Rare Diseases Clinical Research Network

The Porphyrias Consortium

Therapeutic Studies in Porphyria Cutanea Tarda

Study Chair
Karl E. Anderson, M.D.
University of Texas Medical Branch
Porphyria Center
301 University Boulevard
Galveston, TX 77555-1109
Phone: 409-772-4661
Fax: 409-772-6287
Email: kanderso@utmb.edu

Participating Institutions/Investigators Table

Principal Investigator: Robert J. Desnick, PhD, MD

Contact:	Hetanshi Naik, MS, CGC
Institution:	Icahn School of Medicine at Mount Sinai
Address:	Department of Genetics & Genomic Sciences One Gustave L. Levy Place, Box 1497 New York, NY, 10029
Phone:	212-241-7699
Fax:	212-426-9065
Email:	robert.desnick@mssm.edu; hetanshi.naik@mssm.edu
Sub-Investigators:	Manisha Balwani, MD; Lawrence Liu, MD

Principal Investigator: Joseph R. Bloomer, MD

Contact:	Toni Ann Seay
Institution:	University of Alabama Birmingham
Address:	UAB Porphyria Center Liver Center, Division of Gastroenterology and Hepatology, Department of Medicine 1720 2nd Avenue South, BDB 398 Birmingham, Alabama 35294-0012
Phone:	205-934-7332
Fax:	205-975-9777
Email:	jbloomer@uab.edu
Sub-Investigators:	Ashwani Singal, MD; Brendan M. McGuire, MD

Principal Investigator: D. Montgomery Bissell, MD

Contact:	Yuvraaj Kapoor
Institution:	University of California at San Francisco
Address:	UCSF Porphyria Center 350 Parnassus Avenue, Suite 410 San Francisco, CA 94143-0657
Phone:	415-502-5356
Fax:	415-476-0659
Email:	montgomery.bissell@ucsf.edu
Sub-Investigator:	Bruce Wang, MD; Jennifer Lai, MD

Principal Investigator: John Phillips, PhD

Contact:	Sharada Dixit
Institution:	University of Utah
Address:	Porphyria Center Department of Internal Medicine, Division of Hematology

30 North 1900 E, Room # 5C402
Salt Lake City, Utah 84132
Phone: 801-581-6650
Fax: 801-585-3432
Email: john.phillips@hsc.utah.edu
Sub-Investigators: Charles Parker, MD

Principal Investigator: Karl E. Anderson, MD
Contact: Csilla Hallberg, MD
Institution: University of Texas Medical Branch
Address: Porphyria Center
301 University Boulevard
Galveston, TX 77555-1109
Phone: 409-772-4661
Fax: 709-772-6287
Email: kanderso@utmb.edu
Sub-Investigators:

Principal Investigator: Herbert Bonkovsky, MD
Contact: Dee Faust
Institution: Wake Forest
Address: Department of Internal Medicine/Gastroenterology
Wake Forest University School of Medicine
Winston-Salem, NC 27157
Phone: 336-713-7301
Fax: 336-713-7322
Email: hbonkovs@wakehealth.edu
Sub-Investigators:

Principal Investigator: Jeffrey Krischer, PhD
Contact: Carrie Light, Research Compliance Administrator
Institution: Data Management and Coordinating Center (DMCC), Pediatrics Epidemiology Center, University of South Florida
Address: 3650 Spectrum Blvd., Suite 100, Tampa, FL 33612
Phone: 813-396-9248
Fax: 813-910-5997
Email: carrie.light@epi.usf.edu

Table of Contents

1. Protocol Synopsis

Protocol Number:	7206
Protocol Title:	Therapeutic Studies in Porphyria Cutanea Tarda
Study Chair:	Karl E. Anderson, MD
Statistician:	Kristofer Jennings, PhD
Consortium:	Porphyrias Consortium
Participating Sites:	Icahn School of Medicine at Mount Sinai , New York, NY University of Alabama at Birmingham, Birmingham, AL University of California, San Francisco, San Francisco, CA The University of Texas Medical Branch, Galveston, TX University of Utah, Salt Lake City, UT Wake Forest, Winston-Salem, NC
Activation Date:	06/16/2011
Current Status:	Approved
Sample Size:	100 participants with porphyria cutanea tarda (PCT)
Target Enrollment Period:	10 years
Study Design:	Pragmatic Interventional study
Primary Study Objective:	To determine and compare time to remission with treatment with low-dose hydroxychloroquine or repeated phlebotomy in participants with PCT.
Secondary Study Objective(s):	1. To assess the effects of susceptibility factors on responses to treatment of PCT by these methods. 2. To determine and compare rates of recurrence of PCT after treatment with low-dose hydroxychloroquine or phlebotomy.
Study Population and Main Eligibility/ Exclusion Criteria:	Inclusion Criteria: 1. Willing to give informed consent 2. Willing to return to clinic or work with a local physician to have necessary labs done and samples sent to study site 3. Age 18 or greater 4. Women of child-bearing potential must be willing to avoid pregnancy and use an accepted and effective contraceptive method during treatment. 5. Well-documented PCT: i. Clinical features – A history of blistering cutaneous photosensitivity and/or skin fragility. ii. Biochemical findings – a and b are required a. A substantial increase in urinary porphyrins [>450 nmol/24 hours or g of creatinine, or more than 1.5-fold increase (relative to ULN of 300 nmol/24 hours or g of creatinine *or* >300 ug/24 hours or g of creatinine, or more than 1.5-fold increase (relative to ULN of 200 ug/24 hours or g of creatinine)] and/or plasma porphyrins [>2.7 ug/dL, or >3-fold increase (relative to ULN of 0.9 ug/dL), and a

	fluorescence peak at ~619 nm]; a porphyrin pattern in urine or plasma showing a predominance of uroporphyrin and heptacarboxylateporphyrin, and normal or only slightly elevated urine ALA and normal PBG. b. Normal or only slightly elevated erythrocyte porphyrins [<200 ug/dL, or less than 1.5-fold increase (relative to ULN of 80 ug/dL)], unless elevated erythrocyte protoporphyrin are due to a non-porphyric condition in the opinion of the investigator. iii. . Exclusion Criteria 1. Evidence for another cause of blistering skin lesions, such as another type of cutaneous porphyria, or pseudoporphyria 2. Pregnancy 3. Prior treatment by phlebotomy, hydroxychloroquine or chloroquine within one month, unless all appropriate lab results from before treatment was started can be obtained, to document baseline porphyrin levels. 4. Unwillingness to comply with the protocol 5. Previous treatment as a participant in this protocol.
Treatment	
Agent-	Hydroxychloroquine vs. repeated phlebotomy
Dosage, schedule, route of administration-	Hydroxychloroquine 100 mg twice weekly for up to 24 months by mouth vs. phlebotomy 450 mL biweekly until target serum ferritin reached, or up to 24 months.
Safety Issues-	1. Side effects of phlebotomy or hydroxychloroquine, which are the same as in clinical practice.
Primary Outcome Measures:	1. Time to achievement of a normal plasma total porphyrin level. 2. Tolerability and safety of both treatments
Secondary Outcome Measures:	1. Time to disappearance of a plasma fluorescence peak at neutral pH. 2. Time to normalization of urinary total porphyrins. 3. Time to normalization of the urinary total porphyrin pattern by HPLC 4. Effects of susceptibility factors such as hepatitis C, inherited UROD deficiency, etc. on efficacy and safety of the two treatment methods. 5. Rates of recurrence after each type of treatment and the effects of susceptibility factors on recurrence rates.

Statistical Considerations (sample size and analysis plan):	Time to achieving biochemical endpoints will be determined from individual subject data. Outcome measures such as time to remission will be compared using Cox proportional models to study the effects of susceptibility factors on the hazard ratio to compare the two treatments. Additional modeling will assess factors affecting the frequency of recurrence and seasonality effects using logistic regression modeling and log-rank testing, respectively.
Sponsors (federal, state, foundation and industry support):	National Institutes of Health (NIH)

1.A. Abstract: Porphyria cutanea tarda (PCT) is the most common human porphyria and the most responsive to treatment. There is little information on the natural history of the disease, the effects of treatment, how often treatment fails or how often the disease recurs after successful treatment. Two very different approaches to therapy are considered effective and are standard of care, and additional treatments are becoming available. Guidelines for choosing between the two current standard therapies are not in place, and it is not clear how quickly remission can be achieved with either, which makes comparisons with emerging treatments difficult.

Repeated phlebotomy is the most widely used treatment, but has disadvantages that include discomfort, inconvenience and expense. A low-dose regimen of the 4-aminoquinoline antimalarial drugs, either hydroxychloroquine or chloroquine, is more convenient and cost-effective but is less widely used in the U.S. as first line therapy. Before this study, comparisons of these treatments have been lacking. We need to know the times needed to achieve remission with these treatments in order to develop better treatment recommendations and to provide comparative data to evaluate other treatment approaches, such as the new more rapidly effective drugs becoming available for treatment of hepatitis C (found in ~70% of PCT participants in the U.S.).

This study was initiated as a randomized study to compare treatment for PCT with phlebotomy or low-dose hydroxychloroquine, with retention of non-randomized patients in a substudy to provide additional safety data. Prior to this study becoming a Porphyrias Consortium (PC) protocol, 48 participants with well documented PCT and also characterized for the presence or absence of multiple known susceptibility factors were enrolled at one site and treated by phlebotomy or low dose hydroxychloroquine. Unexpectedly, an interim analysis found that fewer than half of the participants were eligible for randomization, and adherence to treatment and study visits was more difficult for study subjects than had been expected. The interim analysis of 30 randomized and nonrandomized patients who were evaluable because their data documented time to remission showed that for both treatments, time to achieving the primary outcome of a normal plasma porphyrin concentration averaged 6-7 months. The original randomized design did not meet the needs of feasibility and analysis for an inclusive, pragmatic study of a rare disease. Also, because follow up was short, the interim analysis did not establish that the two treatments are comparable in all important clinical respects. This revised protocol continues to follow standard of care procedures for these PCT treatments and includes longer follow up and additional treatment outcomes. Strengths of the study are: (1) it aims to enroll all patients who would otherwise undergo treatment for PCT; (2) it assures that all participants are characterized for multiple PCT susceptibility factors, including ethanol use, smoking, hepatitis C, HIV infection, estrogen use, *HFE* mutations and autosomal dominant

inheritance of a partial deficiency of uroporphyrinogen decarboxylase (UROD) due to *UROD* mutations (as in familial PCT); (3) it provides specific eligibility criteria for assignment to either treatment; (4) it randomizes participants eligible for both treatments, to avoid bias; (5) it defines primary and secondary outcomes for remission and aims to continue treatment until all are achieved; and (6) it provides follow up to compare recurrence rates after achieving remission. Visits are scheduled to document primary and secondary treatment outcomes based on the subject's laboratory values at each visit.

The continuing working treatment hypothesis is that low-dose hydroxychloroquine is comparable to phlebotomy in time to remission. The study is conducted under an active IND (IND 66,042). It is likely that this study will define treatment guidelines and improved management of PCT. It also develops standards for time to specific outcomes for phlebotomy and low-dose hydroxychloroquine, which are not currently available and can be used for comparing future treatment approaches for PCT.

1.B Overview

Brief Summary

The purpose of this study is to determine whether a low dose regimen of hydroxychloroquine achieves remission as rapidly and safely as phlebotomy in the treatment of porphyria cutanea tarda (PCT).

Detailed Description

Porphyria cutanea tarda (PCT) is the most common and also the most readily treated form of porphyria. Repeated phlebotomy is the most widely used therapy in the U.S., but has disadvantages that include discomfort, inconvenience and expense. A low-dose regimen of the 4-aminoquinoline antimalarial drugs, either hydroxychloroquine or chloroquine, is also effective, and is more convenient and less expensive, but has not been widely adopted as first line therapy. Prospective data comparing these treatments are lacking, as are guidelines for selecting the most appropriate treatment for individual patients with PCT.

Study Hypothesis: Treatment of PCT by low-dose hydroxychloroquine, which has known advantages in terms of cost and convenience, can achieve remission as rapidly and safely as treatment by phlebotomy, and the durability of treatment is also comparable.

Comparison(s): This study compares treatment with a low-dose regimen of hydroxychloroquine to treatment by repeated phlebotomy. The primary outcome is time to achieving a normal plasma porphyrin concentration. Secondary outcomes include correction of additional porphyrin abnormalities that are expected with complete remission, and recurrence rates during long term follow up.

Significance: The results will guide clinical practice by providing a basis for selecting which of these standard treatments should be used in individuals with PCT. It will also provide data on time to treatment outcomes with both therapies that can be used for evaluating future therapeutic approaches. The pragmatic design of this study will lead to results that are more immediately applicable to clinical practice and relevant to cost-benefit considerations.

