks following treatment will be analyzed by exact two sample Wilcoxon tests or by Fisher's exact test.

Supplementary analyses of the role of demographic and clinical features will be performed.

Adverse events will be coded and analyzed descriptively.

12.4 Sample size determination

The power to detect a treatment-related difference in frequency of attacks and other measurements increases with the magnitude of the differences that are induced by treatment. A prior case series with cross over from a pre-existing

control period to open label use of heme arginate to prevent frequent attacks showed a substantial effect in some patients, but little effect in others. That study suggests that a sample size of 20 should be sufficient. However, the previous study may not be useful in assessing sample size for the current study, which is of a different design. Therefore, an interim analysis is planned in this study after the first 10 subjects. The biostatistician will unblind the data for this analysis, but blinding will be preserved for all others.

The sample size needed to have 80% power to reject the null hypothesis of no effect using the two-sample comparison of proportions at the 5% significance level will be determined based on the initial sample. For instance, if the probabilities of recurrence are 0.8 and 0.2 in the control and treatment groups respectively, the power to detect with 20 patients (10 patients per group) is 80%. However, if the treatment group chance of recurrence is 0.38, the estimated sample size is 40 (20 patients per group). If the estimated sample size is 20 or less the trial will stop after 20 patients have completed; if it is up to ~40, consideration will be given to increasing the number of completed patients to 40; if it greatly exceeds 40 the trial will continue until 20 patients have been completed. If it is not feasible to reach a target sample size, or if it is not even feasible to find enough patients to have 80% power for establishing clinical efficacy, the trial will continue and stop when 20 patients have completed.

12.5 Interim analyses

An interim analysis is planned after completion of 10 subjects, to determine a reasonable estimate of the variance, as stated above in 12.4. Given the change in inclusion criteria, a logistic model will be fit to assess the change in recurrence rate by dose. We do not, however, expect that the dose or regimen effect will be statistically significant or have a substantial effect on the outcome.

13. Ethical considerations

The study will be conducted in accordance with accepted standards for human studies, including the Declaration of Helsinki. The study will be approved by the IRB at UTMB, as will changes made in study documents as appropriate.

13.1 Assessment of Risks

Patients enrolled in this study will have a history of frequently recurring attacks of porphyria and be on preventive treatment with Panhematin™, which is considered a clinical indication for use of this drug. Therefore, the risks from administration of a single dose of Panhematin™ will not be substantially different from their standard treatment.

The following are reported or possible risks related to the products and procedures in this study. How these risks will be minimized is noted.

<u>Risks related to the randomized study design and other study procedures</u>:

- Occurrence of an attack due to randomization to placebo rather than Panhematin™.

This risk is small, because patients will be treated for an attack promptly in a manner at least as beneficial as their treatment before beginning prophylactic treatment. The risks from treatment of an attack occurring after placebo will not be different or greater than for attacks that occur at other times.

Patients on hemin prophylaxis will at some point need to stop treatment to see if it is still needed. An attack that occurs in this study will be clinically beneficial because it provides some useful clinical information, and reinforces the need for continued hemin prophylaxis.

- Blood drawn in this study could contribute to iron deficiency. The volume drawn at each visit will total <30 mL.

This may be somewhat more than would have been drawn if the patient were not enrolled in the study. Iron status will be assessed by serum ferritin measurements and corrected with iron supplements if clinically indicated.

Risks of Panhematin™:

This includes reported effects of Panhematin™ and other human hemin preparations.

- Reversible renal shutdown was observed in a case where an excessive hematin dose (12.2 mg/kg) was administered in a single infusion. Oliguria and increased nitrogen retention occurred although the patient remained asymptomatic [56].

No worsening of renal function has been seen with administration of recommended dosages of hematin [56,73].

- Phlebitis at the site of intravenous infusion is common, which can lead to loss of venous access in patients who require repeated treatment.

