Rare Diseases Clinical Research Network

Porphyrias Consortium

Clinical Study Protocol

A double-blind, randomized, placebo-controlled, parallel group trial on the efficacy and safety of Panhematin™ in the treatment of acute attacks of porphyria

Trial phase: II

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Protocol Synopsis

Interventional Synopsis

Protocol Number:	7203
Protocol Title:	A double-blind, randomized, placebo-controlled, parallel group trial on the efficacy and safety of Panhematin™ in the treatment of acute attacks of porphyria
Study Chair:	Karl E. Anderson, MD
Statistician:	Kristofer Jennings, PhD
Consortium:	Porphyrias Consortium
Participating Sites:	The University of Texas Medical Branch, Galveston, TX
Activation Date:	
Current Status:	Awaiting approval
Sample Size:	Patients to be studied and randomized: 30-40 Patients to be evaluated and screened: 60-80 30-40 patients with well-documented acute porphyria (AIP, HCP or VP)
Target Enrollment Period:	11/1/2013-10/31/2018
Study Design:	Interventional multi-centre, double-blind, randomized, placebo-controlled, parallel group trial
Primary Study Objective:	*Primary Objectives*: • To evaluate the clinical efficacy of Panhematin™ compared to glucose treatment started early for acute attacks of porphyria • To evaluate the safety of Panhematin™, compared to glucose started early for acute attacks of porphyria
Secondary Study Objective(s):	*Secondary Objectives*: • To evaluate the biochemical effects of Panhematin™ in patients treated early for attacks of acute porphyria *Exploratory Objectives*: • To evaluate effects of clinical features, such as sex, age and the factors that precipitate attacks of porphyria on response to Panhematin™ • To evaluate effects of genetic features, including the nature or the PBGD, CPO or PPO mutation on treatment response to Panhematin™ • To evaluate the use of Panhematin™ reconstituted with 25% human albumin in patients with acute attacks of porphyria
Study Population and Main Eligibility/ Exclusion Criteria:	**Inclusion Criteria** 1. Male or female aged ≥18 years 2. Willing to provide written informed consent 3. Acute symptoms (14 days duration or less to time of enrollment) such as abdominal, back and/or limb pain, diagnosed by the investigator as caused by porphyria after initial evaluation has excluded other causes.

	4. Diagnosis of acute porphyria documented by a substantial increase in urinary or serum PBG. 5. Type of acute porphyria confirmed by additional testing (in addition to increased PBG), which may be completed before or after treatment begins using pretreatment samples: a. For AIP: Normal or only slight increases in plasma and fecal porphyrins. Most (~90%) will have deficient activity of erythrocyte PBGD, and almost all (>95%) will have a demonstrable disease-causing PBGD mutation. b. For HCP: Substantial increases in fecal porphyrins (almost entirely coproporphyrin III). In the absence of skin photosensitivity, most will have normal or only slight increases in plasma porphyrins. Almost all (>95%) will have a demonstrable disease-causing CPO mutation. c. For VP: Substantial increases in fecal porphyrins (mostly coproporphyrin III and protoporphyrin), increased plasma total porphyrins and a fluorescence emission maximum of diluted plasma at neutral pH near 626 nm (18, 19, 22). Almost all (~95%) will have a demonstrable disease-causing PPO mutation. Start of treatment will be as soon as possible after enrollment, and may be before Inclusion Criterion 5 is fully met. This approach avoids delay in instituting treatment after a substantial increase in PBG is documented, and is consistent with standard of care. All inclusion criteria must be met for inclusion of a patient in the efficacy analysis. **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. 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 4. Previous randomization in this trial
Treatment	
Agent-	Panhematin™

Dosage, schedule, route of administration-

Panhematin™ dose of 4 mg/kg body weight by intravenous infusion. Representative calculated dosages are shown in the

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 1 vial of Panhematin™ (313 mg hemin) should be used per single dose.

Table, below.

Safety Issues-

Risks related to the randomized study design and other study procedures:

- Progression of symptoms due to initial randomization to placebo and treatment at least initially with glucose rather than Panhematin™. (Treatment is started early, glucose is sometimes sufficient treatment; the study design provides rescue Panhematin™)
- Blood drawn could contribute to iron deficiency. (The volume of blood drawn will total <160 mL if the patient is treated for 4 days, or <260 mL if treatment is required for 10 days; iron status will be assessed and treatment given if needed.)
- Risks of Panhematin™ and other human hemin preparations
  - Reversible renal shutdown with excessive dosage. (Not observed with usual dosages.)
  - Phlebitis at the site of intravenous infusion is common can lead to loss of venous access. (Risk reduced with use of albumin.)
  - Other risks associated with intravenous infusions, such as pain, infiltration and infection.
  - Transient anticoagulant effect. (Risk reduced with use of albumin.)
  - Fever, aching, malaise, headache, migraine
  - Rare hemolysis, anaphylaxis, and circulatory collapse.
  - Infectious agents, such as disease-causing viruses, the Creutzfeldt-Jakob disease (CJD) agent, and unknown infectious agents, since Panhematin™ is made from human blood. (Never reported, and manufacturing process should eliminate such agents.)
- Risks of glucose infusions:
  - Elevated blood glucose

	○ Hyponatremia, seizures and fluid overload • Risks from infusion of albumin: ○ Rare allergic reactions ○ Blood product, may contain infectious agents ○ Blood volume expansion could worsen heart failure, significant chronic anemia or advanced kidney disease. ○ Malaise or headache • Loss of confidentiality
Primary Outcome Measures:	The primary efficacy endpoint will be the change from baseline in pain at 12 hours as assessed by a numeric rating scale (NRS) on a 0-10 scale. Pain will be assessed at other intervals to include 24, 48, 72 and 96 hours after start of treatment are: Primary safety endpoints will include: • Occurrence of phlebitis • Occurrence of coagulopathy ○ Coagulation panel (platelets, prothrombin time and partial thromboplastin time)
Secondary Outcome Measures:	Secondary efficacy endpoints will include: • Pain as assessed by ○ Change from baseline in the NRS score for pain at later time intervals, namely 24, 48, 72 and 96 hours after start of treatment ○ Use of morphine or other opioid in each 24 hour period ○ Time to last administration of opioid • Other symptoms ○ NRS for nausea, vomiting and other symptoms will be recorded on a 0-to-10 scale over each 24 hour period ○ Use of medications for nausea or vomiting in each 24 hour period • Rescue treatment with open label Panhematin™ ○ Rescue treatment given ○ Time to rescue treatment • Biochemical changes (analyzed at the Porphyria Laboratory of the University of Texas Medical Branch) will include: • Serum (or plasma) ALA, PBG and total porphyrins • Urinary ALA, PBG and total porphyrins, including fractionation of individual porphyrins by HPLC • Fecal porphyrins, including fractionation of individual porphyrins by HPLC (if elevated initially) Secondary safety endpoints will include:

	• Symptoms
	• Findings on physical examination including vital signs
	• Routine clinical testing daily for days 1-4 and at day 7-10 to include
	  ○ Complete blood counts
	  ○ Metabolic and liver panels
	<u>Exploratory endpoints</u> are:
	• Length of hospital stay
	• Time to occurrence of next attack

Statistical Considerations (sample size and analysis plan):	The main clinical efficacy variable, change in pain from baseline after commencement of treatment will be analyzed by analysis of covariance with treatment as a factor and baseline value as a covariate. Length of hospitalization and total opioids received will be analyzed by exact two sample Wilcoxon tests. Rescue treatment with hemin within each treatment group will be analyzed by Fisher's exact test. Time to use of human hemin, the time to last administration of opioids and the time to new attack will be analyzed by exact log rank test. Changes in biochemical parameters ($AUC_{PBG\ reduction}$, etc.) will be an exact two sample Wilcoxon test and associated Hodges-Lehmann confidence interval for difference in medians. Changes from baseline to end of treatment will be analyzed by analyses of covariance with baseline value as a covariate. Pain intensity difference based on NRS for current pain will be analyzed by repeated measures analysis of variance. Analyses of signs and symptoms will be descriptive. Safety variables such as physical and laboratory findings will be analyzed descriptively, using shift tables – screening versus end of treatment or by repeated measures analysis of variance. Adverse events will be coded and analyzed descriptively. It is anticipated that there will be large differences in pain and other efficacy parameters in this study, which increases the power to detect a treatment-related difference. An interim analysis is planned after the first 15-20 subjects to determine a reasonable estimate of the variance.
Sponsors (federal, state, foundation and industry support):	U.S Food and Drug Administration Office of Orphan Product Development National institutes of Health (NIH)

Abbreviations:

AE, adverse event ALA, 5-aminolevulinic acidADP 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 CPO, coproporphyrinogen oxidase CRF, case report form CYPs, cytochrome P450 enzymes DMCC, Data Management 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 IRB, Institutional Review Board NRS, numeric rating score, PBG, porphobilinogen PBGD, porphobilinogen deaminase PPO, protoporphyrinogen oxidase RDCRC, Rare Disease Clinical Research Consortium 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.

Introduction

The trial will be conducted in compliance with the protocol, Good Clinical Practice (GCP) and applicable regulatory requirements. This is a single center study at the University of Texas Medical Branch (UTMB) in Galveston, Texas. It is also a study of the Porphyrias Consortium , which is funded by a grant from the National Institutes of Health (NIH), and data will be uploaded to the Porphyrias Consortium database.