2. Background

Porphyria cutanea tarda (PCT), which has also been known as symptomatic porphyria, porphyria cutanea tarda symptomatica, and idiosyncratic porphyria, results from decreased uroporphyrinogen decarboxylase (UROD, EC 4.1.1.37) activity in the liver, and is characterized by blistering skin lesions on sun-exposed areas. Other features include a varying degree of hepatocellular damage and siderosis (1-3). The disease has been classified into three subtypes: type 1 (sporadic), type 2 (familial, autosomal dominant), and type 3 (familial, rare). In addition, there are occasional cases that result from environmental exposure to polyhalogenated chemicals. Type 2 disease is due in part to underlying heterozygous mutations in the *UROD* gene, which result in a partial UROD deficiency that represents a predisposing factor that is inherited as an autosomal dominant trait with low penetrance. Most of the multiple *UROD* mutations that have been found in type 2 disease result in no enzyme activity, and the residual ~50% UROD activity is therefore the product of the normal allele. The inherited ~50% deficiency of UROD affects all tissues and is most readily demonstrated in erythrocytes. Because penetrance is low, relatives with the inherited enzyme deficiency seldom develop PCT. Therefore, type 2 PCT often presents with symptoms as a sporadic condition and with a negative family history for PCT. *UROD* mutations are not found in patients with types 1 or 3 disease, and erythrocyte UROD activity is not deficient. Type 3 disease is a rare familial form in which more than one member of a family is affected, and either other genetic factors, such as hemochromatosis gene (*HFE*) mutations are identified or an underlying genetic mechanism is not established (1, 2).

All three subtypes of PCT have similar clinical features and management. As discussed below, the mechanism of UROD inhibition leading to a profound deficiency of hepatic enzyme activity is probably the same in all types of the disease. Type 1 or “sporadic” PCT is the most common and accounts for about 80% of PCT patients. Type 2 accounts for most of the remaining ~20% of cases. In types 1, 2 and 3, PCT becomes manifest only when the hepatic enzyme level is reduced substantially – i.e. to less than ~20% of normal (2). Iron is essential in causing a substantial reduction in hepatic UROD activity. The effect of iron is not mediated by direct inhibition of UROD, but rather by enabling the oxidation of uroporphyrinogen to uroporphyrin and a UROD inhibitor (see Figure 1). Cytochrome P450 enzymes are also involved in this process. CYP1A2 is especially important, at least in rodents (2, 4). The inhibitor of hepatic UROD has been identified as a uroporphomethene (5). With UROD inhibition, porphyrins (derived from the highly carboxylated porphyrinogens that are substrates and intermediates in the 4-step decarboxylation catalyzed by UROD) then accumulate in large amounts in the liver, and subsequently become markedly increased in the plasma and urine. The distinctive pattern of excess porphyrins in urine and plasma consists primarily of highly carboxylated porphyrins. Fecal porphyrins may be normal or somewhat increased, and an increase in fecal isocoproporphyrins is often prominent, and is readily explained by deficient hepatic UROD activity. UROD is inhibited and amounts of porphyrins in liver, plasma and urine are markedly increased and serve as biomarkers in PCT, and these become normal with treatment and remission. However, it is not known whether hepatic δ-aminolevulinic acid (ALA) synthase (ALAS1), the rate limiting enzyme for hepatic heme biosynthesis, is increased in PCT, and whether this enzyme activity decreases with treatment (1, 2). In the past ALAS1 could only have been assessed if a biopsy was done repeatedly to obtain samples of liver tissue After this project with initiated, it was discovered that hepatic ALAS1 can be assessed noninvasively by measuring ALAS1 mRNA in exosomes that are found in plasma and urine. This provides a closely related new biomarker to assess disease activity and response to treatment in the acute hepatic porphyrias [] and possibly in PCT as well. This study assesses PCT

patients biochemically using previously known biomarkers, and also clinically before and after application of either of the two standard treatments for PCT, and provides a unique opportunity to assess the potential of exosomal ALAS1 mRNA as an additional and closely related biomarker in PCT.

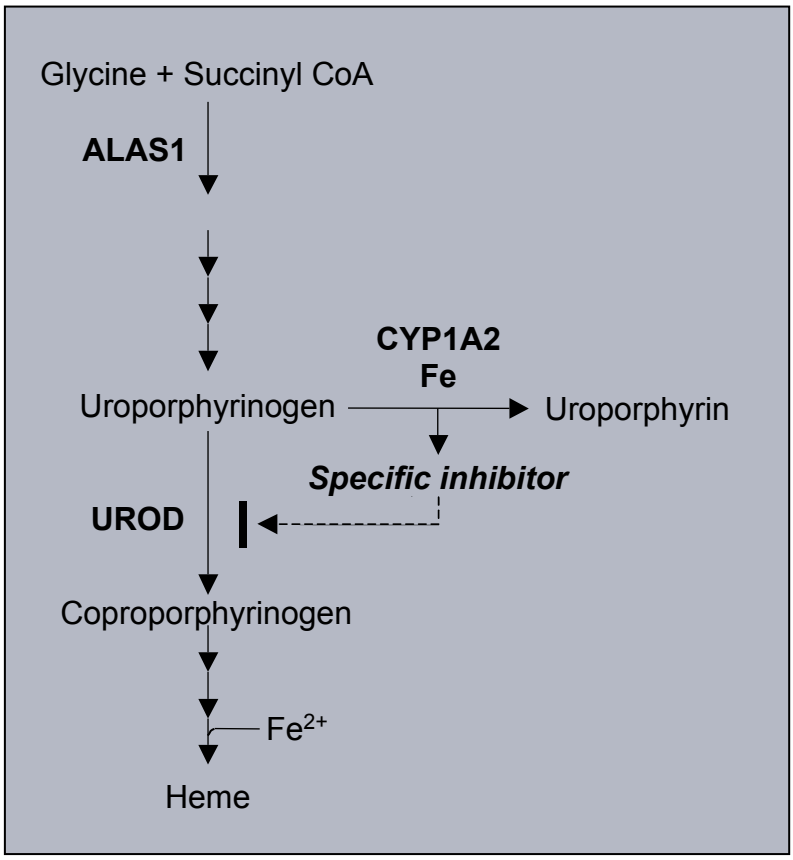


Figure 1. Postulated generation of a UROD inhibitor from uroporphyrinogen in liver in the presence of iron and cytochrome P450 enzymes.

Multiple susceptibility factors can contribute to inhibition of hepatic UROD in all subtypes of the human disease. These factors include alcohol, hepatitis C, estrogen, HIV, smoking and mutations of the *HFE* gene (6-14). Some factors, including *HFE* mutations, alcohol and hepatitis C, can decrease hepatic production of the iron regulatory hormone hepcidin and thereby upregulate iron absorption and contribute to an increase in hepatic iron content (15, 16). More than one of these factors, and often 3-4 or more, are usually present in the individual patient (13, 14). Thus, PCT is a multifactorial condition, and is also heterogeneous, in that combinations of susceptibility factors differ from one patient to another. Hepatitis C is found in up to 80% of PCT cases in some geographic areas. The disease is quite infrequent in some regions, such as Scandinavia where the prevalence in hepatitis C is relatively low. Heavy ethanol use has been recognized as a contributing cause for many years, and smoking has been considered more recently (13, 14). Ethanol, other components of alcoholic beverages, and smoking may contribute to oxidative stress through induction of cytochrome P450 enzymes in the liver. PCT is generally more common in males, possibly due to greater prevalence of some of these susceptibility factors. Some protection may be afforded by menstrual blood loss and consequent less iron retention in women. When PCT occurs in women it is often attributable in part to estrogen-containing oral contraceptives or postmenopausal estrogen replacement (1, 13). Other recently studied factors include functional hepatic biotransformation enzymes (CYP1A2 and GSTM1) and vitamin C deficiency (17, 18).

As reviewed elsewhere (1, 19), some degree of liver damage is present in virtually all patients with untreated PCT, including those without liver-damaging susceptibility factors such as hepatitis C, excess alcohol use or marked iron overload. How marked UROD deficiency might lead to hepatocellular damage is not known, but the massive hepatic porphyrin accumulation that is known to occur in this disease may contribute. Liver dysfunction can improve with treatment of PCT at least in some patients.

Liver biopsy is of value in the management of PCT. The marked excess in porphyrins found in hepatocytes in PCT patients biopsied before treatment is a specific histological finding. But other histopathological features, including excess iron, are quite nonspecific. Because few of the susceptibility factors for PCT are readily evident by liver histopathology, liver biopsy does not contribute greatly to classifying PCT patients. However, liver biopsy may lead to recognition of unsuspected liver conditions, such as nonalcoholic steatohepatitis (NASH), determine the degree of advancement of any associated liver disease and the degree of iron overload with or without *HFE* mutations. A higher prevalence of diabetes and NASH has been reported in some PCT case series (20, 21)

Although PCT is the most common porphyria and occurs worldwide, its prevalence is less well characterized than that of other porphyrias (1, 2). Physicians other than porphyria specialists consider the disease to be rare and are not familiar with issues related to diagnosis and treatment. Commonly cited prevalence estimates for the United States and the Czech Republic/Slovakia are about 1 in 25,000 and 1 in 5,000, respectively (2, 12, 22, 23). Translation of these estimates to the population of the United States of ~300 million indicate that the prevalence of PCT in this country is less than 200,000 cases, as shown in the table below.

Country	Estimated prevalence	Number of cases in a population of 300 million	Reference
United States	1 in 25,000	12,000	(22)
Czechoslovakia	1 in 5,000	60,000	(23)

The yearly incidence of PCT in the United Kingdom was estimated at 2 to 5 per million (2). If this estimate is applicable to the U.S., which has a population of ~300 million, the incidence would be up to 1,500 cases per year. If treated, the disease is likely to be of shorter duration than one year. Therefore, all estimates of which we are aware indicate that the prevalence of PCT is less than 200,000 in the U.S. PCT was reported as prevalent in the Bantus of South Africa in association with iron overload (24), but other risk factors such as hepatitis C may have played a role and were not studied.

Treatment. PCT is the most readily treated human porphyria. Phlebotomy and a low-dose regimen of one of the 4-aminoquinoline antimalarials, chloroquine or hydroxychloroquine, are specific forms of treatment in PCT that are virtually always effective in all PCT subtypes. Either treatment leads to reductions and usually normalization of porphyrin levels, new blisters cease and scarring then resolves more gradually. However, studies comparing these treatments and guidelines for choosing treatment are lacking. *This study addresses the current major issues regarding treatment of PCT in clinical practice.*

These treatments are highly specific for PCT. Other porphyrias, particularly variegate porphyria (VP), as well as hereditary coproporphyria, and even mild cases of congenital erythropoietic porphyria and hepatoerythropoietic porphyria can produce the same cutaneous lesions, but are unresponsive to phlebotomy or 4-aminoquinolines. Therefore, it is important to establish a diagnosis of PCT and exclude other porphyrias such as VP before specific treatment is initiated. As a practical matter, specific treatment can be started after excluding VP by a screening plasma porphyrin determination (including analysis of the fluorescence spectrum at neutral pH) while urine and fecal studies are still pending (25, 26). As noted below, chloroquine and hydroxychloroquine may initially increase porphyrin levels before remission occurs. These drugs are effective in children, in whom phlebotomies may be more difficult (27-29). Other antimalarials, including 8-aminoquinolines, have no effect on the disease.

Patients should cease exposure to alcohol, estrogens, iron supplements, or other exogenous agents that are judged to have contributed to the disease. Estrogen replacement (preferably transdermal) can be resumed in postmenopausal women after successful treatment of PCT (30). Drugs such as barbiturates, phenytoin, and sulfonamides, which are harmful to patients with acute porphyrias, are seldom reported to precipitate PCT, but they may contribute and should be avoided as a precaution (1). Although some patients improve dramatically after the cessation of alcohol (31), the results are generally unpredictable or slow (32). Therefore, it is generally advisable to begin phlebotomy or low-dose chloroquine or hydroxychloroquine as well. PCT may improve when coexisting hepatitis C infection is treated with interferon (33, 34). However, because until recently treatment of hepatitis C had been lengthy and often not effective, it is preferable to treat PCT initially by phlebotomy or low-dose hydroxychloroquine and assess the need for treatment of hepatitis C later. This may change given the newer direct-acting antiviral treatments for hepatitis C, however studies need to be done to assess this.

Each of these standard treatments are described in some detail below, and advantages and disadvantages of each are listed.

Treatment by phlebotomy. Phlebotomy was introduced for treatment of PCT by Ippen in 1961, is still most widely used as standard therapy and can induce remissions in almost all patients (31). This approach was prompted in part because patients with PCT commonly have mild or moderately increased levels of hemoglobin (probably due to chronic pulmonary disease resulting from smoking). The original aim was to normalize the hemoglobin level, stimulate erythropoiesis and perhaps channel excess porphyrins to hemoglobin synthesis in the bone marrow (35). It is now appreciated that the major biochemical pathology in PCT is confined to the liver, and the intermediates that accumulate there and then in plasma are primarily oxidized porphyrins that are not available as reduced porphyrinogens to re-enter the heme biosynthetic pathway. Therefore, the rationale for this treatment has changed. The current rationale is to reduce total body iron stores and liver iron content in order to interrupt oxidative formation of a UROD inhibitor. To gradually reduce excess hepatic iron, about 450 mL of blood can be removed at intervals of ~2 weeks. In one series, an average of 5.4 phlebotomies was required to induce a remission (36). The most valuable guides to the efficacy of phlebotomy therapy are plasma (or serum) levels of ferritin and porphyrins (37, 38). Phlebotomies should be stopped when the serum ferritin reaches the lower limit of normal, and the plasma porphyrin level will then fall to normal, usually within several weeks (19). At this point, skin lesions and friability are usually improved but not fully resolved, and will continue to gradually improve. Hemoglobin or hematocrit levels should be repeated during the course of the phlebotomies to prevent development of significant anemia. The tolerated level of hemoglobin depends upon the initial level and the age and clinical condition of the patient. In most patients the blood hemoglobin should not fall below 10-11 g/dl, and probably should be maintained at a higher level in elderly patients and those with significant concurrent medical conditions.