This is felt to be due to degradation products of hematin, and use of human albumin rather than sterile water for reconstitution of the lyophilized product[43,50] is expected to reduce the risk of this complication in this study. The great majority of patients in this study are expected to have previously implanted chest wall ports to facilitate repeated hemin infusions, which also reduces the risk of phlebitis.

- A transient anticoagulant effect manifested by prolonged PT and PTT and thrombocytopenia is also common, which in one case may have contributed to gastrointestinal bleeding [74].

This transient coagulopathy is thought to be due to degradation products of hematin, which are formed before infusion if the product is reconstituted with sterile water. This side effect, which is not usually sufficient to cause bleeding by itself, can be prevented by stabilizing hematin with human albumin. Patients with preexisting coagulation abnormalities or concurrently on anticoagulant therapy will be excluded if the investigator believes the risk is unacceptable.

- Fever, aching and malaise are sometimes seen [54,55]. Some patients have noted headache or migraine.

These side effects are transient, and may be related to hematin degradation products, although this is not established.

- Very uncommonly reported side effects of hemin (hematin or heme arginate) have included hemolysis, anaphylaxis, and circulatory collapse [54,55].

Patients will be closely monitored for these rare side effects and for any other unanticipated effects.

- Panhematin™ is made from human blood, and theoretically may contain infectious agents, such as disease-causing viruses, the Creutzfeldt-Jakob disease (CJD) agent, and unknown infectious agents. This risk has been reduced by screening blood donors for prior exposure to certain viruses, by testing for the presence of certain current virus infections, and by inactivating certain viruses.

No pre- or postmarketing reports have associated such illnesses with administration of Panhematin™.

Risks of placebo:

There are no known risks from administration of a small volume of 0.9% saline. Risks from randomization to treatment with placebo rather than Panhematin™ are discussed above.

Risks from infusion of albumin.

- Rare allergic reactions.
- Albumin is made from human plasma, and theoretically may contain infectious agents, as described for Panhematin™.
- Albumin may expand the blood volume and could worsen the condition of patients with heart failure, significant chronic anemia or advanced kidney disease. Patients with these conditions will not be excluded from this study but will be observed closely. Hemin for prophylaxis is often reconstituted with albumin in clinical practice, so there would be little or no increased risk compared to their usual ongoing prophylactic treatment with Panhematin™.
- Some patients have complained of malaise or headache lasting for several hours after infusion of albumin with Panhematin™, but it is not clear that this is caused by albumin.

Risks of loss of confidentiality of sensitive medical information. Safeguards to reduce this risk include using unique codes rather than patient identifiers and other procedures to comply with the requirements of the Health Insurance Portability and Accountability Act (HIPAA).

13.2 Assessment of benefits

Patients may derive no immediate benefits from this study, since hemin prophylaxis is available as standard of care. However, demonstration that Panhematin™ is safe and effective as a preventive treatment in a well-designed controlled study will benefit many patients with acute porphyrias, and especially those with frequent attacks. The study may lead to greater recognition and acceptance of this treatment and eventually contribute to broadening of the FDA-approved treatment indications. The study will likely lead to more general acceptance of the use of albumin for reconstitution, which will increase safety of the product when used in clinical practice. For these reasons, overall benefits are considered to outweigh the risks.

13.3 Research consent

Written informed consent will be obtained using a research consent form approved by the IRB at UTMB. The investigators will give subjects the opportunity to ask questions before informed consent is given, and at any time thereafter. Subjects will be given a copy of the signed consent form, and any additional written instructions and information needed.

The research consent form must be signed and dated by the person who conducted the informed consent procedure. The informed consent process will also be documented in the medical record or other source document. All individuals who obtain informed consent must be approved to do so by the IRB.

If information that may be relevant to the subject's willingness to continue participating in the trial becomes available, the investigators must inform the subject in a timely manner, and if appropriate a revised written informed consent prepared and signed.