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 most commonly in adult 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 250 mutations described in

different families. Most known mutations cause the enzyme to be ~50% of normal in all tissues from birth, as 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 (CPO) and protoporphyrinogen oxidase (PPO), 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). 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 PPO 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).

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, 7).

Many patients do well after one or a few attacks. However, some develop frequently recurring attacks and more lasting symptoms, including depression and pain (8). Acute porphyria patients, and especially those with high excretion of urinary ALA and PBG are at increased risk for developing hepatocellular carcinoma, especially after 40-50 years of age (9, 10).

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. The identified mutation can then be sought in relatives to detect those at risk for the disease (11).

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. 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.

The inherited partial deficiencies of PBGD, CPO or PPO 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 (12). Hepatic induction of ALAS1 and CYPs is controlled by similar nuclear receptor-mediated mechanisms (1, 13, 14).

The pathogenesis of the neurological symptoms and signs of the acute porphyrias is poorly understood (1, 4, 15). A neurotoxic effect of ALA or one or more other intermediates or biproducts 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) (7). 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) (11). 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 sometimes needed for diagnosis of HCP and VP, and to differentiate these disorders from AIP (6, 11, 16, 17). 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) (18, 19). 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 in VP (6, 11, 16, 17).

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 (11). 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 acute porphyria – 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. Further testing on the same spot urine sample, and on plasma, feces and erythrocytes (obtained prior to initiating treatment) differentiates AIP, HCP and VP (11).

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 (11).

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 (20). For rapid detection of increased PBG levels in urine, a commercially available kit (Thermo Scientific, 1-800-640-0640), which detects PBG levels at concentrations greater than 6 mg/L and has a color chart for semi-quantitative estimation of higher levels, is recommended (11, 21).

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 immediately 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. All major medical facilities should provide for in-house determination of urinary PBG levels within hours of obtaining the sample, preferably by using the Trace PBG Kit, because life-threatening progression of the disease may occur with a delay of several days in testing. The single-void urine sample is tested, it should be refrigerated or frozen without additives and shielded from light for subsequent quantitative ALA, PBG, and total porphyrin determinations (which can detect 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 (11).

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 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 (11, 19, 22).

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 coproporphyrin excretion (11, 23).

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) (11). 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 (24, 25); 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 (26). 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 (11).

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 (11).

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 human 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 (as human hemin) is regarded as the most effective treatment for acute attacks of porphyria (1, 11). 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)[1] 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

[1] 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.

evident benefit in numerous individual cases and case series, rather than randomized, controlled studies (27-37). One small blinded study of heme arginate, in which treatment was delayed for 2 days, showed biochemical but not clinical efficacy (38). 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 (32).

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 (39-43). 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 (7, 37, 44, 45). This helps preserve peripheral venous access in patients who require repeated courses of hematin. Heme arginate is more stable in solution (46), but is also often reconstituted with albumin (47). Less common reported side effects of hemin have included fever, aching, malaise, hemolysis, anaphylaxis, and circulatory collapse (48, 49). Excessive doses of hematin can cause acute renal tubular damage associated with excretion of heme in urine (50). Clearance of drugs that are metabolized by hepatic CYPs is reduced in some patients with acute porphyrias (51) and rapidly restored after intravenous hemin (52-54).

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 (11). Moreover, clinical response to hemin may be delayed or incomplete when there is advanced neurologic damage, as may occur when treatment is started late (32). Subacute or chronic symptoms, which may reflect persistent neurological damage after repeated or prolonged attacks, are unlikely to respond (29, 55). 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) (11, 32, 56), 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 (57, 58).

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), no statistically significant reduction in mortality was evident with the introduction of treatment with human hemin in 1971 (8). 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 (59).

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 313 mg hemin, 215 mg sodium carbonate and 300 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 43 mL provides the equivalent of approximately 301 mg hematin (7 mg/mL). When reconstituted with 132 mL of 25% human serum albumin instead of sterile water, which is an off-label recommendation, the hemin concentration is 2.4 mg/mL. (44)

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 (37, 44, 45). Phlebitis and coagulapathy 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 (59). 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.”

Because acute porphyrias are rare, there has been concern that an adequately controlled study of treatment of the acute attack was not feasible, and might be unethical. But such a study of recombinant human PBGD for treatment of acute attacks of AIP was recently carried out at multiple centers in Europe and the U.S. There were no ethical concerns raised, and the study was encouraged by leading porphyria experts and patient support groups in many countries. This trial aimed to enroll 36 patients, was able to enroll 26, and demonstrated that intravenous infusion of human genetically recombinant PBGD (Porphozym™, Zymenex) was in fact not effective clinically. Although the scientific rationale for this drug was not considered strong (the enzyme deficiency within hepatocytes was not corrected), the study was well designed and carried out according to accepted industry standards with the aim of obtaining regulatory approval. This study demonstrated that a blinded, controlled study of a treatment for acute attacks is ethical and feasible if multiple centers are involved. Moreover, such a study can provide convincing evidence as to efficacy of a treatment for the acute attack.

Therefore, an adequately powered clinical trial to provide definitive evidence for efficacy of hemin is now considered feasible. We estimate conservatively that enrollment of 30-40 patients, as was intended in the Porphozym™ trial, would probably be required to achieve statistical significance. The most desirable patients for such a study are those who can be treated soon after onset of an attack, as they can be expected to respond quickly to treatment or, if there was no improvement, be provided rescue treatment (with open-label human hemin) after 24-48 hours, or earlier if needed.

This study 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) Product labeling recommends treatment with Panhematin™ only after a trial of glucose for several days is not successful. Current expert opinion is that hemin treatment should be started promptly, without an initial trial of glucose (11). This study will likely provide evidence to support initial treatment with Panhematin™. 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 in men and women with attacks of AIP, HCP or VP either related or 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 (44) 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 narrower dose range of 3-4 mg/kg rather than the 1-4 mg/kg daily recommended in product labeling.

2. Objectives

This blinded, randomized placebo-controlled trial in 30-40 patients with acute attacks of porphyria will compare early treatment with Panhematin™ and glucose with placebo and glucose, with Panhematin™ rescue available for both groups.

Primary Objectives:

- To evaluate the clinical efficacy of Panhematin™ compared to glucose treatment started early for acute attacks of porphyria
- To evaluate the safety of Panhematin™, compared to glucose started early for acute attacks of porphyria

Secondary Objectives:

- To evaluate the biochemical effects of Panhematin™ in patients treated early for attacks of acute porphyria

Exploratory Objectives:

- To evaluate effects of clinical features, such as sex, age and the factors that precipitate attacks of porphyria on response to Panhematin™
- To evaluate effects of genetic features, including the nature or the PBGD, CPO or PPO mutation on treatment response to Panhematin™
- To evaluate the use of Panhematin™ reconstituted with 25% human albumin in patients with acute attacks of porphyria

2.1 Endpoints

The endpoints include primary endpoints that relate to clinical manifestations of the acute attack, which are the main focus of this study. Pain is the primary symptom of the acute attack, and will be recorded every 4 hours and before scheduled or prn opioid dosing. Pain will be assessed in terms of changes from baseline in pain score

and narcotic analgesic requirements. Secondary and exploratory efficacy endpoints will include biochemical changes, genetic features, precipitating factors and other clinical findings, which may correlate with clinical response, and safety endpoints.

The primary efficacy endpoint will be the change from baseline in pain at 12 hours as assessed by a numeric rating scale (NRS) on a 0-10 scale.

Secondary efficacy endpoints will include:

- Pain as assessed by
    - Change from baseline in the NRS score for pain at later time intervals, namely 24, 48, 72 and 96 hours after start of treatment
    - Use of morphine or other opioid in each 24 hour period
    - Time to last administration of opioid
- Other symptoms
    - NRS for nausea, vomiting and other symptoms will be recorded on a 0-to-10 scale over each 24 hour period
    - Use of medications for nausea or vomiting in each 24 hour period
- Rescue treatment with open label Panhematin™
    - Rescue treatment given
    - Time to rescue treatment
- Biochemical changes (analyzed at the Porphyria Laboratory of the University of Texas Medical Branch) will include:
    - Serum (or plasma) ALA, PBG and total porphyrins
    - Urinary ALA, PBG and total porphyrins, including fractionation of individual porphyrins by HPLC
    - Fecal porphyrins, including fractionation of individual porphyrins by HPLC (if elevated initially)

Exploratory endpoints are:

- Length of hospital stay
- Time to occurrence of next attack

Primary safety endpoints will include:

- Occurrence of phlebitis
- Occurrence of coagulopathy
    - Coagulation panel (platelets, prothrombin time and partial thromboplastin time)

Secondary safety endpoints will include:

- Symptoms
- Findings on physical examination including vital signs

- Routine clinical testing daily for days 1-4 and at day 7-10 to include
    - Complete blood counts
    - Metabolic and liver panels
- Unexpected adverse events

Patient characteristics, such as PBGD, CPO or PPO mutations and factors contributing to the attack being treated, will be collected and correlated with treatment endpoints. Patients will be genotyped at the Mt. Sinai Porphyria Center in New York City.