Pretreatment plasma porphyrin levels in PCT are generally 10-25 mcg/dL (normal <0.9 mcg/dL). New skin lesions are unlikely after the plasma porphyrin concentration becomes normal. Friability, hypertrichosis, milia and areas of atrophy, scarring and hyper- or hypopigmentation improve over periods of weeks or months after this. Even scarring and the more extensive scarring and contraction (pseudoscleroderma) that sometimes occur in PCT can disappear (31), but showed little or no improvement in one series (39). Liver function abnormalities can also improve (40). Siderosis, needle-like inclusions, and red fluorescence in liver can be expected to improve or disappear, although other histological abnormalities may not (41, 42). After a remission is obtained, continued phlebotomies are usually not needed even if ferritin levels later increase to typical normal levels. However, it is advisable to follow porphyrin

levels and reinstitute treatment if porphyrin levels begin to rise. Relapses are reported to respond to another course of phlebotomies.

The time to remission with phlebotomy is not well established in the literature to serve as a standard to compare with other treatments, since many studies did not follow ferritin and plasma or urine porphyrin levels during treatment. Reports that phlebotomies for as long as one year are necessary for remission (43, 44) are not consistent with experience at most centers. Our experience and the interim analysis for this study indicate that with good adherence to scheduled phlebotomies the target ferritin can be reached in 3-4 months in most cases, and remission can be achieved in 6-7 months (19, 45).

Most patients with PCT have some degree of hepatic siderosis, but not marked iron overload (36). Therefore, only about 6-8 phlebotomies are needed to induce a remission in most cases. However, if the initial ferritin is markedly increased it can be predicted that more than the usual number of phlebotomies will be needed. As discussed above, many such individuals have hereditary hemochromatosis and are homozygous for the C282Y mutation of the *HFE* gene (especially among individuals of northern European origin) or are C282Y/H63D compound heterozygotes. Therefore it is important to measure serum ferritin, iron, and transferrin saturation, and to screen for the C282Y and H63D mutations in the *HFE* gene prior to starting phlebotomies. Infusions of deferoxamine, an iron chelator, or orally administered iron chelators, may be an alternative approach when phlebotomy is contraindicated, but are much less efficient for iron reduction (46, 47).

Advantages of phlebotomy can be summarized as follows:

1. Successful use for more than 50 years
2. Rationale is strong for treatment of an iron-related disease
3. Frequent visits facilitate monitoring of progress during treatment
4. Target laboratory value (ferritin) for completing therapy is well established

Disadvantages of phlebotomy include:

1. Expense
2. Discomfort, inconvenience and time requirements
3. Visits to clinic or blood bank required
4. Sometimes poorly tolerated (syncope, symptoms of iron deficiency, venous access problems, etc.)

Chloroquine and hydroxychloroquine. A low-dose regimen of these 4-aminoquinoline drugs is considered to be effective and a suitable alternative treatment when phlebotomy is contraindicated or difficult, and when there is not marked iron overload (48), as assessed by serum ferritin, liver biopsy or *HFE* mutations (particularly homozygosity for C282Y). This is the preferred therapy at some centers, especially for patients without marked iron overload (48, 49). These closely related drugs are interchangeable for this purpose, although hydroxychloroquine is considered to be safer in terms of retinal toxicity (50).

It was noted initially that the larger dosages of chloroquine or hydroxychloroquine used for other diseases such as rheumatoid arthritis may, when administered in patients who also have PCT, induce fever, malaise, nausea and marked increases in urinary uroporphyrin and heptacarboxylate porphyrin, plasma porphyrins, serum transaminases, other liver function tests and ferritin levels. In addition, cutaneous manifestations of PCT can be acutely increased.

These are manifestations of acute hepatic damage and the release of large amounts of stored porphyrins from the liver. Indeed, chloroquine administration can unmask previously unrecognized PCT (51, 52). But it was also noted that these adverse effects of 4-aminoquinoline compounds, which are unique to PCT, are transient and followed by complete remission of the disease (53). Chloroquine-induced remission was observed even with continued estrogen treatment for prostate cancer (54). Later, it was found that a low-dose regimen of either chloroquine (e.g., 125 mg by mouth twice weekly) or hydroxychloroquine (e.g. 100 mg by mouth twice weekly) was preferred compared to standard doses, because porphyrins are mobilized from liver more gradually with little or no increase in plasma porphyrins or hepatocellular damage. Treatment may be continued until plasma or urinary porphyrins are normalized (55-58), but the endpoint of treatment is not well established. Some patients have required later treatment with larger doses, and there is at least some risk of retinopathy (58). In a retrospective study of 62 German patients, low-dose chloroquine was effective in all but the 3 patients who were homozygous for the C282Y mutation of the *HFE* gene (48), which suggests that the degree of excess hepatic iron may influence this treatment response.

The mechanism of the effects of these antimalarial drugs in PCT is not established. Liver tissue obtained before and after standard doses of chloroquine showed that acute increases in urinary porphyrins occurred when liver porphyrin concentrations were decreasing, suggesting that mobilization of porphyrins from liver rather than increased porphyrin synthesis accounted for the transient increase in urinary porphyrins (54). These agents ameliorate hexachlorobenzene-induced porphyria in rats, which like human PCT is associated with decreased hepatic UROD and a similar pattern of accumulated porphyrins (59-62). Chloroquine concentrates in liver, and particularly in lysosomes and other acidic intracellular organelles, and may form complexes with many different types of porphyrins that are then more readily mobilized from the liver (62, 63). However, studies by Scholnick and coworkers (63), which are frequently cited to support this mechanism, were in a rat model in which the dicarboxylate porphyrin protoporphyrin accumulates due to inhibition of ferrochelatase, the final enzyme of the heme biosynthetic pathway. By contrast, in PCT primarily uroporphyrin (octacarboxylate porphyrin) and heptacarboxylate porphyrin accumulate due to inhibition of UROD. Studies in animals loaded with hematoporphyrin (also a dicarboxylate porphyrin) did not show an effect of chloroquine to mobilize the administered hematoporphyrin from tissues, and therefore did not support the notion that chloroquine can mobilize a variety of porphyrins from liver and other tissues (64). Interestingly, urinary excretion of heptacarboxylate porphyrin increased in rats treated with hematoporphyrin and chloroquine (65). Moreover, the lack of efficacy of chloroquine in other porphyrias with deficiencies of heme pathway enzymes other than UROD, such as variegate porphyria and congenital erythropoietic protoporphyria (66) indicates that its mechanism of action in PCT is highly specific and not related to nonspecific binding of a variety of different porphyrins. Other mechanisms, such as mobilization of hepatic iron (55, 66) or inhibition of uroporphyrin synthesis (67, 68) have been suggested. Although it is reported that chloroquine does not reduce hepatic siderosis, at least acutely (54), iron excretion may increase in some patients (55, 58) and serum iron markers may improve (48). Low dose chloroquine may decrease hemosiderin deposition in liver, but otherwise has little effect on liver histology (69). If the response to 4-aminoquinolines relates to the degree of excess hepatic iron, as suggested by a report that C282Y homozygotes are treatment-resistant (48), the mechanism of response may indeed involve hepatic iron. However, it seems most likely that because 4-aminoquinolines are taken up by lysosomes they may in the presence of large amounts of porphyrins, disrupt these intracellular organelles, leading to porphyrin release and transient hepatocellular damage, especially with higher doses. But at present this and other mechanisms remain unproven even though the clinical usefulness of these antimalarials is accepted at least as an alternate therapy.

Most reports of efficacy of the 4-aminoquinolines in PCT are single cases or small series of patients. To our knowledge, only one randomized study comparing hydroxychloroquine and phlebotomy for treatment of PCT has been reported (44). In that study 61 patients in Milan, Italy were randomized to phlebotomy or hydroxychloroquine 200 mg twice weekly and studied for one year, and better results were reported with hydroxychloroquine at the end of the study. However, this study has several features that make generalization of the results difficult. For example, only 8 of 31 patients (26%) treated by phlebotomy were improved after one year, which is considerably less than expected for this treatment, and serum ferritin levels were not reported. In our experience, phlebotomy is typically effective within ~6 months with good compliance and if the target ferritin level is attained. Moreover, these investigators used a higher dose of hydroxychloroquine than is now customary for low-dose 4-aminoquinoline treatment in PCT. This study illustrates the need for further comparative studies with more frequent observations and the expectation that complete remissions are likely in ~6 months.

Advantages of 4-aminoquinolines for treatment of PCT include:

1. Efficacy supported by reports for more than 40 years
2. Inexpensive
3. Convenient (oral dosing)
4. No visits required for administration of treatment
5. Twice-weekly dosing helps ensure compliance

Disadvantages of 4-aminoquinoline compounds:

- Off-label indication
- No adequate comparison studies to other therapies
- Dosage form not available; tablets not scored for division
- Difficult or inconvenient to monitor course of treatment
- Target laboratory values not established
- Duration of treatment not established
- Standard doses cause adverse effects in PCT
- Low-dose regimen may cause some increases in liver function tests
- Adverse effects on retina unlikely, but still of concern
- Rationale not established and mechanism not known

This study will test the hypothesis that time to remission with treatment of PCT with low-dose hydroxychloroquine is comparable to that with repeated phlebotomy. Although this is not a mechanistic study, the study provides an opportunity for observations in some participants that may enhance our understanding of how 4-aminoquinolines act in PCT. Such efforts to better understand the underlying mechanism are important because they are likely to enhance acceptance of this form of therapy. For example, patterns of porphyrins in urine will be compared during both types of therapy to determine if there is preferential excretion of heptacarboxylate porphyrin, as we observed in chloroquine-treated rats (64). Serum iron, iron-binding capacity and percent transferrin saturation will be measured at each visit to determine if these serum iron markers are affected by low-dose hydroxychloroquine. We will also compare on an optional research basis urinary excretion of iron in participants on each type of therapy, since there is some evidence that chloroquine can mobilize iron from the liver in PCT (48, 55, 59). Hepatitis C plays an important role in many participants with PCT, and it is of interest to know whether or not 4-aminoquinolines might affect this viral infection during treatment of PCT. Therefore, we will measure hepatitis C viral load (serum HCV RNA levels) on an optional basis in

participants with both PCT and HCV during treatment with hydroxychloroquine or phlebotomy. A change in HCV RNA titer may indicate that treatment alters the production and/or release of this virus. Examination of pilot results in these groups of participants will determine if further observations in additional participants are warranted.

Study initiation. This study was started at UTMB in 2006 with grant support from the FDA Office of Orphan Product Development. It was initially conceived as a randomized noninferiority study, with retention of nonrandomized participants to be treated and followed in a substudy, to provide additional safety data. A planned interim analysis of 48 patients enrolled at UTMB was carried out and published in 2012 (45); 4 additional patients were enrolled after the interim analysis, for a total of 52 enrolled at that site. The study then became a Porphyrias Consortium (PC) study with the same design. Additional subjects have been enrolled, mostly at UTMB. However, the interim analysis and discussions with the DMCC and the DSMB have pointed to a need to amend the study, as in this protocol version.