13.4 Institutional Review Boards

Prior to commencement of the trial, the protocol, any protocol amendments, the research consent form and any other written information to be provided for the subject must be submitted to and approved by the UTMB IRB. Other documents, such as investigators' CVs or Biosketches will also be submitted to the IRB, if required. Resources of the Institute for Translational Sciences (ITS) Clinical Research Center (CRC) will be used, and any additional review requirements will be met. Written final approval must be obtained from the IRB and all other institutional requirements met before starting the study.

During the trial, the Investigator must promptly report new information that affects the risk/benefit ratio to the IRB including unexpected SAEs where a causal relationship cannot be ruled out, amendments to the protocol, notification of administrative changes, any protocol deviations implemented to eliminate immediate hazards to the subjects, new information that may adversely affect the safety of the subjects or the conduct of the trial, annually written summaries of the trial status and other documents as required by the IRB.

Amendments to the protocol or consent form must not be implemented before approval by the IRB, unless urgently necessary to eliminate hazards to the subjects. The Investigator must maintain an accurate and complete record of all submissions made to the IRB.

13.5 Regulatory Authorities

An investigator IND has been submitted to the US Food and Drug Administration (FDA) for study of Panhematin™ at UTMB (IND#13,929), and the protocol, amendments, reports on SAEs, annual reports and other documents will be provided as required by the FDA. The investigators will submit to the FDA all required documents related to ongoing study progress including annual reports and study amendments.

14. Premature Termination of the Trial

The investigators may decide to stop the trial or part of the trial at any time.

If the trial is prematurely terminated or suspended, the PI will promptly inform the IRB and provide a detailed written explanation. The pertinent regulatory authorities will be informed according to national regulations. The investigators will also promptly inform the subjects and ensure appropriate therapy and follow-up.

15. Deviations from the Protocol

If protocol deviations occur, these must be documented, stating the reason and date, the action taken, and the impact for the subject and/or the trial. The implications of the deviation must be reviewed and discussed to help determine whether the deviation needs to be reported to the IRB and other regulatory bodies. The documentation must be kept in the study files.

16. Essential Documents

The following must be maintained in study records for all investigators and study personnel:

- Curriculum vitae of Investigator and sub-investigator(s) (current, dated and signed and/or supported by an official regulatory document)
- Signed and dated agreement of the final protocol
- Signed and dated agreement of any amendment(s), if applicable
- Final written approval from the IRB, with clear documentation of the documents that the IRB has reviewed, which must include the protocol, any amendments, the research consent form, and any other written information to be provided to the subjects during recruitment
- Copies of the IRB approved research consent form and any other written information or advertisements to be used for recruitment
- Signed FDA forms documenting that the site investigators are approved as an investigator in this study by the FDA.

- Any other required regulatory approvals and/or notifications.

17. Reports and Publication

The information obtained during this study is considered confidential and will be used to prepare abstracts, study reports, presentations at scientific meetings and publications. Study subjects will not be individually identified in such presentations and reports.

18. Retention of Clinical Trial Documents

All study records and source documents must be stored for at least 15 years or longer, or for the maximum time period permitted by the institution. No study-related documents should be destroyed before that time without notifying the investigators in advance.

19. Indemnity Statement

This is an investigator-initiated study originating at UTMB that will be conducted by an academic medical center with support from federal and industry grants. The participating institutions will not provide indemnification for the marketed products used in this study, and local institutional policies regarding compensation for research-related injury will apply.

20. Quality Control and Quality Assurance

Monitoring functions for this study will be provided by the PI and others on the study team, as described earlier (see Section **9**).