3. Trial Design

3.1 Type of Trial

The trial is a multi-centre, double-blind, randomized, placebo-controlled, parallel group trial investigating the efficacy and safety of Panhematin™ in the treatment of acute attacks in at least 30 patients with well-documented acute porphyria (AIP, HCP or VP). An interim analysis may be carried out after enrollment of 15-20 patients for possible adjustment of the sample size. The trial consists of the following:

- A screening period lasting up to 4 hours
- A treatment period lasting for 4 days, beginning with the first treatment dose, which may be extended if clinically indicated
- A post-treatment, in-hospital observation period for patients who remain in the hospital after the treatment period and lasting until discharge from the hospital. If hospitalization is prolonged, the observation period may overlap with the follow-up visits.
- Follow-up visits or interviews by telephone
    - 7-10 days after treatment to assess clinical status
    - at 6 months and 1 year to assess time to next attack, if any

The study will consist of two parts. Part One will end after the 7-10-day follow-up visit, as this will complete the collection of data pertinent to efficacy and safety for treatment of the acute attack. Part Two will end after the last visit at 1 year, and will determine if the treatment had any effect on recurrences.

3.2 Rationale for the trial design

The symptoms of acute porphyria, including pain, are highly variable and subjective. Physical signs other than pulse and blood pressure, are also at least somewhat subjective. Therefore, a double-blind study design is important for evaluate clinical efficacy of a treatment for acute porphyria. A parallel control group is important because the acute porphyrias are conditions with intermittent symptoms, and attacks can resolve without treatment with glucose or hemin. In this parallel study, one group will be treated with glucose, 300g daily, and Panhematin™ and the other with glucose and placebo. Thus both groups will receive what can presently be considered standard treatment (i.e. glucose and/or human hemin). Pretreatment with glucose prior to Panhematin™ is consistent with product labeling. Rescue treatment with open-label Panhematin™, which can be started based on criteria

described in this protocol or at the decision of the treating physician or the patient, is also consistent with current product labeling for Panhematin™. The study is expected to show that initial treatment with hemin and glucose is more effective than glucose alone, and if so will increase the quality of the evidence for efficacy, perhaps from 1C to 1B.

Patients with frequently recurring attacks are most likely to be available for this study, and for many reasons are also most suitable. 1) These patients can be evaluated and enrolled in advance and optimal biochemical, genetic and clinical characterization assured. 2) Their recurrent attacks are usually predictable, and patients can be brought to a study site in advance of an attack, so that early treatment can be assured. 3) These patients are among the most severely affected by their disease, and it is reasonable to extrapolate evidence of efficacy to patients with less frequent attacks. 4) Most will have had prior experience with hemin treatment and will be favorable to participation. Other patients may participate if the diagnosis of acute porphyria is well documented and they present within 14 days of onset of symptoms. All documented patients registered or followed at participating centers or contacted through the American Porphyria Foundation will be contacted in advance and informed of the study, and will be asked to come to the center hospital as soon as their symptoms suggest that an attack is beginning.

3.3 Treatment of Subjects and Rationale for Treatment

Panhematin™ 4 mg/kg will be reconstituted with 25% human albumin (44) and infused over a 1 hour period once daily for 4 days. Product labeling suggests that Panhematin™, after reconstitution with sterile water, be infused within 15 minutes. An infusion time of 1 hour is based on guidelines for infusion of the amount of human albumin used for reconstitution, which is based on achieving a 1:1 molecular ratio for hemin and albumin (37, 44). The longer infusion time of 60-90 minutes is acceptable given the enhanced stability of hemin in the presence of albumin. Experience indicates that a single infusion site can be used 4 or more times if Panhematin™ is reconstituted with albumin.

Glucose will be infused intravenously as a 10% solution to total 300 grams of glucose daily (3 liters daily). Although Panhematin™ product labeling suggests an initial trial of glucose at a higher dose of 400 grams daily, a dose of 300 grams daily as 10% glucose is generally accepted (11, 60, 61) and a higher dose given as 10% glucose would 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 (62).

Symptomatic treatment will be provided, including opioids, as needed to control pain and other symptoms of the attack. Amounts of individual and daily doses of all drugs administered during the attack will be recorded.

4. Trial Population

4.1 Number of patient and sites

Patients to be studied and randomized: 30-40

Patients to be evaluated and screened 60-80

Approximately 2 patients will need to be evaluated for every patient found suitable for study and randomization. Patients who do not complete the Part 1 of the study will need to be replaced.

Study sites: One site at the University of Texas Medical Branch (UTMB) is participating in this study. This study is a project of the Porphyrias Consortium. All current sites participating in the PC (8 additional sites to UTMB) will refer suitable patients to UTMB for this study. Additionally, the American Porphyria Foundation (APF) is an active patient support group and is a supporting partner in the Porphyrias Consortium that will also refer potential participants for this study. The Porphyrias Consortium is one of many NIH-funded consortia that comprise the Rare Disease Clinical Research Network (RDCRN). Funds for the Porphyrias Consortium are provided by the NIH Office of Rare Diseases as well as the National Institute for Diabetes, Digestive and Kidney Diseases (NIDDK).

4.2 Recruitment of Subjects

Investigators will contact patients known to them who are likely to be eligible for the trial by phone or letter. Additional patients will be referred by the American Porphyria Foundation (APF), which has been an important referral source for previous porphyria studies, and by the Porphyrias Consortium. Those patients not previously known to the investigators or the APF 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 as well as other centers. A Screening Log will be kept of all subjects who are contacted by phone or letter. Subjects will be enrolled in advance of an attack, especially if they have frequent, predictable attacks, or at the time of presentation to the study site.

4.3 Inclusion Criteria

1. Male or female aged ≥18 years
2. Willing to provide written informed consent
3. Acute symptoms (14 days duration or less to time of enrollment) such as abdominal, back and/or limb pain, diagnosed by the investigator as caused by porphyria after initial evaluation has excluded other causes.
4. Diagnosis of acute porphyria documented by a substantial increase in urinary or serum PBG.
5. Type of acute porphyria confirmed by additional testing (in addition to increased PBG), which may be completed before or after treatment begins using pretreatment samples:
    a. For AIP: Normal or only slight increases in plasma and fecal porphyrins. Most (~90%) will have deficient activity of erythrocyte PBGD, and almost all (>95%) will have a demonstrable disease-causing PBGD mutation.
    b. For HCP: Substantial increases in fecal porphyrins (almost entirely coproporphyrin III). In the absence of skin photosensitivity, most will

have normal or only slight increases in plasma porphyrins. Almost all (>95%) will have a demonstrable disease-causing CPO mutation.

c. For VP: Substantial increases in fecal porphyrins (mostly coproporphyrin III and protoporphyrin), increased plasma total porphyrins and a fluorescence emission maximum of diluted plasma at neutral pH near 626 nm (18, 19, 22). Almost all (~95%) will have a demonstrable disease-causing PPO mutation.

Start of treatment will be as soon as possible after enrollment, and may be before Inclusion Criterion 5 is fully met. This approach avoids delay in instituting treatment after a substantial increase in PBG is documented, and is consistent with standard of care. All inclusion criteria must be met for inclusion of a patient in the efficacy analysis.

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

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 rescue treatment with open label Panhematin™ are not withdrawn from the study. Rescue treatment is completed as part of the study.

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

An intent to treat approach will be used. All data acquired prior to termination for the reasons listed below will be included in the primary analysis unless patient withdraws consent. Every effort will be made to conduct a final study visit with the participant and participants will be followed clinically until, if applicable, all adverse events resolve.

- Withdrawal of consent
- Withdrawal by the participant
- Withdrawal by the investigator
- Intercurrent illness or event that precludes further visits to the study site or ability to evaluate disease (e.g.-mental status change, large pleural effusion).

4.6 Subject Replacement

Subjects who are enrolled initially and then found not to meet inclusion criterion will be replaced in order to provide 30-40 patients eligible for randomization.

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 Pharmaceuticals, and reconstituted with 132 mL of 25% human serum albumin (37, 44).

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 one vial 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. An existing central venous port may be used. The venous access may be used for other medications and fluids, but only 0.9% sodium chloride should be infused simultaneously with reconstituted Panhematin™, as described below.

Procedure for reconstitution of Panhematin™ (44).

The following materials are needed:

1. One 313-mg vial of Panhematin™.
2. One 150-mL sterile empty glass bottle for infusion
3. Three 50-mL vials of 25% albumin (only 132 mL will be used)
4. One 5-micron filter needle
5. One vent needle.

To prepare Panhematin™ for infusion:

1. Reconstitute the 313-mg vial of Panhematin™ 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.

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 1 vial of Panhematin™ (313 mg hemin) should be used per single dose.

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. After reconstitution, the hemin concentration is 2.4 mg/mL. The volume required to deliver the desired dose (usually 3 to 4 mg/kg of body weight) should be calculated according to representative volumes for corresponding body weights (see **Table**).
4. Withdraw the required dose into a syringe by using a 5-micron filter needle.
5. Inject the dose into a 150-mL empty sterile bottle.
6. Label the bottle. The label will be provided for the study.
7. Place the bottle in an amber bag to protect the mixture from light. Also place a vented spike adapter in the bag. Affix a label provided. [Customarily, a yellow Medication Administrations Recording blood products label (for both albumin and Panhematin™) would be attached to the amber bag. This should be done only if the treatment is unblinded, i.e. for open label Panhematin™ rescue treatment.] Then place the amber bag inside a STAT-labeled bag.
8. Hand-deliver the bag to the clinical unit immediately. The infusion should be started within 1 hour or less of preparation. 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 also 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 (37, 44). 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. Before and after the heme-albumin infusion, the infusion site can be used for infusion of 10% glucose, other fluids and electrolytes and intravenous medications needed for treating symptoms.