Results of the interim analysis. We analyzed results from 48 consecutive patients with well-documented PCT who were also well characterized for susceptibility factors and were prospectively treated by phlebotomy (450 mL, every 2 weeks until they had serum ferritin levels of 20 ng/mL) or low-dose hydroxychloroquine (100 mg orally, twice weekly, until at least 1 month after they had normal plasma levels of porphyrin) (45). Unexpectedly (1) fewer than half of the patients could be randomized, such that more patients were assigned to phlebotomy than hydroxychloroquine, (2) many treatment and study visits were missed, especially with phlebotomy, and this delayed remission, and (3) due mostly to missed study visits near the time of remission, time to achieve a normal plasma porphyrin concentration (the primary outcome) was evaluable in only 30 of 48 subjects (17 treated with phlebotomy and 13 with hydroxychloroquine, see **Figure 2**). Medium times to the primary endpoint were comparable – between 6 and 7 months for both treatments, when either all of the 30 evaluable patients or only the 17 randomized evaluable patients were analyzed. Noninferiority was not established, and numbers were insufficient to assess effects of individual susceptibility factors on treatment response. Compliance was better with hydroxychloroquine than phlebotomy. It was noted that when plasma porphyrin levels became normal, other measurements, such as urine porphyrin levels and porphyrin patterns, were often still abnormal, suggesting that a normal plasma

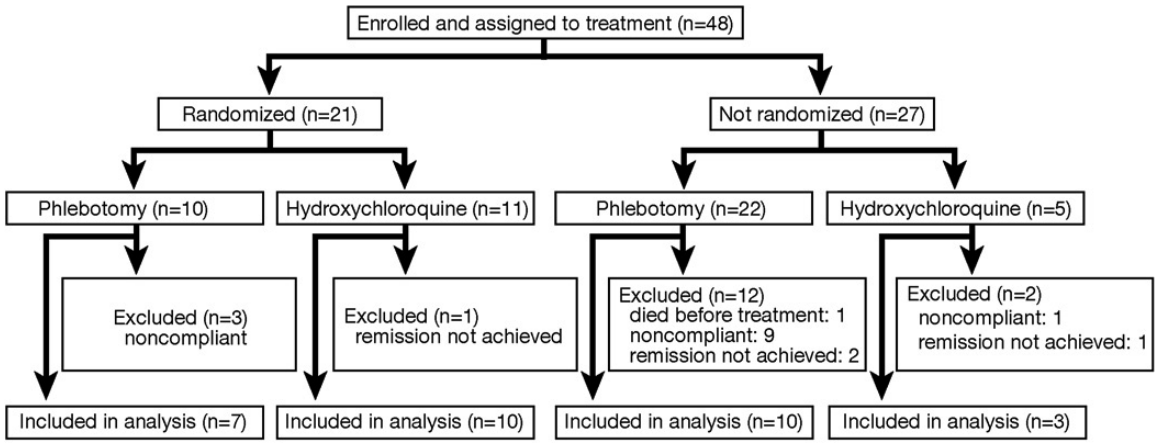


Figure 2. Enrollment, assignment to treatment with or without randomization of 48 study patients with PCT in the interim analysis, and inclusion of the 30 patients who achieved a normal plasma porphyrin concentration in the analysis comparing time to remission with phlebotomy and low-dose hydroxychloroquine.

porphyrin level may not be a sign of full remission. Therefore, continuing treatment with

hydroxychloroquine until these additional outcome measures become normal may be beneficial. There were no significant side effects with either treatment. Follow up was insufficient to compare recurrence rates with these treatments.

Based on these interim findings we concluded that (1) the study should continue with changes to better assess response and durability with these two standard treatments. (2) Time to achieving treatment endpoints in addition to normal plasma porphyrin levels should be assessed. (3) All available PCT patients who require treatment should be enrolled, if possible, and assigned treatment based on medical indications, preference, or randomization (for patients suitable for either treatment). (4) A noninferiority design is not appropriate, because most subjects are not randomized, and outcomes should be compared using descriptive methods. (5) Power analyses to assess effects of clinical features and susceptibility factors should emphasize those of greatest clinical interest. We consider that age and sex are the clinical features of greatest interest, and alcohol use, hepatitis C and *UROD* mutations are susceptibility factors of greatest interest. (6) Longer follow up is needed to assess durability of treatment, and whether this relates to susceptibility factors. (7) The single primary outcome was evaluable in ~2/3 of patients in the interim analysis. Inclusion of additional outcomes is likely to provide evaluable outcomes for a greater proportion of patients. (8) This study also will provide serial data and samples on a cohort of PCT participants in whom treatment responses and complete remission are rigorously documented, which will be valuable for future cross sectional and longitudinal research on the pathogenesis and natural history of this disease.

3. Specific aims

The primary working hypothesis for this study is that time to remission with low-dose hydroxychloroquine is comparable to phlebotomy for treatment of PCT. Because of the very different features of the treatments, the study will be unblinded, but the major outcomes in terms of efficacy will be measurements that are objective. The study follows standard of care procedures for treating PCT, but assures that all participants have well-documented PCT, are characterized for multiple susceptibility factors, are assigned to treatment based on consistent criteria, and documents remission and recurrence of the disease, which are aspects often not accomplished in clinical practice.

The specific aims for testing the hypothesis are:

1. Enroll up to 100 participants, including those enrolled to date, in a prospective, unblinded study comparing treatment by phlebotomy with treatment by low-dose hydroxychloroquine. Specified eligibility criteria will be used for treatment assignment and participants eligible for both treatments will be randomized, to avoid bias. Tolerability and safety of both treatments will be assessed by interviews and questionnaires.

2. Times to defined primary and secondary treatment outcomes will be compared and subjects will be followed for up to 5 years to assess durability of treatment.

3. Characterize all participants for the presence or absence of known susceptibility factors for PCT, including ethanol use, smoking, hepatitis C, HIV infection, estrogen use, *HFE* mutations and an inherited partial deficiency of UROD (as in familial, type 2 PCT), the latter as assessed by measuring erythrocyte UROD activity and DNA studies for *UROD* mutations. Some of these factors (e.g. heavy ethanol use, *HFE* mutations) will influence treatment assignment, and all will be used to determine if individual susceptibility traits influence treatment response and durability.

Use of hydroxychloroquine for treatment of PCT is an off label indication. The results of this study will contribute to future efforts to modify product labeling for hydroxychloroquine to include a specific dosing regimen for treatment of PCT.

4. Research design and methods

This study was initially conceived as a randomized noninferiority study, with inclusion of nonrandomized participants to be treated and followed in a substudy. But as described above, an interim analysis found that more than half the participants were either not medically eligible for both treatments or preferred to choose their treatment, and therefore were not eligible for randomization (45). The results found no major demographic or clinical differences between randomized and nonrandomized participants, and treatment responses were also similar. For reasons noted above, the study will continue with inclusion of all treatment-eligible participants as a more pragmatic study relevant to clinical practice, with assignment to treatment based on uniform criteria and randomization only of participants eligible for both treatments, to avoid unnecessary bias. A descriptive approach to data analysis will be employed, with clinically relevant comparisons of the two treatments. A larger number of subjects will allow assessment of effects of susceptibility factors on treatment response and durability.

Study visits are scheduled at 2-4 week intervals to document achieving a target ferritin concentration in subjects treated by phlebotomy, and for both treatments achieving a normal plasma porphyrin concentration. Visits may be less frequent than 2-4 weeks early in the course of treatment if, for example, during a course of phlebotomies the serum ferritin is still well above the target concentration, or if after phlebotomies are complete or during hydroxychloroquine treatment the plasma porphyrin concentrations are still quite high and not yet approaching normal.

Once a normal plasma porphyrin concentration is achieved visits will then be scheduled at 4-8 week intervals to document achieving the additional treatment outcomes and complete remission of PCT. Less frequent visits may be scheduled if these outcomes are not yet approaching normal, although the time course of improvements in these outcome parameters can be difficult to predict. The expected order of achieving these outcomes with each treatment is as follows:

1. Phlebotomy only: Target ferritin (<20 ng/mL) – expected ~2 weeks after the last phlebotomy. (This requirement can also be met by a ferritin of 25 ng/mL <u>on the same day</u> as the last phlebotomy.)
2. Normal plasma porphyrin concentration
3. Absence of a fluorescence peak in plasma at neutral pH
4. Normal urine total porphyrins
5. Normal pattern of urine porphyrins by HPLC

How quickly each of these targets and outcomes will be achieved will vary greatly among patients. Therefore, levels need to be followed closely to anticipate when visits are needed to document treatment outcomes. If less frequent visits than recommended are contemplated, site investigators should review laboratory results within 3 days of receiving the reports and determine when the next visit and the next set of tests should be done in order to document all primary and secondary outcomes.

<u>To be considered an evaluable outcome, time to achieving each of the study outcomes listed above (e.g. time to achieving a normal plasma porphyrin level) will be documented by an abnormal finding (e.g. a high plasma porphyrin level) followed by normalization of that finding</u>

(e.g. achieving a normal plasma porphyrin level) within a specified time interval, which is referred to as the *documentation window* for achieving that outcome. That interval/documentation window will be 4 weeks for achieving a normal plasma porphyrin concentration, and 8 weeks for achieving the later biochemical outcomes. Therefore, a single missed visit that is not within one of these windows should not lead to a missed verification of a treatment outcome.

The following study practices are recommended to document the major study outcomes:

1. A phlebotomy patient should return every 2 weeks after the ferritin falls to 60 ng/mL, in order to either 1) document a ferritin of 25 ng/mL on the day of the last phlebotomy, or if serum ferritin was not measured on the day of the last phlebotomy, 2) to document a ferritin level of <20 ng/mL ~2 weeks after the last phlebotomy.

2. Once the plasma porphyrin level drops to less than ~4 mcg/dL, this test should be repeated biweekly to ensure that, even if a visit is missed, normalization is captured within the 4-week documentation window, which will properly document time to this primary treatment outcome.

3. After the plasma porphyrin concentration is normal (<0.9 mcg/dL), visits should be scheduled at 4 week intervals until the other outcomes are achieved. If visits are missed intermittently, this will still allow capturing a change from abnormal to normal within an 8-week documentation window.

Participant adherence to the treatment regimen and study visits is tracked as a study objective, as this was found to significantly influence time to remission in the interim analysis. As in the interim analysis (45), noncompliance will be recorded in days. For example, missing a biweekly phlebotomy will be recorded as 14 days of noncompliance. Missing treatment for medical reasons (e.g. hematocrit too low for phlebotomy) will be recorded as medically-related delays rather than as noncompliance, and so described in the CRFs.

Noncompliance from missing a dose of hydroxychloroquine will be recorded as the time in days between the day of the missed dose and the next dose of study drug. With the twice weekly regimen for hydroxychloroquine, a half tablet is taken at an interval of 3 or 4 days. It is permissible to take a twice weekly dose up to one day late, and this should be recorded, and will not be regarded as a day of noncompliance. If a dose is not taken for longer than one day after a missed dose, that dose should be omitted and recorded as noncompliance. The next dose should be taken 3 days later or as scheduled, with an interval no greater than 3 days.

Follow up visits after all treatment outcomes are achieved are to detect biochemical or clinical evidence of recurrence. Additional visits may be scheduled for events such as side effects or early recurrence of PCT.

4.A. Treatment sites and laboratory resources. Treatment sites are the six centers in the Porphyrias Consortium. Other sites with suitable resources and expertise may also be approved.

Samples can be sent to the Porphyria Laboratory at UTMB for all biochemical determinations needed to document the diagnosis of PCT and study outcomes. This laboratory is part of the CLIA- and CAP-approved clinical laboratories for the UTMB Hospital. Samples are sent to this laboratory for diagnostic testing on a daily basis from many physicians and medical

centers in the U.S. The laboratory uses published methods and reports results with a clinically useful interpretation (70-72). Other laboratories can be used if equivalent results can be obtained. Although results from different laboratories are likely to be comparable, this has not been verified. Normal ranges are likely to be less comparable between laboratories than elevated abnormal values. It is desirable to use the same laboratory for porphyrin testing throughout the study. In general, Quest, LabCorp, ARUP or Mayo are suitable for urine and plasma porphyrin measurements. However plasma porphyrin fluorescence peak testing is only done at UTMB and ARUP. The treatment outcomes are based on criteria stated in this protocol rather than on normal ranges stated by different laboratories, which may vary considerably.

Molecular analyses to identify *UROD* mutations are provided by the Genetic Testing Laboratory at the Icahn School of Medicine at Mount Sinai in New York City.

4.B. Design. This is an open-label comparative study of the two standard treatments for PCT. Participants will be assigned to the treatment for which they are eligible, as determined by strict medical criteria as outlined in this protocol. Participants eligible and willing to receive either treatment will be randomized across sites; however the majority of participants are expected to be assigned a treatment based on their medical indications and preference. Based on many decades of clinical experience, both treatments are accepted as effective and safe. Therefore, comparing efficacy and safety will not be an objective of these standard of care treatments. Rather, the main objectives will be to compare time needed to achieve remission, as assessed by documented primary and secondary biochemical and clinical outcomes, and the durability (i.e. lack of disease recurrence) after each treatment. Since phlebotomy and low dose hydroxychloroquine are both considered accepted alternatives for treatment of PCT, costs will be either considered standard of care and charged to insurance or supported by the Porphyrias Consortium grant for those participants without insurance.

4.C. Treatment definitions. For participants assigned to Group 1, the treatment regimen will be hydroxychloroquine 100 mg twice weekly until at least one month after plasma porphyrin levels are normal and other biochemical outcomes (absence of a plasma fluorescence peak at ~619 nm, a normal total urinary porphyrin level and a normal pattern of individual urinary porphyrins by HPLC) are achieved, with a maximum of 24 months of treatment.

Hydroxychloroquine is available only as 200 mg unscored tablets. Participants in Group 1 will be supplied with half tablets containing hydroxychloroquine 100 mg and instructed to take one half tablet twice weekly, and on a specific schedule, such as every Monday and Thursday, or Tuesdays and Friday. Participants will be supplied with tablets that have been halved with a razor blade by the Pharmacy at UTMB and supplied to each of the study sites. The FDA CDER has suggested that with regard to the proposed strength of the half tablet, we follow the procedures outlined for the physical test for the “Uniformity of Dosage Limits” found in the USP, as follows:

- Weigh and record the weight of each whole tablet
- Split the tablet and reweigh each tablet half and record those weights
- Follow the USP physical test for Uniformity of Dosage Units, such that each tablet half should be within a range of 85-115% of the intended 100 mg

This procedure will better assure uniformity of the half tablets used in this study. The UTMB Investigational Drug Pharmacy will supply half tablets to be dispensed to patients at the other sites.