21. References cited

1. Anderson KE, Sassa S, Bishop DF, Desnick RJ. Disorders of heme biosynthesis: X-linked sideroblastic anemias and the porphyrias. In: Scriver CR, Beaudet AL, Sly WS, Valle D, Childs B, Vogelstein B, eds. *The Metabolic and Molecular Basis of Inherited Disease, 8th edition.* Vol II, Chapter 124. 8th Edition ed. New York: McGraw-Hill; 2001:2991-3062.
2. Sassa S. ALAD porphyria. *Semin Liver Dis.* 1998;18(1):95-101.
3. Akagi R, Kato N, Inoue R, Anderson KE, Jaffe EK, Sassa S. δ-Aminolevulinate dehydratase (ALAD) porphyria: The first case in North America with two novel ALAD mutations. *Mol Genet Metab.* 2006;87:329-336.
4. Deybach J-C. Acute intermittent porphyria (Chapter 86). In: Kadish KM, Smith K, Guilard R, eds. *Porphyrin Handbook, Part II.* Vol 14. San Diego: Academic Press; 2003:23-41.
5. Badminton MN, Elder GH. Molecular mechanisms of dominant expression in porphyria. *J Inherit Metab Dis.* 2005;28(3):277-286.
6. Meissner P, Hift RJ, Corrigall A. Variegate porphyria (Chapter 89). In: Kadish KM, Smith K, Guilard R, eds. *Porphyrin Handbook, Part II.* Vol 14. San Diego: Academic Press; 2003:93-120.
7. Whatley SD, Puy H, Morgan RR, et al. Variegate porphyria in Western Europe: identification of PPOX gene mutations in 104 families, extent of

allelic heterogeneity, and absence of correlation between phenotype and type of mutation. *Am J Hum Genet.* 1999;65(4):984-994.
8. Lamoril J, Puy H, Whatley SD, et al. Characterization of mutations in the CPO gene in British patients demonstrates absence of genotype-phenotype correlation and identifies relationship between hereditary coproporphyria and harderoporphyria. *Am J Hum Genet.* 2001;68(5):1130-1138.
9. Anderson KE, Bloomer JR, Bonkovsky HL, et al. Recommendations for the diagnosis and treatment of the acute porphyrias. *Ann Intern Med.* Mar 15 2005;142(6):439-450.
10. Harper P, Wahlin S. Treatment options in acute porphyria, porphyria cutanea tarda, and erythropoietic protoporphyria. *Curr Treat Options Gastroenterol.* Dec 2007;10(6):444-455.
11. Jeans JB, Savik K, Gross CR, et al. Mortality in patients with acute intermittent porphyria requiring hospitalization: a United States case series. *Am J Med Genet.* 1996;11:269-273.
12. Andersson C, Bjersing L, Lithner F. The epidemiology of hepatocellular carcinoma in patients with acute intermittent porphyria. *J Intern Med.* Oct 1996;240(4):195-201.
13. Andant C, Puy H, Bogard C, et al. Hepatocellular carcinoma in patients with acute hepatic porphyria: frequency of occurrence and related factors. *J Hepatol.* 2000;32(6):933-939.
14. Meissner P, Adams P, Kirsch R. Allosteric inhibition of human lymphoblast and purified porphobilinogen deaminase by protoporphyrinogen and coproporphyrinogen. A possible mechanism for the acute attack of variegate porphyria. *J Clin Invest.* Apr 1993;91(4):1436-1444.
15. Anderson KE, Freddara U, Kappas A. Induction of hepatic cytochrome P-450 by natural steroids: relationships to the induction of δ-aminolevulinate synthase and porphyrin accumulation in the avian embryo. *Arch Biochem Biophys.* 1982;217:597-608.
16. Peyer AK, Jung D, Beer M, et al. Regulation of human liver delta-aminolevulinic acid synthase by bile acids. *Hepatology.* Dec 2007;46(6):1960-1970.
17. Podvinec M, Handschin C, Looser R, Meyer UA. Identification of the xenosensors regulating human 5-aminolevulinate synthase. *Proc Natl Acad Sci U S A.* Jun 15 2004;101(24):9127-9132.
18. Meyer UA, Schuurmans MM, Lindberg RLP. Acute porphyrias: pathogenesis of neurological manifestations. *Semin Liver Dis.* 1998;18(1):43-52.
19. Soonawalla ZF, Orug T, Badminton MN, et al. Liver transplantation as a cure for acute intermittent porphyria. *Lancet.* Feb 28 2004;363(9410):705-706.
20. Seth AK, Badminton MN, Mirza D, Russell S, Elias E. Liver transplantation for porphyria: who, when, and how? *Liver Transpl.* Sep 2007;13(9):1219-1227.