The study drug is administered once daily for 4 days at a dose of 4 mg/kg body weight. The infusion is given over one hour (44), 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 container that is not visible to study personnel or the patient. One research nurse will have responsibility for the infusion and 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 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. At completion of the one-hour infusion, the unblinded nurse will remove the IV set and other materials to another location for disposal. To further reinforce blinding, 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. If a patient does not wish to be blinded (e.g. due to claustrophobia), this will be recorded, but he or she will not be excluded. Visitors will not be allowed in the room during a blinded infusion. 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 started as soon as possible after enrollment and eligibility are determined. Panhematin™ is FDA approved at the dosage level used in this study. It is not necessary to time subsequent doses at exactly 24 hour intervals. The second dose should be administered on the second study day and at least 12 hours after the first dose. For example if the first dose is given in the afternoon or evening of the first day, the second dose can be given during the morning of the second day. Panhematin™ or placebo administration on the third and fourth study days should be at about the same time as on the second day, but may be given at a later time if necessary. Timing of all dose administrations will be recorded on the CRFs.

5.4 Glucose administration

All patients who are randomized to receive either Panhematin™ or placebo will also be treated intravenously with glucose 300 grams daily, which is considered the standard dosage for glucose loading (7, 11, 60, 61, 63). Although larger amounts are sometimes recommended, this results in larger volumes of intravenous fluid and increased risk for fluid overload and hyponatremia.

Glucose (10% solution) will be supplied in the usual manner by the hospital and 3 liters (300 grams glucose) will be infused daily during the 4-day treatment period. Electrolytes may be added to correct imbalances or for other clinical indications. The same intravenous access site may be used for glucose and for Panhematin™ or placebo, in which case the glucose infusion will be stopped and replaced with a 0.9% saline infusion during the time required for infusion of Panhematin™ or placebo.

The amount of 10% glucose can be reduced if there are clinical findings such as hyponatremia or if needed to prevent severe hyperglycemia in diabetics, based on clinical judgment. The amounts of glucose administered daily will be recorded on the CRFs.

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 daily, 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. Pharmacy personnel (1-2 individuals) and one study nurse who will administer the drug will not be blinded. The Pharmacy personnel will deliver the study drug or placebo in a non-transparent container to the unit and the unblinded study nurse who will administer the drug. This nurse will be responsible for the infusion and for maintaining blinding at the bedside, but will not otherwise be involved in the patient's care or in collecting data. The drug or saline placebo will be administered through an already established intravenous access, with drapes to prevent the patient and blinded staff from viewing the drug administration. Also, the patient will be blind-folded from before the study drug is delivered to the unit until after all administration material is removed from the unit. 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 unblinding of additional personnel is required (e.g. for venous access problems), this will be recorded in the Case Report Forms. If rescue treatment with Panhematin™ is required less than 12 hours after a blinded infusion, the first rescue infusion will also be blinded. This will allow for the first rescue infusion to be saline, if the previous treatment infusion within the previous 12 hours was active drug, without compromising blinding of the assigned treatment. Any difficulties that might compromise blinding will be recorded.

5.6 Rescue treatment

Rescue treatment for this study will be Panhematin™ 4 mg/kg body weight daily for 4 days, or longer if clinically indicated. Panhematin™ will be reconstituted with human albumin, as described above, since this is now considered optimal (11). If rescue treatment is given, blinding will be maintained, unless doing so would compromise patient safety.

If rescue treatment is necessary, it is not necessary to wait until the next day after the last dose of Panhematin™ or placebo. 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 (11). When two doses are given, an interval of at least 6-8 hours is customary. Provision is made in this study to avoid giving two doses of Panhematin™ within a period of 12 hours.

If rescue treatment with Panhematin™ is required less than 12 hours after a blinded infusion, the first rescue infusion will also be blinded, and this will be so recorded on the CRFs. This will avoid unblinding of the treatment given before rescue treatment. Subsequent rescue doses will not need to be blinded, since they will be administered at least 12 hours apart.

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 in this study, since they will be needed in varying amounts by all study patients.

5.7 Randomization

Randomized treatment in this is double-blind, symmetrically randomized, parallel group trial study will be assigned by the Data Management and Coordinating Center (DMCC) of the RDCRN at the University of South Florida in Tampa. The randomization numbers will be a different series from the study enrollment numbers. Randomization will be done during the screening visit after the inclusion and exclusion criteria are satisfied. Subjects will be randomized through an online data management system at the DMCC, and a subject is considered on therapy as soon as randomized. Randomization can be accomplished automatically anytime by the research pharmacistvia the RDCRN website. .

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. This is most likely to be indicated if there is an allergic or other adverse reaction that might influence starting rescue treatment unless it is known whether the initial treatment was hemin or placebo. 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. The Data Management and Coordinating Center (DMCC) may unblind the data at any time during the study without unblinding others involved in the study.

The site may decide to break the code at any time if this is necessary for benefit of the subject or to reduce undue risk. Reasons for breaking the code are recorded in detail on the CRF. Unblinded data will be identified, and may be excluded from the data analysis.

6. Methods and Assessments

This study will be carried out in an inpatient setting and will consist of a brief period of screening and enrollment (designated Visit 1a), a treatment period of 4 days or longer (Visits 1b, etc), an optional period of observation until discharge (Visit 2), a short term observation period of 7-10 days after discharge (Visit 3), and long term follow-up at 6 months (Visit 4) and one year (Visit 5).

6.1 Visit Procedures

Visits are designated as shown below to facilitate scheduling of procedures and recording of study-related data.

Visit 1	a	Screening, 0-4 hours
	b	Treatment Day 1
	c	Treatment Day 2
	d	Treatment Day 3

	e	Treatment Day 4
	f, etc.	Additional treatment days, beyond the standard treatment of 4 days, may be added if clinically indicated, at the discretion of the investigator.
Visit 2	a, etc.	Post-treatment observation period until discharge from the hospital (duration will depend upon need for continued hospitalization, and will be omitted if discharge occurs at completion of treatment or may overlap with Visit 3 if hospitalization is prolonged)
Visit 3		7-10 days after treatment
Visit 4		6 months after treatment
Visit 5		One year after treatment

6.1.1 Visit 1a Screening

Eligible patients will be fully informed, orally and in writing, about the purposes and procedures of the study, and asked to sign a research consent form approved by the IRB, which describes study procedures and risks and potential benefits of the study.

Procedures:

After informed consent is obtained 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.
   Demographic Information, to include:
   - Date of Birth
   - Sex
   - Race and ethnicity
   History, to include:
   - Year of first porphyria attack
   - Number of attacks during the past year
   - 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 present attack
   - Previous treatment with Panhematin™
2. Concomitant illnesses
3. History of allergy
4. Concomitant medications
5. Use of opioid agonists during the previous 24 hours
6. Physical Examination, including:
   a. Body height and weight

b. Vital Signs

7. Vital capacity measurement
8. Electrocardiogram, if clinically indicated
9. Recording of porphyria-related signs and symptoms
10. Completion of NRS for pain and other symptoms
11. Blood samples will be drawn for testing, to include erythrocyte PBGD, serum or plasma ALA, PBG and porphyrins, complete blood counts, metabolic and hepatic panels, and serum progesterone (females only)
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)
15. Urine pregnancy tests (for females of childbearing potential only).

6.1.2 Visit 1b: Treatment Day 1:

Procedures:

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

1. Concomitant medications, and any changes not recorded previously
2. Vital Signs
3. Recording of porphyria-related signs and symptoms
4. Vital capacity measurement
5. Completion of NRS for pain and other symptoms
6. Begin continuous recording of use of opioids preceded by assessment of current pain
7. Urine, blood and fecal samples for ALA, PBG and porphyrins (pretreatment)
8. Recording of adverse events
9. Administration of study treatment

6.1.3 Visit 1c, d, etc.: Treatment Days 2-4 etc.

Procedures:

These will be the same as for Visit 1b. Additional treatment days may be added if clinically indicated, at the discretion of the investigator.

6.1.4 Visit 2a, b, etc.: Daily observation after treatment, until discharge

Procedures:

These will be the same as for Visits 1b, c, d, etc., except that there will be no study treatment. Additional observation days may be added if clinically indicated, at the discretion of the investigator.

6.1.5 Visit 3: Follow-up 7-10 days after completion of treatment

Procedures:

These will be the same as for Visits 2b, c, d, etc., except that biochemical measurements are not required. Unless clinical indications require the patient to come to the site, this visit can be completed by telephone. Additional observation days may be added if clinically indicated, at the discretion of the investigator, and labeled 3a, etc.

6.1.6 Visit 4. Follow-up 6 months after completion of treatment

Procedures:

These will be the same as for Visit 3

6.1.7 Visit 5. Follow-up one year after completion of treatment

Procedures:

These will be the same as for Visits 3 and 4.

6.2 Assessments for Efficacy

6.2.1 Clinical improvement

These assessments will be performed at each visit.