For Group 2, treatment by phlebotomy will consist of removal of one unit (450 ml) of whole blood at intervals of ~2 weeks or as tolerated until a target serum ferritin concentration of ≤20 ng/ml is reached. A serum ferritin of ≤25 ng/ml *on the same day as the last phlebotomy* will also be regarded as a satisfactory target ferritin concentration. The time to achieving this target ferritin level will depend on the initial ferritin level and the participant's compliance with the scheduled treatment visits. Suitable sites for phlebotomies may include 1) an outpatient clinic, 2) a blood bank that performs therapeutic phlebotomies or 3) a clinical research center. The phlebotomy site must provide documentation of the procedure to the site investigator. A site that can obtain a sample for a serum ferritin concentration is most desirable.

4.D. Recruitment and availability. Participants with PCT will be obtained primarily by referral to the participating centers. Physicians in primary care and specialty clinics such as dermatology and gastroenterology are made aware through personal contacts and notices of this treatment study and asked to refer participants. Notices are also sent to primary physicians and specialists in the referral area for each center in order to attract additional participants. We anticipate enrolling ~60 additional participants over the second 5-year cycle of funding of the Porphyrias Consortium to bring the total enrollment to at least 100. Thus the total target enrollment period will be 10 years.

Feasibility of recruitment is based on data from UTMB, which has been studying PCT and capturing all patients seen at that institution for many years. On average, we expect to enroll 8-10 patients with new onset or recurrent PCT per year at UTMB. Over the second 5-year period of Porphyrias Consortium funding we can expect to enroll a total of 40-50 patients at UTMB. With contribution from the other 6 centers, we expect to achieve the recruitment objective of ~60 new patients.

4.E. Enrollment and treatment assignment. Subjects will be enrolled if they meet the inclusion and exclusion criteria shown in Table 1, which should allow inclusion of the great majority of individuals who present with PCT. PCT patients are often unsponsored, and may be unable to afford either of the standard treatments in this study or to attend scheduled study visits. To assure both study adherence and optimal management, treatment and study costs will be covered either by participants' insurance or other funding if the participant does not have insurance, at the discretion of the study site. Reimbursement for travel and time lost for study participation will be at the discretion of each study site. This will help to enable all who need therapy for PCT to be treated within the study.

A history and physical examination will be carried out, which will help document the diagnosis and identify susceptibility factors such as alcohol and estrogen use and smoking, which will then be assessed in more detail by alcohol and smoking questionnaires. The results of the history, physical examination and screening laboratory tests will be recorded on Case Report Forms (CRFs) prepared for this study.

PCT will be documented by appropriate laboratory measurements carried out in the Porphyria Laboratory at UTMB or another reliable clinical laboratory. This can be done before or after enrollment in an outpatient clinic or at an initial visit to a Clinical Research Center (CRC). Participants are considered to be enrolled after they complete the informed consent process, and will be assigned a Patient Number. Subjects found to be not eligible after enrollment will be recorded as screening failures to be replaced. A recruit to replace approach will be utilized to achieve final recruitment goals. A relapse during follow up can be treated within the study, but will not be part of the primary analysis.

Table 1. Inclusion and exclusion criteria for initial and continued enrollment in the study.

Inclusion Criteria:

1. Willing to give informed consent

2. Willing to return to clinic at time points deemed appropriate by the investigator or work with a local physician to have necessary labs done and samples sent to study site

3. Age 18 or greater

4. Women of child-bearing potential must be willing to avoid pregnancy and use an accepted and effective contraceptive method during treatment.

5. Well-documented PCT:
   i. Clinical features- A history of blistering cutaneous photosensitivity and/or skin fragility. Subjects with subclinical PCT who have diagnostic porphyrin elevation but do not have symptoms may also be included.
   ii. Biochemical and molecular findings – a and b are required
      a. A substantial increase in urinary porphyrins [>450 nmol/24 hours or g of creatinine, or more than 1.5-fold increase (relative to ULN of 300 nmol/24 hours or g of creatinine *or* >300 ug/24 hours or g of creatinine, or more than 1.5-fold increase (relative to ULN of 200 ug/24 hours or g of creatinine)] and/or plasma porphyrins [>2.7 ug/dL, or >3-fold increase (relative to ULN of 0.9 ug/dL), and a fluorescence peak at ~619 nm], with a predominance of uroporphyrin and heptacarboxylateporphyrin in urine or plasma, and normal or only slightly elevated urine ALA and normal PBG.
      b. Normal or only slightly elevated erythrocyte porphyrins [<200 ug/dL, or less than 1.5-fold increase (relative to ULN of 80 ug/dL)], unless elevated erythrocyte protoporphyrin are due to a non-porphyric condition in the opinion of the investigator. This is to exclude mild cases of CEP and HEP, which may have urine findings similar to PCT.

   iii.

Exclusion Criteria:

1. Evidence for another cause of blistering skin lesions, such as another type of cutaneous porphyria, or pseudoporphyria
2. Pregnancy
3. Prior treatment by phlebotomy, hydroxychloroquine or chloroquine within one month, unless all appropriate lab results from before treatment was started can be obtained, to document baseline porphyrin levels.
4. Unwillingness to comply with the protocol and required visits
5. Previous treatment as a participant in this protocol.

Pregnant women will not be included in the study, because it is generally recommended that treatment of PCT be delayed in pregnant women until 1-2 months after delivery. Phlebotomies may induce borderline iron status during pregnancy, which may compromise fetal health. Usage of 4-aminoquinoline compounds during pregnancy should be avoided because these drugs cross the placenta and accumulate in ocular tissues of the fetus.

In very rare instances a subject that is an excellent candidate for the study may not meet all inclusion/exclusion criteria. If the investigator feels the subject may still be a good candidate for the study, entrance to the study will be reviewed with the Study Chair and the site's IRB to determine participation. Reasons for the exception must be recorded on the CRF. The objective is to include in the study all patients who need to be treated and obtain information and experience to better define future treatment guidelines.

Documentation of PCT will include consistent cutaneous symptoms and signs, increases in urinary total porphyrins with a predominance of highly carboxylated porphyrins (uroporphyrin, hepta-, hexa- and pentacarboxylate porphyrin) and increased plasma total porphyrin with a plasma fluorescence emission spectrum at neutral pH at 619~620, which is consistent with PCT. Participants with subclinical PCT (i.e. without skin manifestations) and biochemical documentation can also be included. Additional testing will exclude other blistering cutaneous porphyrias such as variegate porphyria, and erythrocyte total porphyrin measurement will exclude congenital erythropoietic porphyria, which rarely mimics PCT. Samples required for these tests prior to treatment include a 24 hour urine collection or a spot urine sample, a blood sample; a random fecal sample is optional but recommended. Testing and laboratory methods will include the following:

- Plasma total porphyrins (25, 26, 64)
- Urinary δ-aminolevulinic acid and porphobilinogen (73)
- Urinary porphyrins (total, and separation by high performance liquid chromatography) (64, 74)
- Erythrocyte protoporphyrin/total porphyrins (75)
- Erythrocyte uroporphyrinogen decarboxylase (76)
- Fecal porphyrins (total, and separation by high performance liquid chromatography) (64, 77) – desirable but not required at baseline

DNA studies to identify heterozygous *UROD* mutations, which are found in ~20% of PCT participants and represent a genetic susceptibility factor, will be done at baseline at the molecular resource for the Porphyrias Consortium at the Icahn School of Medicine at Mount Sinai.

Susceptibility factors that will be assessed and their prevalence observed previously in a series of 143 patients (mean age 52 years, 66% male, 88% Caucasian) seen at UTMB(14) are listed in Table 3.

Table 2. Susceptibility factors to be identified in this study and their prevalence reported in a previous study at UTMB of 143 patients with PCT.

Susceptibility factors	Prevalence(14)
1. Ethanol usage, including amount, duration and how recent (alcohol consumption questionnaire)	87%
2. Smoking history, including amount, duration and how recent (tobacco use questionnaire)	81%
3. Estrogen use, including type, dose, duration and how recent	66% of females
4. Hepatitis C (serum anti-HCV)	69%
5. HIV (serum anti-HIV)	13%
6. *HFE* mutations (C282Y and H63D)	53% (14% with 2 mutations)
7. Inherited UROD deficiency (a) by measuring erythrocyte UROD activity, or	13%

(b) by screening for *UROD* mutations	20%*
**UROD* mutations were found in 25 of 126 UTMB PCT patients (unpublished)	

Given the high prevalence of many of these susceptibility factors, it is not surprising that 3 or more were identified in 70% of patients. HCV infection was significantly associated with other behavior-related factors such as ethanol use (odds ratio [OR], 6.3) and smoking (OR, 11.9) (14). However, there is a biological rationale for increasing susceptibility with each of these behavior-related factors, and it is unlikely that some are confounding.

As shown in Table 3, gene sequencing has been found to be more reliable than measuring erythrocyte UROD for identifying patients with *UROD* mutations, since the erythrocyte enzyme activity is subject to change; it is higher in younger erythrocytes and can be increased with unapparent stimulation of erythropoiesis.

In assessing the effects of susceptibility factors and required sample sizes in this study, we will focus on those of most clinical interest, namely alcohol, hepatitis C and UROD mutations, with estimated prevalence of 87, 69 and 20%, respectively.

Other testing. Because PCT participants typically have abnormalities in liver function tests, other causes of liver disease such as hepatitis B, diabetes mellitus, etc. will be looked for by appropriate laboratory testing as standard of care, and charged to health insurance.

1. Hepatitis B will be screened for by HBsAg testing.
2. Fatty liver disease will be screened for by hepatic ultrasound, if clinically indicated, recognizing that increased echogenicity is consistent with but not specific for hepatic steatosis and lacks sensitivity.
3. Liver biopsy will be recommended, if clinically indicated.
4. Conditions sometimes associated with PCT, such as end stage renal disease and myelofibrosis or other bone marrow disorders impairing erythropoiesis, will be screened for as reasons for selecting therapy.

Additional testing will be done, unless results are already available from within the previous month, to include complete blood counts, serum ferritin, iron, iron binding capacity, transferrin percent saturation and a screening chemistry panel (to include sodium, potassium, chloride, carbon dioxide, blood urea nitrogen, creatinine, total protein, albumin, total bilirubin, glutamic oxaloacetic transaminase [aspartate aminotransferase], serum glutamic pyruvic transaminase [alanine aminotransferase], glucose, uric acid, calcium, and phosphorous).

Liver ultrasound and biopsy will be recommended to participants based on clinical indications per the investigator, these tests will not be done solely for the study. If completed at an outside institution records will be obtained. Tissue will be examined microscopically, and iron content will be measured if possible. Evaluation of liver histology is not required for this study because the results are usually not essential for diagnosis or classification of PCT participants, identifying risk factors, or deciding on treatment options. However, liver biopsy is the best means of assessing liver iron concentration, and is often recommended for assessing the degree of pretreatment damage to the liver associated with hepatitis C, alcohol, steatosis, etc. Results of liver biopsy, when available, will be recorded on the CRFs and tabulated and analyzed with other clinical information that pertains to PCT.

Treatment assignment. Before assigning treatment, subjects will be evaluated for the contraindications to treatment with hydroxychloroquine and phlebotomy listed in Table 3 and 4.

If one of these treatments is contraindicated, the reason(s) will be recorded, and the subject will be assigned to the other treatment. Participants eligible for both will be assigned by randomization.

Table 3. Contraindications to treatment with hydroxychloroquine.

1. Unwillingness to consider this form of treatment
2. Lactating women
3. Psoriasis
4. Significant retinal disease
5. Glucose-6-phosphate dehydrogenase deficiency
6. Recent and continued heavy use of alcohol (defined as 28 or more drinks per week)
7. Recent and continued use of hepatotoxic drugs such as acetaminophen, isoniazid or valproic acid
8. Marked abnormalities in liver function tests at baseline, such as serum bilirubin >3mg/dL, serum alanine aminotransferase >200 U/L, or prothrombin time >16 seconds (or INR >1.4)
9. Advanced renal disease, with serum creatinine >3 mg/dL
10. Poor tolerance or poor response to this treatment in the past
11. *HFE* genotypes C282Y/C282Y or C282Y/H63D and serum ferritin above the normal range (>336 ng/mL)
12. Substantial iron overload, with serum ferritin >500 ng/mL, in the absence of these *HFE* genotypes
13. Marked iron overload (3+ or 4+) on liver biopsy, if available

Continued heavy use of alcohol is unusual in most PCT participants. Most were moderate rather than heavy drinkers, and in our experience most discontinue or greatly reduce their intake of alcohol when so advised during the study. Therefore, we seldom need to exclude hydroxychloroquine treatment in PCT because of heavy use of alcohol. Most participants also smoke cigarettes, and are less successful in reducing or stopping this addiction.