21. Wahlin S, Harper P, Sardh E, Andersson C, Andersson DE, Ericzon BG. Combined liver and kidney transplantation in acute intermittent porphyria. *Transpl Int.* Jun 2010;23(6):e18-21.
22. Hift RJ, Davidson BP, van der Hooft C, Meissner DM, Meissner PN. Plasma fluorescence scanning and fecal porphyrin analysis for the diagnosis of variegate porphyria: precise determination of sensitivity and specificity with detection of protoporphyrinogen oxidase mutations as a reference standard. *Clin Chem.* May 2004;50(5):915-923.
23. Kuhnel A, Gross U, Jacob K, Doss MO. Studies on coproporphyrin isomers in urine and feces in the porphyrias. *Clin Chim Acta.* 1999;282(1-2):45-58.
24. Longas MO, Poh-Fitzpatrick MB. A tightly bound protein-porphyrin complex isolated from the plasma of a patient with variegate porphyria. *Clin Chim Acta.* 1982;118:219-228.
25. Poh-Fitzpatrick MB. A plasma porphyrin fluorescence marker for variegate porphyria. *Arch Dermatol.* 1980;116:543-547.
26. Mauzerall D, Granick S. The occurrence and determination of δ-aminolevulinic acid and porphobilinogen in urine. *J Biol Chem.* 1956;219:435-446.
27. Deacon AC, Peters TJ. Identification of acute porphyria: evaluation of a commercial screening test for urinary porphobilinogen. *Ann Clin Biochem.* 1998;35(Pt 6):726-732.
28. Poh-Fitzpatrick MB, Lamola AA. Direct spectrophotometry of diluted erythrocytes and plasma: a rapid diagnostic method in primary and secondary porphyrinemias. *J Lab Clin Med.* 1976;87:362-370.
29. Gibson PR, Grant J, Cronin V, Blake D, Ratnaike S. Effect of hepatobiliary disease, chronic hepatitis C and hepatitis B virus infections and interferon-alpha on porphyrin profiles in plasma, urine and faeces. *J Gastroenterol Hepatol.* 2000;15(2):192-201.
30. Chen C-H, Astrin KH, Lee G, Anderson KE, Desnick RJ. Acute intermittent porphyria: identification and expression of exonic mutations in the hydroxymethylbilane synthase gene: an initiation codon missense mutation in the housekeeping transcript causes "variant acute intermittent porphyria" with normal expression of the erythroid-specific enzyme. *J Clin Invest.* 1994;94:1927-1937.
31. Whatley SD, Roberts AG, Llewellyn DH, Bennett CP, Garrett C, Elder GH. Non-erythroid form of acute intermittent porphyria caused by promoter and frameshift mutations distant from the coding sequence of exon 1 of the HMBS gene. *Hum Genet.* 2000;107(3):243-248.
32. Anderson KE, Sassa S, Peterson CM, Kappas A. Increased erythrocyte uroporphyrinogen-I-synthetase, δ-aminolevulinic acid dehydratase and protoporphyrin in hemolytic anemias. *Am J Med.* 1977;63:359-364.
33. Watson CJ, Pierach CA, Bossenmaier I, Cardinal R. Postulated deficiency of hepatic heme and repair by hematin infusions in the "inducible" hepatic porphyrias. *Proc Natl Acad Sci USA.* 1977;74(5):2118-2120.