6.2.1.1 Pain related to the attack of porphyria

Current pain and average pain over the last 24 hours will be assessed during the trial using a numerical rating scale (NRS) ranging from 0-10. The source(s) of pain will also be documented on the CRF. Study personnel will be educated on completion of the NRS.

Current pain will be assessed by the NRS and recorded every 4 hours and before scheduled or prn opioid dosing.

For average pain over the last 24 hours the patient will be asked how much pain he/she has experienced as an average during the past 24 hours, and this will be recorded using the NRS.

6.2.1.2 Use of opioids during the past 24 hours

Use of potent opioid agonists during the last 24 hours will be recorded during 24 hours prior to enrollment and during each day in Visits 1-3 or until discharge from the hospital. The generic and trade names, dose, route of administration and time of each dose will be recorded.

In past studies of treatment of acute porphyries it has not been possible to use one opioid drug, such as morphine, for all patients, because some patients have side effects from morphine but not certain other opioids. A standard opioid conversion table will be used to express data in morphine equivalents, in order to combine data for all patients. The results of calculating morphine equivalence will be entered on the CRF page after collection of the page from the site and prior to data entry. The major treatment-related comparison will be change in opioid requirements, rather than comparing requirements between patients.

6.2.1.3 Other porphyria-related signs and symptoms

Other signs and symptoms will be recorded at the times stated above, and rated for severity using a NRS of 0 to 10. These will include nausea, vomiting, constipation and specified neurological and psychiatric symptoms.

6.2.2 Rescue treatment

Rescue treatment will be given when needed and recorded as part of the trial. Rescue treatment can be started if one of the following criteria is met, in the judgment of the investigators:

- no improvement in symptoms after 48 hours
- worsening in symptoms after 24 hours
- development or worsening of hyponatremia
- other findings that place the patient at undue risk
- the patient requests rescue treatment due to worsening symptoms

No improvement after 48 hours is an indication for rescue treatment because continuing placebo beyond this time is considered to place the patient at undue risk. Patients with hyponatremia may be started on blinded treatment, but rescue treatment will be started if there is worsening of hyponatremia. Blinding up to rescue treatment will be maintained until the end of the study, and rescue treatment will be continued for the standard 4 days, or for a shorter period if there is rapid improvement, at the judgment of the study physician. Rescue treatment may be continued longer than 4 days, if clinically indicated. Patients will remain in the study during and after rescue treatment.

6.2.3 Biochemical measures of improvement

Biochemical measures, to include urinary ALA, PBG and porphyrins, plasma PBG and porphyrins and fecal porphyrins will be measured daily during Visits 1 and 2, and at Visits 3-5 only if the patient visits a study site. Analyses will be performed at the Porphyria Laboratory of the University of Texas Medical Branch. Procedures for obtaining, handling and storage of samples will be described in a trial laboratory manual. All results will be provided to the RDCRN Data Management and Coordinating Center during the trial.

Baseline biochemical measurements obtained before initiation of treatment with Panhematin™ or placebo will not be blinded and will be recorded as usual in the subject's medical record. Biochemical data during blinded treatment will be remain blinded until the end of the study, and will not be provided to the medical record. These results will be maintained in the laboratory separately from other laboratory results to avoid inadvertent unblinding of other study personnel. This blinded laboratory data will be entered into the database at the end of the study and only whether the sample was collected or not will be recorded on the CRFs at the time of the study visit.

6.2.4 Recurrent attacks and continued symptoms

Occurrences of new attacks and continued symptoms will be recorded at Visits 3-5.

6.3 Safety Assessment

6.3.1 Symptoms

Any new symptoms not related to porphyria will be recorded during and after treatment.

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 daily during Visits 1 and 2, and at Visits 3-5 only if the patient visits a study site. Height will be recorded 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 daily during Visits 1 and 2, and at Visits 3-5 only if the patient visits a study site. Analyses will be performed either by the hospital laboratory or a central 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 the site, 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 the site at Visit 1. Since pregnancy is not a reason for avoiding treatment with hemin or glucose, a positive pregnancy test will not exclude a patient. Pregnancy will be recorded as a concomitant condition.

6.3.6 Laboratory data flow

The DMCC will provide the site with on-line forms and/or electronic data exchange mechanisms - depending on their capabilities and needs - to enter, update and obtain relevant data. On-line forms exist to perform specimen receipts, report specimen issues and submit test results for specimens. The preferred method to exchange data electronically is through the Specimen Management System Web Service. The Web Service allows laboratories to obtain specimen shipment information, receive individual specimens or specimen shipments, report specimen issues and communicate specimen aliquots in a secure manner (test result submission is planned). The DMCC will also support uploading of files electronically. All transactions are logged and validated for both methods. As noted above, laboratory data that would result in premature unblinding will be entered at the end of the study.

6.3.7 Adverse Events

Adverse Events will be recorded during Visits 1-5.

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 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/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
Unexpected Adverse Event	Any adverse experience…the specificity or severity of which is not consistent with the risks of information described in the protocol.
Expected Adverse Event	Expected adverse events are those that are identified in the research protocol as having been previously associated with or having the potential to arise as a consequence of participation in the study.
* 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.

In the present trial a prolongation of hospitalization caused by lack of effect of the trial drug should not be reported as an SAE, unless one of the other criteria for an SAE is fulfilled.

All reported adverse events will be classified using the current version of the Common Terminology Criteria for Adverse Events (CTCAE) developed and maintained by CTEP at National Cancer Institute.

Reporting Timeline

- Within **24 hours** (of learning of the event), investigators must report any reportable Serious Adverse Event (SAE) that:
  - Is considered life-threatening/disabling or results in death of subject

  -OR-
  - Is Unexpected/Unanticipated
- Investigators must report all other reportable SAEs within **5 working days** (of learning of the event).
- All other (suspected) reportable AEs must be reported to the RDCRN within **20 working days** of the notification of the event or of the site becoming aware of the event.

Local institutional reporting requirements to IRBs, any CTSA oversight committee, the Data and Safety Monitoring Board (DSMB) and the FDA, if appropriate, remain the responsibility of the treating physician and the Study Chair.

RDCRN Adverse Event Data Management System (AEDAMS)
Upon entry of a serious adverse event, the DMCC created Adverse Event Data Management System (AEDAMS) will immediately notify the Study Chair, the Medical Review Officer, and any additional agencies (if applicable- industry sponsor, CTEP, etc) of any reported adverse events via email.

Serious adverse events: The NIH appointed Medical Review Officer (MRO) reviews the site investigator's report and determines causality (definitely not related, probably not related, possibly related, probably related, definitely related) of the adverse event. The MRO may request further information if necessary and possibly request changes to the protocol or consent form as a consequence of the adverse event. A back-up notification system is in place so that any delays in review by the MRO beyond a specified period of time are forwarded to a secondary reviewer. Any follow up reports or requested additional participant data will be entered into the AEDAMS system by the reporting site and reviewed by the MRO. Completed AE reviews by the MRO will sent to Study Chair, site PIs, and the appointed NIH officers.

If warranted, the MRO may request an ad hoc call with the DSMB to review the adverse event. All reported AE's will be reviewed during the regularly scheduled DSMB call.

The Adverse Event Data Management System (AEDAMS) maintains audit trails and stores data (and data updated) and communication related to any adverse event in the study.

Non-serious expected adverse events: Except those listed above as immediately reportable, non-serious expected adverse events that are reported to or observed by the investigator or a member of his research team will be submitted to the DMCC in a timely fashion (within 20 working days). The events will be presented in tabular form and given to the MRO and RDCRN DSMB on a bi-annual basis. Local site investigators are also required to fulfill all reporting requirements of their local institutions.

The DMCC will post aggregate reports of all adverse events (serious/not serious and expected, unexpected) for site investigators and IRBs and for review by the DSMB.

8. Case Report Forms

Case Report Forms (CRFs) will be prepared at the Coordinating Center at the University of Texas Medical Branch and supplied to other participating centers.

Data Entry

Data collection for this study will be accomplished with online electronic case report forms. Using encrypted communication links, on-line forms will be developed that contain the requisite data fields.

Registration

Registration of participants on this protocol will employ an interactive data system in which the clinical site will attest to the participant's eligibility as per protocol criteria and obtain appropriate informed consent. IRB approval for the protocol must be on file at the DMCC before accrual can occur from the clinical site.

The DMCC will use a system of coded identifiers to protect participant confidentiality and safety. Each participant enrolled will be assigned a local identifier by the enrollment site. This number can be a combination of the site identifier (location code) and a serial accession number. Only the registering site will have access to the linkage between this number and the personal identifier of the subject. When the participant is registered to participate in the study, using the DMCC provided web-based registration system, the system will assign a participant ID number. Thus each participant will have two codes: the local one that can be used by the registering site to obtain personal identifiers and a second code assigned by the DMCC. For all data transfers to the DMCC both numbers will be required to uniquely identify the subject. In this fashion, it is possible to protect against data keying errors, digit transposition or other mistakes when identifying a participant for data entry since the numbers should match to properly identify the participant. In this fashion, no personal identifiers would be accessible to the DMCC.

8.1 Rules for Completing CRFs

CRFs may be completed by investigators, coordinators and study nurses at each site. 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.

Corrections necessary after the CRFs have been removed from the Investigator's site must be documented on a Query Resolution Form, and can be approved only by the Investigator.