Participants with serum ferritin concentrations >500 ng/mL or with marked iron overload on liver biopsy will not be considered for treatment with hydroxychloroquine, since they will be considered clinically to likely have substantial iron overload and require treatment by phlebotomy. Participants with the *C282Y/C282Y or C282Y/H63D HFE* genotypes often have serum ferritin concentrations in the normal range, do not have iron overload and will be eligible for either treatment. Those with these genotypes and ferritins >336 ng/mL (the upper limit of normal) will not be randomized, since we consider that phlebotomy is indicated with these genotypes when iron overload is evidenced by a high ferritin level, and PCT participants with the *C282Y/C282Y* genotype and high ferritin are reported to be resistant to low dose 4-aminoquinolines (48).

Table 4. Contraindications to treatment with phlebotomy.

1. Unwilling to consider this form of therapy
2. Severe bone marrow dysfunction manifested by anemia
3. Poor venous access
4. Poor tolerance in the past, such as frequent syncopal episodes, or poor response

Most participants with HIV infection and PCT will be eligible for either treatment. HIV participants with PCT generally tolerate phlebotomy and can be assigned to this treatment

unless there is evidence of bone marrow failure, in which case they would be eligible for treatment with low-dose hydroxychloroquine.

Participants with chronic hepatitis C (HCV) and PCT will be eligible for either treatment. Prospective participants will be evaluated for inclusion in PC7210 for HCV treatment first. If they are not eligible or decline participation in PC7210, they will be offered enrollment in PC7206, Patients who are eligible for PC7210 will include PCT patients >18 years old with HCV genotype 1. Major exclusion criteria for this study include patients who are currently receiving PCT treatment by phlebotomy or hydroxychloroquine, alcohol abuse, amiodarone use, other comorbid conditions.

4.F. Treatment Phase. The treatment phase of this study will continue until treatment is completed *and all primary and secondary outcomes have been achieved*.

Group 1: Assigned treatment with low-dose hydroxychloroquine. Half tablets of hydroxychloroquine (100 mg for each half tablet) will be prepared as described above in the UTMB Investigational Drug Pharmacy and supplied to the other sites.

Participants assigned to Group 1 will be instructed to record when they take each half tablet on a diary sheet that is provided to them (see Appendix B), and to bring the record and the bottle of half tablets back to the study site at the time of each visit. Compliance will be assessed at the time of each visit by the dosing record and by counting the half tablets remaining in the medication bottle. Given the convenience of this regimen, we expect good adherence to the regimen, but this will be monitored.

As noted above, it is permissible to take a twice weekly dose of hydroxychloroquine up to one day late, and this should be recorded. The next dose should be taken 3 days later or as scheduled, with an interval no greater than 3 days. If a dose is not taken for longer than one day, that dose should omitted and recorded as noncompliance.

Group 2: Assigned treatment with phlebotomy. Participants in Group 2 will undergo repeated phlebotomies with removal of one unit (~450 ml) of whole blood at intervals of ~2 weeks at a suitable facility at the study site or elsewhere, such as 1) an outpatient clinic, 2) a blood bank that performs therapeutic phlebotomies or 3) a clinical research center. Efforts will be made to identify a suitable facility that is most convenient for the individual subject, in part to encourage compliance with the regimen. It is advantageous to choose a study site that can obtain samples for serum ferritin measurements.

The phlebotomy site must issue a report to document the date of each phlebotomy and the volume of blood removed, and provide a copy to the study site investigator. The information will be recorded in the CRF. If a scheduled phlebotomy is not done (e.g. hematocrit is below the permissible level), the reason should be recorded on the report to the site investigator. It must be determined in advance for each participant how documentation of each phlebotomy will be provided, as customary practices may vary among blood banks and other facilities that do therapeutic phlebotomies. Copies of this documentation will be retained at each site as source documents to verify each phlebotomy. Source documentation will need to be specified if phlebotomies are performed at other sites, such as CRCs and outpatient clinics. If such documentation is not received at the site, the coordinator will reach out to the outside facility to follow up appropriately. If an outside facility is being used for phlebotomies and does not provide documentation of the procedures, the participant will be given such a form to have completed by medical staff at the facility and will be responsible for sending this to the site

coordinator (see Appendix C). The cost of the phlebotomies will be considered standard of care and covered by the patient's insurance or by the Porphyrias Consortium if the patient has no insurance.

At each session, a hematocrit or hemoglobin determination will be done prior to the phlebotomy to assure this is high enough for tolerating phlebotomy. The permitted lowest value for each patient will be specified in advance and prerecorded, and will be no more than 10 points below the starting hematocrit, and no less than ~33. This value will be adjusted upward if there are concurrent medical conditions that need to be considered. The permitted hematocrit value is specified in advance to enhance safety and is not a therapeutic target.

During the course of treatment by phlebotomy, samples for measuring serum ferritin and plasma total porphyrins will be obtained at baseline and at intervals of 2 weeks, or as needed to document achieving the target serum ferritin of ≤20 ng/mL. Documenting a serum ferritin of ≤25 ng/mL *on the same day as the last phlebotomy* will satisfy this requirement. Ferritin levels may not be needed every 2 weeks early in the course of treatment, but should be measured most frequently (biweekly) once the ferritin falls below 60 ng/mL and is approaching the target value of ≤20 ng/mL.

Samples for tests such as blood counts and serum chemistries will be obtained at baseline, during the treatment phase of the study only when clinically indicated, during the post treatment phase when clinically indicated, and at the end of the follow up phase. This is the standard approach to the treatment of PCT by repeated phlebotomy in the U.S. and other countries.

It is best if therapeutic phlebotomies can be done at a site that can also send blood samples for ferritin, plasma porphyrins, blood counts and chemistries and urine porphyrins, when needed. Otherwise, additional visits will need to be arranged for monitoring these test results.

Phlebotomies will be discontinued once the target serum ferritin (≤20 ng/mL) is reached, which is before porphyrin levels and patterns normalize. The ferritin level should be repeated 2 weeks after the last phlebotomy, *unless a ferritin of ≤25 ng/mL was documented on the same day as the last phlebotomy*. Porphyrin levels will continue to be monitored after phlebotomies have been discontinued in order to document times to each treatment outcome.

Most facilities that do phlebotomies require that a consult request or orders be provided. Suggested wording for such requests is as follows, which will need to be modified for each site and permitted hematocrit (or hemoglobin) values specified for each participant:

Please start repeated phlebotomies at intervals of approximately 2 weeks for treatment of porphyria cutanea tarda (PCT). The treatment target is a serum ferritin <u>from the previous visit</u> of less than 25 ng/mL.

1. Please call the patient to schedule the first visit.
2. At each visit, please
    a. obtain an hematocrit
    b. send a blood sample to Clinical Chemistry for serum ferritin and
    c. obtain a blood sample for total plasma porphyrins (green top tube – call extension xxxx for pickup).
3. At each visit, if the hematocrit is less than xxxx, omit the phlebotomy and just obtain the above samples. Ask the patient to return in 1-2 weeks for the next

phlebotomy. By that time the hematocrit should increase to or above xxxx, and allow phlebotomies to continue until the target ferritin is reached.
4. If the serum ferritin from the previous visit is less than 25 ng/mL (i.e. the target ferritin), omit the phlebotomy and just obtain the above samples. After this target ferritin is reached, schedule or ask the patient to schedule a return visit to xxxx Clinic to see Dr. xxxx in 2 weeks.
5. For each patient visit, please send a report recording the date and the amount of blood removed. If a phlebotomy was not done, please state the reason on the report. Reports should be sent to xxxx at xxxx.

Note that the target ferritin for this treatment is ≤20 ng/mL. However, a serum ferritin of ≤25 ng/mL can satisfy this requirement *if it is obtained on the same day as the last phlebotomy*, because it can be assumed that an effect of the last phlebotomy will be to decrease the ferritin further by at least 5 ng/mL. If a ferritin level is not obtained on the same day as the last phlebotomy, a ferritin level should be obtained ~2 weeks later to document a level of ≤20 ng/mL. If the target is not reached, another phlebotomy should be scheduled.

Treatment failure. A complete lack of response to either treatment is unlikely. In the interim analysis (45), 30 participants with adequate numbers of observations achieved a normal plasma porphyrin concentration, and only 4 (2 on each therapy) did not. In 3 of these, partial but still substantial reductions in plasma porphyrin levels and symptomatic improvement occurred; their incomplete remissions were associated with continued use either of estrogen, a known susceptibility factor (1 patient treated with hydroxychloroquine), or carisoprodol, an inducer of hepatic heme and porphyrin synthesis, for chronic back pain (2 patients treated by phlebotomy). The fourth patient, who had HIV infection, anemia, and concurrent treatment with antiretroviral drugs, did not improve with hydroxychloroquine and died of an acquired immune deficiency syndrome–related infection. Therefore, *it is important to continue to record concomitant medications and clinical features during the study and determine if they correlate with incomplete responses to treatment*.

Change of treatment. Treatment may need to be changed due to incomplete response or to side effects or intolerance of the initial treatment. Change of treatment will be a clinical decision by the investigator and the patient, and the reasons will be recorded on the CRFs. The second treatment will be monitored as part of the study with an additional set of the treatment-related CRFs in the same manner as the first treatment. Data collected after a change in treatment will be analyzed descriptively and separately from data collected during the first treatment.

Treatment of recurrences. Patients will be followed after completion of treatment in order to determine the frequency of recurrent disease, as indicated by increases in porphyrin levels with or without recurrent cutaneous manifestations. These recurrences will be monitored as part of the study. Data collected during treatment of recurrences will be analyzed descriptively and separately from data collected during the first treatment. These provisions will provide information about all treatment that is needed for this disease, since repeated courses of treatment greatly increase the impact of the disease.

4.G. Study visits during the Treatment Phase. During the Treatment Phase, participants will be scheduled to return to the study site or be contacted by the study site by telephone at 2 week intervals. A study visit can be scheduled after longer than 2 weeks based on careful review of data from the last visit. If this is contemplated, the investigative team should evaluate clinical features and laboratory results within 1 week, to be sure that a longer visit interval will not impair

capturing timely data within a documentation window. Sometimes study information can be collected by telephone, including recording of patient-reported skin manifestations, and by special arrangements study samples collected locally and shipped to the study site.

The treatment outcomes for this study are documentation of times to normalization of several porphyrin laboratory measurements, which occur in sequence at different times during the Treatment Phase. Study performance will be assessed by whether or not primary study outcomes are documented within specified time windows. *Documenting the time to each outcome requires an abnormal result followed by a normal result within a specified time window.* The windows of documentation for each treatment outcome are shown in Table 5. Experience to date indicates that these outcomes are usually achieved in the order shown. All outcome measures may not be documented in all patients, but with four rather than one outcome measure it is more likely that each patient will achieve documentation of at least some of these outcomes.

Table 5. Treatment outcomes and windows for documentation

Treatment Outcomes	Windows for Documentation of Outcomes
Normal plasma porphyrin level	4 weeks
Disappearance of plasma fluorescence peak at ~619 nm	8 weeks
Normal urine total porphyrins	8 weeks
Normal pattern of urinary porphyrins	8 weeks

Time to achieving each of these treatment outcomes and the location of each of these outcome windows in time during the Treatment Phase will vary among individual participants. For this reason, regular visits should be scheduled based on these windows, and less frequent visits scheduled only after examination of the most recent laboratory results.

4.H. End of treatment. The maximum duration of treatment will be 24 months, but is expected to be shorter for most participants. Treatment by phlebotomy will end when the target ferritin value is reached (≤20 ng/mL, or ≤25 ng/mL on the day of the last phlebotomy). Therefore, it is advantageous, if possible, to obtain ferritin measurements on the days of phlebotomy. Porphyrin levels will start to decrease, but will not be normal when phlebotomies are ended. Therefore the Treatment Phase of the study extends beyond the last phlebotomy until all treatment outcomes are documented. Factors that may lengthen the duration of phlebotomy treatment include marked iron overload, some loss of venous access and difficulty adhering to treatment visits.

Hydroxychloroquine treatment will end after the plasma total porphyrin concentration and other porphyrin abnormalities have been normal for at least one month (i.e. at least one month after all treatment outcomes are achieved). Treatment may be stopped for poor tolerance or other medical reasons, and these reasons will be recorded. For hydroxychloroquine, the desirable length of this treatment and factors that delay response are less well understood, and will be clarified by the results of this study. For this protocol, the duration of hydroxychloroquine treatment will not exceed 24 months.

Either treatment will be discontinued if a participant no longer meets the inclusion criteria or develops one of the exclusion criteria for the assigned treatment. A treatment will also be stopped if an adverse event occurs that is regarded as a reason for stopping the current

treatment or in the judgment of the investigators on clinical grounds is a reason for stopping the current treatment in the best interest of the patient. The reasons for discontinuing treatment will be recorded on the CRFs. If a participant is discontinued from one treatment, they may be switched to the alternate treatment if deemed appropriate by the investigator. Should this happen their information will still be collected according to the schedule described in this protocol and analyzed as part of this study.