34. Watson CJ, Pierach CA, Bossenmaier I, Cardinal R. Use of hematin in the acute attack of the "inducible" hepatic porphyrias. *Adv Intern Med.* 1978;23:265-286.
35. Lamon JM, Frykholm BC, Hess RA, Tschudy DP. Hematin therapy for acute porphyria. *Medicine.* 1979;58:252-269.
36. Pierach CA, Bossenmaier I, Cardinal R, Weimer M, Watson CJ. Hematin therapy in porphyric attacks. *Klin Wochenschr.* 1980;58:829-832.
37. Kostrzewska E, Gregor A, Tarczynska-Nosal S. Heme arginate (Normosang) in the treatment of attacks of acute hepatic porphyrias. *Mater Med Pol.* Oct-Dec 1991;23(4):259-262.
38. Mustajoki P, Nordmann Y. Early administration of heme arginate for acute porphyric attacks. *Arch Int Med.* 1993;153:2004-2008.
39. Nordmann Y, Deybach JC. [Acute attacks of hepatic porphyria: specific treatment with heme arginate]. *Ann Med Interne.* 1993;144(3):165-167.
40. Kostrzewska E, Gregor A. Acute hepatic porphyrias. Detection, prophylaxis and treatment. *Mater Med Pol.* Jan-Mar 1996;28(1):5-7.
41. McColl KEL, Moore MR, Thompson GG, Goldberg A. Treatment with haematin in acute hepatic porphyria. *Quart J Med.* 1981;198:161-174.
42. Mustajoki P, Tenhunen R, Tokola O, Gothani G. Haem arginate in the treatment of acute hepatic porphyrias. *Br Med J.* 1986;293:538-539.
43. Bonkovsky HL, Healey JF, Lourie AN, Gerron GG. Intravenous heme-albumin in acute intermittent porphyria: evidence for repletion of hepatic hemoproteins and regulatory heme pools. *Am J Gastroenterol.* 1991;86:1050-1056.
44. Herrick AL, McColl KEL, Moore MR, Cook A, Goldberg A. Controlled trial of haem arginate in acute hepatic porphyria. *Lancet.* 1989;1:1295-1297.
45. Green D, Reynolds N, Klein J, Kohl H, Ts'ao CH. The inactivation of hemostatic factors by hematin. *J Lab Clin Med.* 1983;102:361-369.
46. Goetsch CA, Bissell DM. Instability of hematin used in the treatment of acute hepatic porphyria. *N Engl J Med.* 1986;315:235-238.
47. Green D, Furby FH, Berndt MC. The interaction of the factor VIII/von Willebrand factor complex with hematin. *Thromb Haemost.* 1986;56:277-282.
48. Jones RL. Hematin-derived anticoagulant. Generation in vitro and in vivo. *J Exp Med.* 1986;163:724-439.
49. Green D, Tsao CH. Hematin: effects on hemostasis. *J Lab Clin Med.* 1990;115:144-147.
50. Anderson KE, Bonkovsky HL, Bloomer JR, Shedlofsky SI. Reconstitution of hematin for intravenous infusion. *Ann Intern Med.* Apr 4 2006;144(7):537-538.
51. Siegert SW, Holt RJ. Physicochemical properties, pharmacokinetics, and pharmacodynamics of intravenous hematin: a literature review. *Adv Ther.* Sep 2008;25(9):842-857.
52. Mustajoki P, Tenhunen R, Pierach C, Volin L. Heme in the treatment of porphyrias and hematological disorders. *Semin Hematol.* 1989;26:1-9.