8.3 CRF Review and Data Entry

The original CRFs are reviewed by the Monitor at the time of monitoring site visits, and photocopies made for storage at the study site and the Coordinating Center. The Monitor removes the original CRFs after no further corrections or amendments to the content are expected.

The Coordinating Center will enter data from the original CRFs into the RDCRN DMCC database. After all necessary database verification, the original CRFs will be archived at a secure archiving location. Other copies may be destroyed after a comprehensive Clinical Trial Report has been finalized, and in accordance with practices approved at each site.

9. Monitoring Procedures

Study Oversight

The Study Chair has primary oversight responsibility of this clinical trial. The NIH appointed Data (Observational) Safety Monitoring Board (D/OSMB) has oversight responsibility of the Data Safety Monitoring Plan (DSMP) for this clinical trial. The D/OSMB will review accrual, patterns and frequencies of all adverse events, protocol compliance every 6 months. The D/OSMB makes recommendations to the NIH regarding the continuation status of the protocol.

Each site's Primary Investigator and their research team (co-Investigators, research nurses, clinical trial coordinators, and data managers) are responsible for identifying

adverse events. Aggregate report- detailed by severity, attribution (expected or unexpected), and relationship to the study drug/study procedures – will be available from the DMCC for site review. Adverse events will be reviewed at least every 3 months by the research team. A separate report detailing protocol compliance will also be available from the DMCC for site review on a monthly basis. The research team will then evaluate whether the protocol or informed consent document requires revision based on the reports.

Safety Monitoring Plan

The study protocol has been reviewed and approved by the National Institutes of Health (NIH) and a DSMB formed by NIH to oversee all Porphyrias Consortium studies. Participant enrollment may only begin after IRB approval of consent forms and other study documents.

This is an interventional phase II study that meets the federal definition of low risk. The risk level for this study is judged to be low, since the treatment modalities are already part of clinical practice, 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 **13.1** below). Although this is not a Phase III clinical trial, the DSMB formed by NIH will oversee the study.

Recruitment, enrollment, retention, adverse events, and study procedures will be monitored carefully by the PI and the investigator at each site. The investigators 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 weekly investigators meetings or conference calls, and discuss any instances of adverse events or unexpected problems encountered regarding patient safety or data collection. The DSMB will review data safety and data collection records at least every 6 months.

Plan for Adverse Event (AE) Reporting. See Section 7. The PI and site investigator will be notified immediately if an AE occurs, and a medical member of the team will evaluate the patient and enter a note into the medical chart. 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, the DSMB and others as required, within 24 h of occurrence. The investigators, the DSMB 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 will be immediately suspended. Studies will not resume until modifications are made that are deemed to result in an acceptable risk/benefit ratio. Aggregate reports of adverse events will be prepared on an annual basis and forwarded to the IRB and others as required. Aggregate reports will be provided to the DSMB at six-month intervals. 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.

Data monitoring for this Phase II study will be carried out by a Monitor from the Data Management Coordinating Center (DMCC) of the RDCRN, who will visit the trial sites at regular intervals and be available for discussions by telephone. The purposes of these visits are to verify that the rights and well-being of study subjects are protected, reported trial data are accurate, complete, and verifiable from source documents and that the conduct of the trial is in compliance with the currently approved protocol and any amendments, and with applicable regulatory requirements.

The Monitor must be given direct access to source documents (original documents, hospital charts – including electronic medical records, and other pertinent data and records). Direct access includes permission to examine, analyze and verify any records and reports that are important to the evaluation of the clinical trial. If these are removed from the site, identifying information other than initials and date of birth must first be removed.

The following items must be verifiable in source documentation other than the CRF:

- 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 medication
- Blood pressure, pulse, weight and height
- Reason for exclusion or withdrawal

For all other items the data recorded in the CRF are considered as source data. The Monitor will ensure that the CRFs are collected.

10. Data Management

Data Management will be through the DMCC of the NIH RDCRN, which will design a secure web-entry database for this study. Data will be entered at the Porphyria Center at the University of Texas Medical Branch or other sites. Data downloaded from the database for further analysis will identify subjects by study number, without personal identifiers. The identity of subjects will be excluded from all presentations and publications.

Data Quality and Monitoring Measures
As much as possible data quality is assessed at the data entry point using intelligent on-line data entry via visual basic designed screen forms. Data element constraints, whether independent range and/or format limitations or 'relative' referential integrity limitations, can be enforced by all methods employed for data input. QA reports assess data quality post-data entry. As we note, data quality begins with the design of the data collection forms and procedures and incorporates reasonable checks to minimize transcription and omission errors. Of the more important quality assurance measures are the internal validity checks for reasonableness and consistency.

- Data Monitoring: The RDCRN DMCC identifies missing or unclear data and generates a data query to the consortium administrator contact.
- Data Delinquency Tracking: The Data Management and Coordinating Center will monitor data delinquency on an ongoing basis.

All study data will be collected via systems created in collaboration with the RDCRN DMCC and will comply with all applicable guidelines regarding patient confidentiality and data integrity.

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 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 Biostatistics, 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 differences between measurements for the two treatment groups. The primary outcome will be change in pain score. In addition, secondary outcomes will be assessed, including use of opiods for pain, other symptoms, use of rescue treatment, length of hospital stay and time to next attack. Biochemical parameters, such as urine and serum levels of PBG, will be analyzed also, because it is recognized that hemin is almost always associated with biochemical improvement. However, it is known that these biochemical changes do not necessarily predict clinical improvement.

12.2 Variables for statistical analyses

12.2.1 Efficacy variables

The primary efficacy endpoints is:

- Change in NRS pain score at 12 hours

Secondary efficacy endpoints are:

- Change in NRS pain score at later time points
- Use of opioid for pain
  - Use of morphine or other opioid in each 24 hour period
  - Time to last administration of opioid
- Other symptoms
  - Scores for nausea, vomiting and other symptoms will be recorded on a 0-to-10 scale over each 24 hour period
  - Use of medication for nausea, vomiting or other symptoms in each 24 hour period
  - Time to last administration of medication for such symptoms
- Rescue treatment with open label Panhematin™
  - Rescue treatment given or not given
  - Time to rescue treatment
- Length of hospital stay
- Time to occurrence of next attack

The main clinical efficacy variable, which is also the basis for sample size projection, is the change from baseline to 12 hours after commencement of treatment in NRS score for pain intensity. Secondary comparisons will include differences from baseline at 24, 48, 72 and 96 hours and the sum of pain intensity differences (SPID). Baseline is the pre-treatment score just before starting therapy. Values recorded after starting rescue Panhematin™ will not be part of the analysis comparing blinded study drug and placebo. But if rescue treatment occurs before 12 hours, the last pain score before rescue treatment and at least 6 hours after starting treatment will be used in the analysis. The analysis will be based on intent to treat, excluding post-rescue scores.

Secondary efficacy pain-related variables include pain scores at later time intervals, total daily dose of morphine, time to last administration of morphine, time to rescue treatment with open label hemin, time to discharge from the hospital and time to the next attack.

Opioids may decrease pain intensity for up to four hours and therefore have a lowering effect on NRS scores during that time. Therefore, NRS scores are recorded immediately prior to each administration of morphine throughout the trial. But it may not always be possible to avoid giving opioids for a four hour period before a NRS score is recorded. We will examine two methods for dealing with these occurrences. 1) NRS scores for pain recorded within 4 hours after

administration of an opioid will be excluded from the analyses. 2) Scores within 4 hours after an opioid will be imputed based on an earlier NRS score recorded within the previous 4 hours and not preceded by opioid administration within the preceding 4 hours. This second method will be used only if imputed scores are distributed evenly between the two treatment groups.

Differences in scores at 12, 24, 48, 72 and 96 hours will be analyzed statistically as described below. Also, the sum of pain intensity differences (SPID) will be derived and subjected to statistical analysis, as the time weighted pain intensity differences:

$$SPID = \sum_{k=1}^{6} (NRS_0 - NRS_k)*(T_k - T_{k-1}),$$

where NRS_k and T_k are the NRS scores and times respectively for k= 0, 12, 24, 48, 72 and 96 hours after treatment.

Secondary efficacy and exploratory endpoints also include biochemical effects of treatment, time to hospital discharge and to next attack, and the effects of genetic and clinical features on treatment response.

The following biochemical endpoints will be analyzed:

- Serum (or plasma) ALA, PBG and porphyrins
- 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)

The area under the 'reduction in PBG relative to baseline' versus time curve ($AUC_{PBG\ reduction}$) will be determined by the linear trapezoid rule for each 24 hour period during treatment.

The relative plasma PBG reduction (% reduction relative to baseline, R) at time t is calculated as one hundred multiplied by one minus the plasma PBG at time t (PBG_t) over the plasma PBG at time zero (immediately before dosing):

$$R = 100 * (1 - (PBG_t/PBG_0)) (\%),\ R \leq 1.$$

Note that R may be negative if PBG exceeds the baseline level.

A few sporadically missing values will be disregarded and replaced by linear interpolation for application of the trapezoid rule. Any other options considered for handling missing values will be finalized before unveiling the treatment allocation. Changes in ALA and porphyrins will be analyzed in the same way as for PBG.