4.I. Follow Up Phase. Determining durability of treatment after achieving a complete biochemical remission of PCT is an important objective of this study. Therefore, subjects will be observed long term during the Follow Up Phase for clinical and biochemical recurrences of PCT. This phase will begin after treatment is completed and all treatment outcomes are achieved. These visits will occur, either on site or remotely, every 3-6 months for up to 5 years, or possibly longer, depending on the availability of funding for the study. Symptoms will be monitored, and porphyrin levels measured for early detection of recurrence. Determinations on these visits will include total plasma and urinary porphyrin concentrations, plasma porphyrin neutral pH fluorescence emission peak, urinary porphyrin HPLC, ferritin and liver function tests. These samples may be obtained at the study site or elsewhere as part of standard of care. This will provide information on both clinical and subclinical biochemical recurrence after remission with both types of treatment. If a subject has a recurrence of symptoms their treatment will be followed clinically and they will continue with this follow up visit schedule.

It is important to assess both symptoms and porphyrin levels during follow up. In our experience patient reports of recurrence of skin manifestations are not always reliable, and can be discounted if evaluation at the time finds that porphyrin levels remain normal and no objective skin changes consistent with PCT are present. Conversely, biochemical recurrence with small or large increases in porphyrins can occur in the absence of symptoms. It is suspected that subclinical PCT may be especially common in patients with *UROD* mutations, but this is not well documented.

4.J. Study duration and end of study. The duration of the study including the Follow Up Phase is at least five years. It is possible that the study will be extended further depending on results and the availability of funding. Subjects will enter the study needing treatment, and require up to 24 months of treatment and observation to document achieving the treatment outcomes, followed by at least 3 years of follow-up. Therefore, the most frequent study visits will occur during the Treatment Phase and generally the first ~12 months of the study, which includes active treatment and the treatment outcome windows to document biochemical remission. Based on the interim analysis (45) it is anticipated that the first outcome (a normal plasma porphyrin concentration) will be reached within 6-7 months, on average. Other treatment outcomes should be achieved within ~12 months, although this is uncertain at present, since such observations were not part of the interim analysis.

4.K. Treatment outcomes and schedule of events. A schedule of events in tabular form and a study flow diagram are shown below. This study compares time to reaching certain efficacy outcomes with the two treatments and documents durability of treatment in terms of absence of increases in porphyrins and recurrence of symptoms.

The primary efficacy outcome is achieving a normal plasma porphyrin concentration (<0.9 mcg/mL). Secondary efficacy outcomes include achieving (1) absence of a fluorescence peak in diluted plasma at neutral pH; (2) a normal level of total urinary porphyrins; (3) a normal pattern of individual porphyrins in urine (defined as total highly carboxylated porphyrins less than coproporphyrin); (4) times to achieving intermediate improvements in these porphyrin

measures, which will be defined during data analysis; (5) absence of new skin lesions for at least 3 months; and (6) effects of susceptibility factors such as hepatitis C, inherited UROD deficiency, etc. on achieving these treatment outcomes.

Durability of treatment (i.e. absence of recurrence) is recorded as beginning with the time that treatment achieves the treatment outcomes, and extends to the time of repeat documentation of a normal plasma porphyrin concentration, assuming there have been no interim recurrences of skin manifestations.

4.L. Safety. Safety parameters are based on known side effects of both treatments and include suggestions of the UTMB IRB and the FDA CDER. Participants will be evaluated at the beginning of the study to ensure that they meet the entry criteria and are informed about risks and precautions. They will then be monitored at study visits for assessment of safety. Close monitoring of hemoglobin or hematocrit may occur as a part of standard treatment by phlebotomy. This is not the case for hydroxychloroquine, because initiation of treatment in clinical practice requires only one visit to prescribe the drug, and continued visits are not needed to administer treatment. Therefore, safety monitoring will be as good or better than in clinical practice.

1. History and physical examination

History and physical examination (including height and weight) will be performed at the beginning and end of the study. Results will be recorded on CRFs.

Schedule of Events

	Baseline (Day -60 to Day -1)	Treatment Period (including all treatment outcomes)	Follow Up Period (to monitor for recurrence)
Visit frequency	One visit or as needed	Every 2-4 weeks until plasma porphyrin concentration normal, then every 4-8 weeks	Every 3-6 months
Medical History and physical examination	X		Annually & End of study
Documentation of PCT & porphyrin testing- urine total porphyrins, plasma total porphyrins[#], erythrocyte protoporphyrin, UROD mutation testing, erythrocyte UROD, urine ALA & PBG, fecal porphyrins (optional)	X		
Lab assessment for susceptibility factors- serum anti-HCV (with follow up HCV RNA if positive), serum anti-HIV, HFE mutation testing	X		
Screening Lab tests- serum ferritin, complete metabolic panel[%], CBC with Diff, hemoglobin A1C, Erythrocyte glucose-6-phosphate dehydrogenase screening, PT, aPTT, serum iron & TIBC	X		
Alcohol & Tobacco Use Questionnaires	X		
History of Estrogen Use (type, dose, duration, how recent)	X		
PCT Symptom Assessment	X	X	X

Liver ultrasound and/or liver biopsy (if clinically indicated)	X		
Ophthalmologic Evaluation (only for HCQ participants)	X	Annually	
Plasma and urine (24 hour or spot sample) total porphyrins, quantified & plasma porphyrins fluorescent peak[#]		X	X
Urine pregnancy test	X	X	
Follow Up Lab tests- Hepatic Function Panel with reflex to PT and aPTT if abnormal, HCV RNA if positive at baseline		X	X
Serum Ferritin[@] (follow up needed only for phlebotomy participants)	X	X	X
Dispense HCQ or begin phlebotomies		X	
HCQ twice weekly or ~biweekly phlebotomies[$]		X	
Investigator review of labs to determine next visit		PRN	X
Collection of urine for research testing	X	X	X

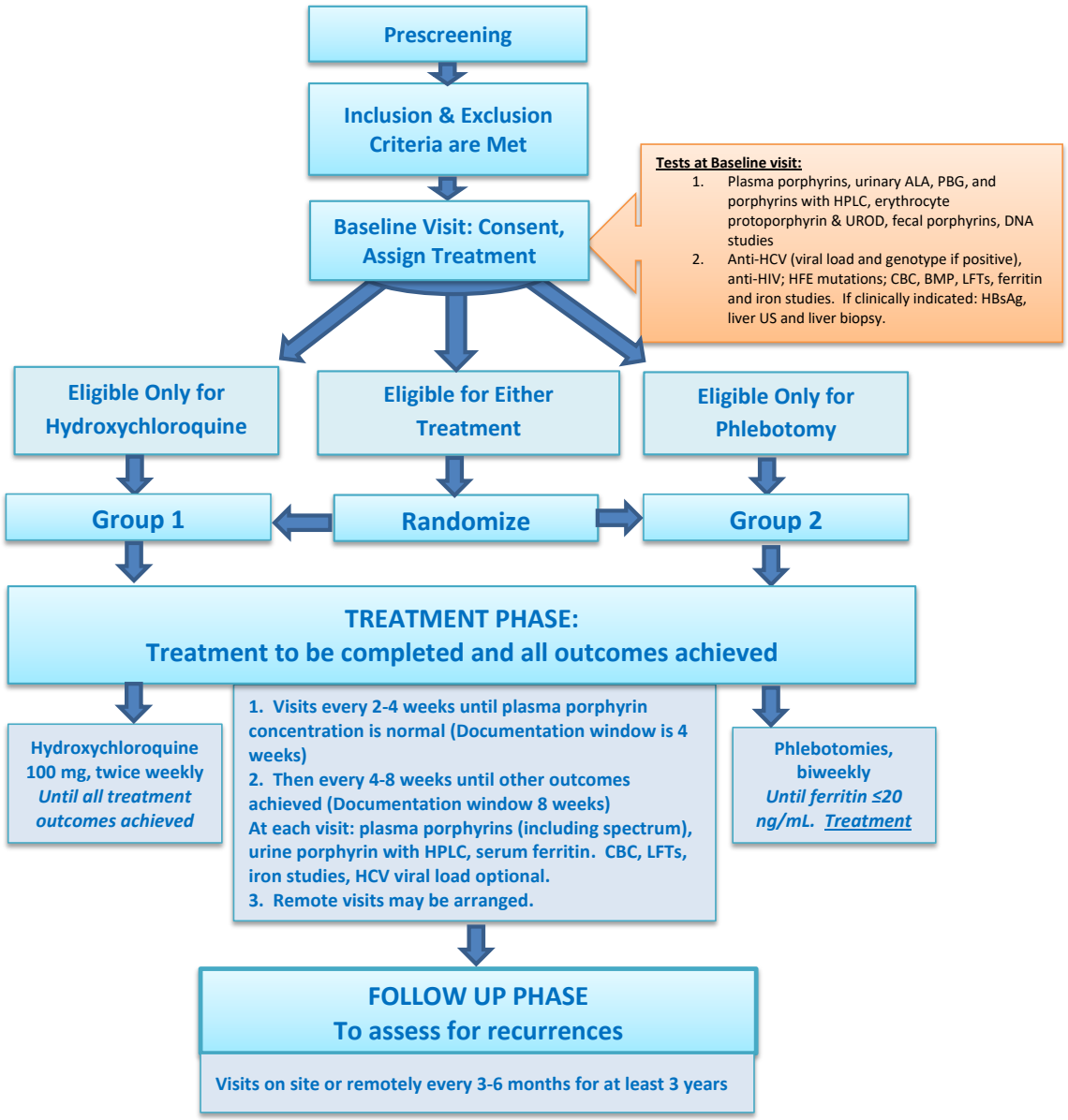
#Plasma porphyrin scan at neutral pH can be done at UTMB or ARUP, urine and plasma porphyrin measurements can be done at UTMB, ARUP, Mayo, Quest or LabCorp. During treatment phase once plasma levels drop to less than ~5 mcg/dL the test should be repeated biweekly

%Complete Metabolic Panel should include- sodium, potassium, chloride, carbon dioxide, blood urea nitrogen, creatinine, total protein, albumin, total bilirubin, glutamic oxaloacetic transaminase [aspartate aminotransferase], serum glutamic pyruvic transaminase [alanine aminotransferase], glucose, uric acid, calcium, and phosphorous

@ Serum ferritin should be done biweekly once the level drops below 60 ng/mL, until the target of ≤20 ng/mL is reached

$ For participants on HCQ the coordinator will need to complete compliance forms monthly. For participants on phlebotomy the phlebotomy visit sheet will need to completed at every phlebotomy visit

Study flow diagram:



2. Laboratory evaluations

The tests listed below are required to assess safety of treatment or detect concurrent conditions that might affect treatment indications. Examples of the latter include advanced liver disease, chronic renal disease and myelofibrosis and other bone marrow disorders. The serum pregnancy test is needed in women of child-bearing potential to exclude pregnancy, because this is a reason for exclusion at the start of the study or for stopping treatment during the study.

a. The following will be measured by the hospital clinical laboratory.

i) Tests done at baseline

• Erythrocyte glucose-6-phosphate dehydrogenase activity

- Prothrombin time
- Partial thromboplastin time
- Total protein
- Albumin
- Total bilirubin
- Aspartate aminotransferase (glutamic oxaloacetic transaminase)
- Alanine aminotransferase (glutamic pyruvic transaminase)
- Iron
- Iron binding capacity
- Transferrin percent saturation
- Ferritin
- Complete blood cell count (to include hemoglobin, hematocrit, red blood cell count, red blood cell indices, white blood cell count, differential white blood cell count and platelet count)
- Sodium
- Potassium
- Chloride
- Carbon dioxide
- Blood urea nitrogen
- Creatinine
- Glucose

ii) Tests done at subsequent visits

- Repeat baseline tests that were abnormal, or are clinically indicated
- Total bilirubin
- Aspartate aminotransferase (glutamic oxaloacetic transaminase)
- Alanine aminotransferase (glutamic pyruvic transaminase)
- Iron
- Iron binding capacity
- Transferrin percent saturation
- Ferritin
- Prothrombin time and partial thromboplastin time if liver function tests are abnormal

A urine or serum pregnancy test will be done at baseline and every 2 months until the end of treatment in premenopausal females; pregnancy is a reason for exclusion at any time during the study.

Hydroxychloroquine will be discontinued if there is worsening of liver disease, as determined by a rise in alanine aminotransferase to >300 U/L, or a two-fold increase from baseline in either total bilirubin or prothrombin time. Participants will also be monitored at each visit for signs or symptoms of worsening liver disease. Phlebotomy will be discontinued if participants are unable to tolerate this treatment at least monthly. Randomized patients discontinued from either treatment may be treated with the alternative method, since an objective of this pragmatic study is to achieve remission in all patients.

3. Ophthalmologic examinations will be done in all participants treated with hydroxychloroquine at the beginning and end of treatment. This is consistent with the recommendations of the American Academy of Ophthalmology as updated in 2011 (Table 7) [78]. Because retinal damage from 4-aminoquinolines is very rare (especially with hydroxychloroquine), it is not expected that ocular effects will be observed in this small study

employing a low dose regimen of relatively short duration. The risk of retinal damage is very low even with the much higher doses used for prolonged treatment of rheumatoid disorders, with the risk increasing if treatment is continued for longer than 5 years or if the cumulative dose is high. In this study, the dose of 100 mg twice weekly averages to a daily dose of less than 0.5 mg/kg for a 70 kg patient, and the duration of treatment will be less than 5 years. Therefore, a second ophthalmological examination will be done at the end of treatment rather than 5 years. Each participating institution will have their own standard practices established by ophthalmology departments based on the guidelines shown in Table 7.