53. European Porphyria Initiative. Treatment of the acute attack. *Accessed at http://www.porphyria-europe.com/02-for-healthcare/treatment.asp on May 18, 2014.*
54. Khanderia U. Circulatory collapse associated with hemin therapy for acute intermittent porphyria. *Clin Pharmacy.* 1986;5:690-692.
55. Daimon M, Susa S, Igarashi M, Kato T, Kameda W. Administration of heme arginate, but not hematin, caused anaphylactic shock. *Am J Med.* 2001;110(3):240.
56. Jeelani Dhar G, Bossenmaier I, Cardinal R, Petryka ZJ, Watson CJ. Transitory renal failure following rapid administration of a relatively large amount of hematin in a patient with acute intermittent porphyria in clinical remission. *Acta Med Scand.* 1978;203:437-443.
57. Anderson KE, Alvares AP, Sassa S, Kappas A. Studies in porphyria. V. Drug oxidation rates in hereditary hepatic porphyria. *Clin Pharmacol Ther.* 1976;19:47-54.
58. Herrick A, McColl KEL, McLellan A, Moore MR, Brodie MJ, Goldberg A. Effect of haem arginate therapy on porphyrin metabolism and mixed function oxygenase activity in acute hepatic porphyria. *Lancet.* 1987;2:1178-1179.
59. Mustajoki P, Himberg JJ, Tokola O, Tenhunen R. Rapid normalization of antipyrine oxidation by heme in variegate porphyria. *Clin Pharmacol Ther.* 1992;51:320-324.
60. Mustajoki P, Mustajoki S, Rautio A, Arvela P, Pelkonen O. Heme arginate infusion and drug metabolism in variegate porphyria. *Netherlands J Med.* 1993;42:A42.
61. Bissell DM. Treatment of acute hepatic porphyria with hematin. *J Hepatol.* 1988;6:1-7.
62. Tenhunen R, Mustajoki P. Acute porphyria: treatment with heme. *Semin Liver Dis.* 1998;18(1):53-55.
63. Anderson KE, Egger NG, Goeger DE. Heme arginate for prevention of acute porphyric attacks (abstract). *Acta Haematologica.* 1997;98, Supplement 1:120.
64. Anderson KE, Collins S. Open-label study of hemin for acute porphyria: clinical practice implications. *Am J Med.* Sep 2006;119(9):801 e819-824.
65. Sood G, Anderson KE. Porphyrias. In: Crowther MA, Ginsberg J, Schunemann H, Meyer RM, Lottenberg R, eds. *Evidence-Based Hematology*. Hoboken: Wiley; 2008.
66. Lamon JM, Frykholm BC, Bennett M, Tschudy DP. Prevention of acute porphyric attacks by intravenous haematin. *Lancet.* 1978;2:492-494.
67. Anderson KE, Spitz IM, Bardin CW, Kappas A. A GnRH analogue prevents cyclical attacks of porphyria. *Arch Int Med.* 1990;150:1469-1474.
68. Bonkovsky HL, Barnard GF. The Porphyrias. *Curr Treat Options Gastroenterol.* 2000;3(6):487-500.

69. Anderson KE. The porphyrias (Chapter 229). In: Goldman L, Ausiello D, eds. *Cecil Medicine*. 23rd ed. Philadelphia: Saunders Elsevier; 2008:1585-1593.
70. Anderson KE. The porphyrias (Chapter 217). In: Goldman L, Schafer AI, eds. *Goldman's Cecil Medicine*. 24th ed. Philadelphia: Elsevier Saunders; 2012:1363-1371.
71. Badminton MN, Elder GH. Management of acute and cutaneous porphyrias. *Int J Clin Pract.* May 2002;56(4):272-278.
72. Tschudy DP, Valsamis M, Magnussen CR. Acute intermittent porphyria: clinical and selected research aspects. *Ann Intern Med.* 1975; 83:851-864.
73. Pierach CA. Hematin therapy for the porphyric attack. *Semin Liver Dis.* May 1982;2(2):125-131.
74. Morris DL, Dudley MD, Pearson RD. Coagulopathy associated with hematin treatment for acute intermittent porphyria. *Ann Intern Med.* 1981;95:700-701.