12.2.2 Safety variables

As noted earlier, safety endpoints will include:

- Symptoms
- Finding 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, change in pain from baseline after commencement of treatment will be analyzed by analysis of covariance with treatment as a factor and baseline value as a covariate. Data for the post randomization exclusions will be listed and the possible impact assessed.

Length of hospitalization and total dose of opioids received will be analyzed by exact two sample Wilcoxon tests. The use of rescue treatment with hemin within each treatment group will be analyzed by Fisher's exact test, and the time to use of human hemin, the time to last administration of opioids and the time to new attack will be analyzed by exact log rank test. The analysis of time to next attack will be done separately in the groups of subjects with attacks related or not related to the menstrual cycle.

Efficacy analyses for changes in biochemical parameters ($AUC_{PBG\ reduction}$, etc.) will be an exact two sample Wilcoxon test and associated Hodges-Lehmann confidence interval for difference in medians. Changes from baseline to end of treatment will be analyzed by analyses of covariance with baseline value as a covariate.

Supplementary analyses of the role of clinical features, such as attack relationship to the menstrual cycle will be performed.

Pain intensity difference based on NRS for current pain will be analyzed by repeated measures analysis of variance.

Analyses of signs and symptoms will be descriptive.

Safety variables such as physical and laboratory findings will be analyzed descriptively, using shift tables – screening versus end of treatment or by repeated measures analysis of variance.

Adverse events will be coded and analyzed descriptively.

12.4 Sample size determination

The primary outcome measure in this study will be the difference in NRS pain score. The power to detect a treatment-related difference in pain and other measurements increases with the magnitude of the difference in, for example, pain intensity before and after treatment.

A minimum clinically relevant difference between the means of NRS pain scores on a 0-10 scale is judged to be 1.5 (64). A sample size of 20 in each group will have 80% power to detect a difference in means of 1.5 assuming that the common

standard deviation is 1.65 using a two group t-test with a 0.050 two-sided significance level.

12.5 Interim analyses

An interim analysis is planned after the first 15-20 subjects, since there are a number of uncertainties related to variance in treatment outcomes and the potential effects of factors such as opioid administration and rescue treatment on outcome measures. An interim analysis is appropriate in a phase 2 study such as this that has an intentionally flexible study design.

It is anticipated that there will be large differences in pain and other efficacy parameters in this study, but differences in NRS pain scores may be reduced by opioids needed for symptom control. Rescue treatment may also reduce the power of the observations. Only one adequately powered double-blind placebo-controlled study of treatment of acute attacks of porphyria (with recombinant human PBGD) has been done previously, and that study did not show a positive treatment effect (unpublished); variance in outcomes from that study have not been made available (7). Therefore, an interim analysis will be important in this study to consider adjustment in sample size and possibly even treatment outcomes.

The sample size needed to have 80% power to reject the null hypothesis of no effect by the appropriate test at the 5% significance level will be determined based on this minimum clinically relevant difference. If the estimated sample size is smaller than 30 the trial will stop after 30 patients have completed; if it is 30-40, the trial will continue to 40 patients; if it exceeds 40 the trial will continue until this sample size has been reached, if that is feasible. 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 either biochemical or clinical efficacy, the trial will continue and stop when 40 patients have completed.

A statistician will unblind the data for this analysis sufficiently to separate the two treatment groups, but blinding will be preserved for all others. An interim analysis is planned after completion of 15-20 subjects, to determine a reasonable estimate of the variance, as stated above in 12.4.

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 each participating center, and changes made in study documents as needed to achieve these approvals.

13.1 Assessment of Risks

Patients enrolled in this study have clinical indications for treatment with Panhematin™. Therefore, the risks from this study will not be substantially different from standard treatment, which would likely include Panhematin™.

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>:

- Progression of symptoms due to initial randomization to placebo and treatment at least initially with glucose rather than Panhematin™.

This risk will be minimized, because all patients are provided with intravenous glucose treatment, which may be sufficient treatment in some cases, and the study design allows for rescue treatment with Panhematin™. Patients will be treated early in an attack, so any delay in rescue treatment will not be prolonged. Moreover, some patients who might benefit from Panhematin™ which they might not have been given as part of standard care, and others who respond to glucose will not be at risk from side effects of Panhematin™.

- Blood drawn in this study could contribute to iron deficiency. The volume of blood drawn will depend on the length of treatment with Panhematin™ or placebo. We expect that the great majority of patients will be treated for 4 days. The volume drawn will total <160 mL if the patient is treated for 4 days, or <260 mL if treatment is required for 10 days.

This may be somewhat more than would have been drawn during standard treatment, but safety assessments may provide for earlier detection and correction of complications, such as electrolyte imbalances. Iron status will be assessed by serum iron, iron-binding capacity and ferritin measurements and corrected with iron supplements if clinically indicated.

Risks of Panhematin™:

This includes reported effects of 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 (50).

No worsening of renal function has been seen with administration of recommended dosages of hematin (50, 65).

- 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 (37, 44) is expected to reduce the risk of this complication in this study.

- 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 (66).

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. Therefore, it seems unlikely that hematin reconstituted with albumin will worsen a pre-existing coagulopathy, although to our knowledge this has not been studied. Preexisting coagulation abnormalities or concurrent anticoagulant therapy will not be reasons for exclusion if the investigators and medical team agree that treatment with hemin is indicated clinically and the potential benefits of treatment outweigh the risks.

- Fever, aching and malaise are sometimes seen (48, 49). 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 (48, 49).

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™.

- Iron overload can occur after repeated administration of hemin. Amounts of hemin administered in this acute study are insufficient to cause iron overload, but might worsen pre-existing iron loading from previous courses of hemin treatment. Serum ferritin will be measured in all patients to assess iron status, and if found to have iron overload they will be advised regarding management by repeated phlebotomies after recovery from the attack.

<u>Risks of placebo</u>:

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

<u>Risks of glucose infusions</u>:

- Elevated blood glucose

This represents a risk in patients with preexisting diabetes mellitus, and will be avoided by initial screening for elevated blood glucose or a history of diabetes. In patients with diabetes, blood glucose will be monitored and treated with insulin as clinically indicated.

- Hyponatremia, seizures and fluid overload

Hyponatremia and the syndrome of inappropriate antidiuretic hormone secretion (SIADH) are common complications of acute porphyric attacks, and may lead to seizures if not recognized and treated. Serum electrolytes will be monitored daily during treatment. If hyponatremia is present initially, this will be treated according to clinical indications, which may include saline infusions and/or fluid restriction. The amounts of 10% glucose administered will be reduced if clinically indicated. Development or worsening of hyponatremia despite these measures is indications for rescue with Panhematin™.

<u>Risks from infusion of albumin</u>.

- 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.
- 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).

Certificate of Confidentiality

To help protect participant privacy, a Letter of Confidentiality has been obtained from the National Institutes of Health (NIH). With this Certificate, the researchers cannot be forced to disclose information that may identify a study participant, even by a court subpoena, in any federal, state, or local civil, criminal, administrative, legislative, or other proceedings. The researchers will use the Certificate to resist any demands for information that would identify a participant, except as explained below.

The Certificate cannot be used to resist a demand for information from personnel of the United States Government that is used for auditing or evaluation of Federally funded projects or for information that must be disclosed in order to meet the requirements of the federal Food and Drug Administration (FDA).

Even with the Certificate of Confidentiality, the investigators continue to have ethical and legal obligations to report child abuse or neglect and to prevent an individual from carrying out any threats to do serious harm to themselves or others. If keeping information private would immediately put the study participant or someone else in danger, the investigators would release information to protect the participant or another person.

Department of Health and Human Services (DHHS) personnel may request identifying information for purposes of performing audits, carrying out investigations of DHHS grant recipients, or evaluating DHHS funded research projects.

13.2 Assessment of benefits

Patients may derive no immediate benefits from this study, since both treatments are available as standard of care. However, demonstration that Panhematin™ is safe and effective 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 lead 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 from each participant before any study-specific procedures or assessments are done and after the aims, methods, anticipated benefits, and potential hazards are explained. The participant's willingness to participate in the study will be documented in writing in a consent form, which will be signed by the participant with the date of that signature indicated. The investigator will keep the original consent forms and signed copies will be given to the participants. It will also be explained to the participants that they are free to refuse entry into the study and free to withdraw from the study at any time without prejudice to future treatment. Written and/or oral information about the study in a language understandable by the participant will be given to all participants.

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 IRB. Other documents, such as investigators' CVs or Biosketches will also be submitted to the IRB, as required. Since resources of a Clinical and Translational Science Award (CTSA) will be used at some sites, all documents should be jointly submitted for approval to the CTSA as well as the IRB, as appropriate. Written final approval must be obtained from 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 and, if required, the CTSA 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 application for an investigator IND has been obtained from the US Food and Drug Administration (FDA) for this study by the Coordinating Center (IND#13,929), and the protocol, amendments, reports on SAEs, annual reports and other documents will be provided as required by the FDA. The Coordinating Center will submit to the FDA all required documents related to the participation of each site, and copies will be provided to each site for submission to local IRBs.

14. Premature Termination of the Trial

The Coordinating Center may decide to stop the trial or part of the trial at any time. A site may decide to withdraw from study participation at any time, and the site and

Coordinating Center must agree on procedures to be followed for withdrawal from the study.

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

The NIH, the DSMB and local IRB's (at their local site) have the authority to stop or suspend this trial at any time. This study may be suspended or closed if:

- Early stopping rules have been met
- Accrual has been met
- The study objectives have been met
- The Study Chair / Study Investigators believe it is not safe for the study to continue
- The DSMB suspends or closes the trial
- The NIH suspends or closes the trial
- The FDA suspends or closes the trial

15. Deviations from the Protocol

If protocol deviations occur, the Investigator must inform the Coordinating Center and the Monitor, and each deviation 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 of the site Investigator and the Coordinating Center.

16. Essential Documents

Before the Investigator starts the trial (i.e. obtains research consent from the first subject) the following documents must be provided to the Coordinating Center:

- 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 title and protocol version date, 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 Investigator is 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 by the participating group of investigators is considered confidential and will be used to prepare a joint Clinical Trial Report, a joint publication and possibly joint presentations of the study results at scientific meetings. The investigators at all sites who enrolled patients in the study will be offered co-authorship. The investigators agree that they will not individually submit data for publication or for presentation at scientific meetings until a joint publication has been accepted for publication, after which each investigator will have the right to publish results obtained at that site.

18. Retention of Clinical Trial Documents

All study records and source documents must be stored at each site for at least 15 years or longer, or for the maximum time period permitted by the institution. The Coordinating Center must be informed at study initiation the policy on storage that will be followed at each site. No study-related documents should be destroyed before that time without notifying the Coordinating Center in advance.

19. Indemnity Statement

This is an investigator-initiated study that will be conducted by academic medical centers with support from federal 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 DMCC of the NIH RDCRN as described earlier (see Section 9). Details will be provided prior to study initiation.

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*. 8th Edition ed. Vol. II, Chapter 124. 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):277-86.
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. **Harper P, Wahlin S.** Treatment options in acute porphyria, porphyria cutanea tarda, and erythropoietic protoporphyria. *Curr Treat Options Gastroenterol*. 2007;10(6):444-55.
8. **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;11:269-73.
9. **Andersson C, Bjersing L, Lithner F.** The epidemiology of hepatocellular carcinoma in patients with acute intermittent porphyria. *J Intern Med*. 1996;240(4):195-201.
10. **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):933-9.
11. **Anderson KE, Bloomer JR, Bonkovsky HL, et al.** Recommendations for the diagnosis and treatment of the acute porphyrias. *Ann Intern Med*. 2005;142(6):439-50.
12. **Anderson KE, Freddara U, Kappas A.** Induction of hepatic cytochrome P-450 by natural steroids: relationships to the induction of d-aminolevulinate synthase and porphyrin accumulation in the avian embryo. *Arch Biochem Biophys*. 1982;217:597-608.
13. **Peyer AK, Jung D, Beer M, et al.** Regulation of human liver delta-aminolevulinic acid synthase by bile acids. *Hepatology*. 2007;46(6):1960-70.
14. **Podvinec M, Handschin C, Looser R, Meyer UA.** Identification of the xenosensors regulating human 5-aminolevulinate synthase. *Proc Natl Acad Sci U S A*. 2004;101(24):9127-32.
15. **Meyer UA, Schuurmans MM, Lindberg RLP.** Acute porphyrias: pathogenesis of neurological manifestations. *Semin Liver Dis*. 1998;18(1):43-52.
16. **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*. 2004;50(5):915-23.
17. **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.
18. **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.
19. **Poh-Fitzpatrick MB.** A plasma porphyrin fluorescence marker for variegate porphyria. *Arch Dermatol*. 1980;116:543-547.
20. **Mauzerall D, Granick S.** The occurrence and determination of ∂-aminolevulinic acid and porphobilinogen in urine. *J Biol Chem*. 1956;219:435-446.
21. **Deacon AC, Peters TJ.** Identification of acute porphyria: evaluation of a commercial screening test for urinary porphobilinogen. *Ann Clin Biochem*. 1998;35(Pt 6):726-32.

22. **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.

23. **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.

24. **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.

25. **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):243-8.

26. **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.

27. **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.

28. **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.

29. **Lamon JM, Frykholm BC, Hess RA, Tschudy DP.** Hematin therapy for acute porphyria. *Medicine*. 1979;58:252-269.

30. **Pierach CA, Bossenmaier I, Cardinal R, Weimer M, Watson CJ.** Hematin therapy in porphyric attacks. *Klin Wochenschr*. 1980;58:829-832.

31. **Kostrzewska E, Gregor A, Tarczynska-Nosal S.** Heme arginate (Normosang) in the treatment of attacks of acute hepatic porphyrias. *Mater Med Pol*. 1991;23(4):259-62.

32. **Mustajoki P, Nordmann Y.** Early administration of heme arginate for acute porphyric attacks. *Arch Int Med*. 1993;153:2004-2008.

33. **Nordmann Y, Deybach JC.** [Acute attacks of hepatic porphyria: specific treatment with heme arginate]. *Ann Med Interne*. 1993;144(3):165-7.

34. **Kostrzewska E, Gregor A.** Acute hepatic porphyrias. Detection, prophylaxis and treatment. *Mater Med Pol*. 1996;28(1):5-7.

35. **McColl KEL, Moore MR, Thompson GG, Goldberg A.** Treatment with haematin in acute hepatic porphyria. *Quart J Med*. 1981;198:161-174.

36. **Mustajoki P, Tenhunen R, Tokola O, Gothani G.** Haem arginate in the treatment of acute hepatic porphyrias. *Br Med J*. 1986;293:538-539.

37. **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.

38. **Herrick AL, McColl KEL, Moore MR, Cook A, Goldberg A.** Controlled trial of haem arginate in acute hepatic porphyria. *Lancet*. 1989;1:1295-1297.

39. **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.

40. **Goetsch CA, Bissell DM.** Instability of hematin used in the treatment of acute hepatic porphyria. *N Engl J Med*. 1986;315:235-238.

41. **Green D, Furby FH, Berndt MC.** The interaction of the factor VIII/von Willebrand factor complex with hematin. *Thromb Haemost*. 1986;56:277-282.

42. **Jones RL.** Hematin-derived anticoagulant. Generation in vitro and in vivo. *J Exp Med*. 1986;163:724-439.

43. **Green D, Tsao CH.** Hematin: effects on hemostasis. *J Lab Clin Med*. 1990;115:144-147.

44. **Anderson KE, Bonkovsky HL, Bloomer JR, Shedlofsky SI.** Reconstitution of hematin for intravenous infusion. *Ann Intern Med*. 2006;144(7):537-8.

45. **Siegert SW, Holt RJ.** Physicochemical properties, pharmacokinetics, and pharmacodynamics of intravenous hematin: a literature review. *Adv Ther*. 2008;25(9):842-57.

46. **Mustajoki P, Tenhunen R, Pierach C, Volin L.** Heme in the treatment of porphyrias and hematological disorders. *Semin Hematol*. 1989;26:1-9.

47. **European Porphyria Initiative.** Treatment of the acute attack. *Accessed at http://www.porphyria-europe.com/02-for-healthcare/treatment.asp on July 30, 2008*.

48. **Khanderia U.** Circulatory collapse associated with hemin therapy for acute intermittent porphyria. *Clin Pharmacy*. 1986;5:690-692.

49. **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.

50. **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.

51. **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.

52. **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.

53. **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.

54. **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.

55. **Bissell DM.** Treatment of acute hepatic porphyria with hematin. *J Hepatol*. 1988;6:1-7.

56. **Tenhunen R, Mustajoki P.** Acute porphyria: treatment with heme. *Semin Liver Dis*. 1998;18(1):53-5.

57. **Anderson KE, Egger NG, Goeger DE.** Heme arginate for prevention of acute porphyric attacks (abstract). *Acta Haematologica*. 1997;98, Supplement 1:120.

58. **Anderson KE, Collins S.** Open-label study of hemin for acute porphyria: clinical practice implications. *Am J Med*. 2006;119(9):801 e19-24.

59. **Sood G, Anderson KE.** Porphyrias. In: Crowther MA, Ginsberg J, Schunemann H, Meyer RM, Lottenberg R, eds. *Evidence-Based Hematology*. Hoboken: Wiley; 2008.

60. **Bonkovsky HL, Barnard GF.** The Porphyrias. *Curr Treat Options Gastroenterol*. 2000;3(6):487-500.

61. **Anderson KE.** The porphyrias (Chapter 229). In: Goldman L, Ausiello D, eds. *Cecil Medicine*. 23rd ed. Philadelphia: Saunders Elsevier; 2008:1585-1593.

62. **Badminton MN, Elder GH.** Management of acute and cutaneous porphyrias. *Int J Clin Pract*. 2002;56(4):272-8.

63. **Tschudy DP, Valsamis M, Magnussen CR.** Acute intermittent porphyria: clinical and selected research aspects. *Ann Intern Med*. 1975; 83:851-864.

64. **Breivik H, Borchgrevink PC, Allen SM, et al.** Assessment of pain. *Br. J. Anaesth.* 2008:aen103.

65. **Pierach CA.** Hematin therapy for the porphyric attack. *Semin Liver Dis*. 1982;2(2):125-31.

66. **Morris DL, Dudley MD, Pearson RD.** Coagulopathy associated with hematin treatment for acute intermittent porphyria. *Ann Intern Med*. 1981;95:700-701.