Table 7. Recommendations of the American Academy of Ophthalmology on screening patients treated with chloroquine and hydroxychloroquine [78].	
Timeline	Baseline examination within first year of use
	Annual screening after 5 yrs of use
Recommended Screening Procedures	
Ocular examination	Dilated retinal examinations are important for detection of associated retinal disorders, but should *not* be relied on for screening (low sensitivity).
Automated visual field	White 10-2 threshold testing. Interpret with a low threshold for abnormality, and retest if abnormalities appear.
In addition, if available, perform one or more of the following objective tests	
SD-OCT	Rapid test that can be done routinely; can show abnormalities very early, even before field loss
mfERG	Valuable for evaluation of suspicious or unreliable visual field loss; may show damage earlier than visual field testing
FAF	May validate other measures of toxicity; can show abnormalities earlier than field loss
Not Recommended for Screening	
Fundus photography	Recommended for documentation, especially at baseline, but not sensitive for screening
Time-domain OCT	Insufficient resolution for screening
Fluorescein angiography	Use only if corroboration of pigmentary changes is needed
Full-field ERG	Important for evaluation of established toxicity, but not for screening
Amsler grid	Use only as adjunct test
Color testing	Use only as adjunct test
EOG	Questionable sensitivity
Abbreviations: EOG = electro-oculogram; FAF = fundus autofluorescence; mfERG = multifocal electroretinogram; SD-OCT = spectral domain optical coherence tomography.	

The recommendation in the product labeling for hydroxychloroquine for ophthalmological examination before and at 3 month intervals during treatment for lupus or rheumatoid arthritis is considered outdated and leads to unnecessary health care costs (50), and less frequent examinations are recommended in the recently revised guidelines of the American Academy of Ophthalmology (78).

Participants will be questioned at each visit about specific eye symptoms, such as reading and seeing difficulties, missing objects or parts of objects, missing or blacked out areas, blurred vision, halos around lights, photophobia, light flashes or streaks. Evidence of ophthalmic complications of hydroxychloroquine, although unlikely, will be reason for discontinuing the drug. The patient may then be treated by phlebotomy.

4. Neuromuscular symptoms. Participants will be questioned at each visit regarding neuromuscular symptoms such as sensory changes and weakness, and examined for sensory changes, depression of deep tendon reflexes and loss of muscle strength, which are reported side effects of hydroxychloroquine.

5. Precautions for children. Participants taking hydroxychloroquine will be warned to keep the medication out of the reach of children.

6. Harmful exposures. Participants will be advised to avoid exposure to ultraviolet light (sunlight, tanning parlors, etc.) and alcohol, smoking, iron supplements, estrogens or other substances known to exacerbate PCT. This is part of standard clinical advice given to participants with PCT.

4.M. Efficacy. Efficacy will be assessed primarily by laboratory results. Information on symptoms and signs will also be recorded.

1. Laboratory Tests

The primary measures of efficacy will be plasma total porphyrin concentration, the plasma fluorescence emission spectrum at neutral pH, and urinary porphyrins (total as well as fractionated). The following will be measured at each visit.

- Plasma total porphyrins
- Plasma fluorescence emission spectrum at neutral pH
- Urinary porphyrins (total, and separation of individual porphyrins by high performance liquid chromatography-HPLC)
- Plasma and urinary exosomal ALAS1 mRNA

The following will be done on an optional research basis based on preliminary results from the interim analysis (45):

- Samples will be collected for measurement of urinary iron excretion will be done on a research basis, with precautions to avoid contamination, at baseline and at 1, 3 and 6 months of treatment by graphite furnace atomic absorption mass spectroscopy or inductively coupled plasma mass spectrometry (80, 81).
- Serum HCV RNA – quantitative – will be determined on an optional basis before and at 1, 2, 3, and 6 months (total = 5) in participants with PCT and hepatitis C on either therapy.

2. Cutaneous manifestations

Symptoms related to PCT, such as appearance of new blisters or milia, as well as other symptoms that might result from concomitant conditions such as hepatitis C or from treatment, will be recorded on CRFs at each visit.

At each visit, the number of new lesions, especially blisters and milia, will be counted. The backs of both hands will be photographed, if possible, and kept in the participants study chart to document any new lesions and improvement in the number of lesions.

Pain may occur as a result of cutaneous lesions, but is otherwise not a prominent or characteristic feature of PCT. If participants have pain or other symptoms that can be graded, the following scale will be used. This information will be recorded on CRFs.

Scale	Descriptions for pain and other symptoms
Absent	No pain
Mild	Awareness of pain but easily tolerated.
Moderate	Pain not easily tolerated and interferes with usual activities.
Severe	Pain incapacitating and prevents usual activities.

4.N. Concomitant therapy. Drugs other than hydroxychloroquine will not be allowed in this study for treatment of PCT. Other drugs will be permitted if needed for symptomatic relief or other medical conditions, and will be recorded at the time of each study visit. Estrogens will be stopped until a remission is achieved (30). Iron-containing preparations will be discontinued. Participants will be recommended to stop use of alcohol and tobacco, as is standard for management of PCT. There will be no dietary restrictions. Compliance with these recommendations will be monitored during the study.

4.O. Adverse experiences. At the time of each visit it will be determined whether any adverse experiences have occurred. The participants will be questioned in a general way and no specific adverse symptoms will be suggested, except in regard to the symptoms to be recorded on the CRFs or symptoms that are of particular importance in the clinical management of an individual patient. Any clinical adverse experiences that have occurred will be recorded on the CRFs, and a judgment made regarding whether or not the adverse experience was related to study treatment.

Any serious adverse experience (SAE), including death, due to any cause and occurring during the course of this investigation, whether or not related to the study treatments or procedures will be reported immediately to the Institutional Review Board (IRB) at the site, the IRB at UTMB, the Clinical Research Center at UTMB and the site, if required, and the US Food and Drug Administration.

A SAE with respect to clinical experience includes any experience that is fatal or life-threatening, is permanently disabling, or either requires or prolongs inpatient hospitalization.

4.P. Patient withdrawal. Every reasonable effort will be made to keep participants in the study. If a patient wishes to leave the study or is removed by the PI, a complete final evaluation will be performed. A narrative description of the reason(s) for withdrawal from the study will be recorded in the source documents.

The following are justifiable reasons for removing a patient from the study:

1. Development of a study exclusion criterion, as previously listed
2. If continuing the study presents an unacceptable risk or discomfort to the patient.
3. Violation of the study protocol.
4. The patient is uncooperative or noncompliant in study-related observations or in administration of study medication.
5. Consent is withdrawn.
6. The study is terminated.

4.Q. Treatment failure or intolerance. Participants who fail therapy are expected to be few. Participants who fail or develop contraindications to the treatment assigned will be switched to the alternate treatment and continue to be followed in the study. Reasons for changing treatment will be recorded within the study. Participants who relapse and require retreatment will also remain in the trial. Data from second or subsequent treatments will be collected, but analyzed separately from data obtained during the first treatment in the trial. Rare patients who are not eligible for either treatment will continue to be followed, anticipating that their eligibility may change and treatment will be possible. There is no specific standard-of-care as to when participants are switched to alternate therapy, because failure of treatment is highly unusual and poorly documented with either type of therapy, except when there is noncompliance or other reasons for not completing the therapeutic procedures as planned. The study will provide important new information on patients who need to change therapy for PCT. Participants also have the option of withdrawing from the study at this or any other time.

4.R. Data analysis and statistical considerations. This was originally designed as a randomized noninferiority clinical trial, with inclusion of participants who could not be randomized in a substudy, but with the same treatment and follow up for all participants. We had anticipated that 90% of participants could be randomized, and the intent was to include only randomized participants in the noninferiority analysis. A planned interim analysis, which has been published (45) found that more than half the participants could not be randomized due to medical reasons or patient preference; therefore the interim data was analyzed for the randomized participants and for all participants. Although the treatments appeared equivalent in terms of time to remission, statistical significance was not achieved. Therefore, after formation of the Porphyrias Consortium (PC), the study was continued as a PC study with the goal of enrolling enough participants for a statistically significant result. Based on the interim analysis, the PC investigators identified additional value in continuing this study to enroll a larger number of participants. 1.) Like the PC Longitudinal Study (LS), PC7201, this study enrolls participants with well documented PCT who are characterized for all of the known susceptibility factors, which are multiple but different for individual participants. But unlike the LS, this study carefully documents treatment response and remission, and allows assessment of treatment response and durability as related to specific, multiple susceptibility factors, some of which may change over time. 2.) This study will allow examination of the importance of newly-identified or postulated susceptibility factors, not only for contributing to development of PCT but for affecting treatment responses. 3.) This study is developing for the first time reference information on time to remission for phlebotomy, presently for comparison with hydroxychloroquine. But additional treatment arms may be added to the study as new therapeutic approaches are developed in the future.

The study will continue as a more pragmatic study relevant to clinical practice, with assignment to treatment based on uniform criteria and randomization only of participants eligible for both treatments, to avoid unnecessary bias. Therefore, it will be a two-group study with no substudy. A descriptive approach to data analysis will be employed, with clinically relevant comparisons of the two treatments, rather than a noninferiority analysis.

Inclusion of all available participants rather than just the minority that can be randomized has a number of advantages. Participants eligible for both treatments (and therefore eligible for randomization) might respond differently than those who are not, since some of the reasons that make participants ineligible for one treatment relate to certain susceptibility factors and to prior treatment experience. Exclusion of a majority of participants from treatment comparisons also would make the study less pragmatic and less clinically relevant. In the interim analysis, randomized and nonrandomized participants did not differ in terms of clinical and demographic

features, but such important comparisons would be more meaningful with a larger number of participants. In the amended study, participants who are eligible for both treatments are to be randomized as a method for choosing between treatments that is needed in the study to avoid bias, but not as a study design feature. Bias might arise, for example, from existing site-specific treatment preferences.

A total of at least 100 participants will be assigned, based on medical criteria, preference, or (if eligible for both treatments) by randomization, to one of these standard treatment groups. CRFs will be completed for each patient, including porphyrin levels, and data entered into the study database. This database is designed by and housed at the Data Management and Coordinating Center (DMCC) at the University of South Florida in Tampa. The DMCC is supported by the NIH Rare Diseases Clinical Research Network and serves as the secure repository for de-identified data for all consortia in the Network. Upon approval from the UTMB IRB and the Porphyrias Consortium DSMB, data from patients previously enrolled in this study at UTMB starting in 2006 before the study was a RDCRN study will be entered into the database. Subjects will be contacted to give their consent for this data transfer to the DMCC and storage in a federal data repository. For subjects who are now deceased consent will be obtained from next of kin. For subjects who cannot be re-contacted or where the next of kin cannot be re-contacted, approval of the UTMB IRB and the Porphyrias Consortium DSMB will be sought for this data transfer. Transferring this previously collected de-identified data is not expected to cause any increased risks to subjects. Data will be verified and edited where necessary for completeness and consistency by comparison with source documents. Study outcomes, such as time to achieving biochemical endpoints, will be determined from this data. As was found in the interim analysis, assignment to the two groups is unlikely to be equal, and more will be assigned to phlebotomy than to hydroxychloroquine. As in the interim analysis, times to achieving measures of remission are not expected to be substantially different for the two treatment groups. Effects of multiple susceptibility factors and other clinical and demographic features on treatment response and durability will be further examined. Outcome measures such as time to remission will be compared using Cox proportional models to study the effects of susceptibility factors on the hazard ratio to compare the two treatments. All patients who start treatment will be included in the analysis. Safety measures will be compared more descriptively where the adverse effects of each type of treatment are different.

The following susceptibility factors for PCT will be summarized and tabulated, and examined for balance across treatment groups. Differences in response based on susceptibility factors will be examined by multivariate analysis. The following susceptibility factors will be emphasized in this analysis:

1. Alcohol use (presence or absence)
2. Smoking (presence or absence)
3. Estrogen use (presence or absence)
4. Hepatitis C (presence or absence)
5. HIV (presence or absence)
6. UROD mutations (presence or absence)
7. HFE mutations (presence or absence of C282Y and H63D, and specific genotype combinations)

The analysis regarding susceptibility factors will take into consideration that some (e.g. homozygotes for HFE mutations with high ferritin levels) are medical reasons for assignment to one treatment group and not the other. We recognize that because the number of participants in subgroups based on susceptibility factors or combinations of susceptibility factors will be

relatively small, and it may not be possible to detect or exclude statistically significant differences for all susceptibility factors. The data is expected to support continuing this study with larger numbers of participants, or to suggest whether other studies should be designed to clarify the effects of these factors on treatment response and durability.

Additional modeling will assess factors affecting the frequency of recurrence and seasonality effects using logistic regression modeling and log-rank testing, respectively.

Sample Size and Power. A sample size calculation was performed to assess power for a study assessing the effect of factors on time to remission. Extremely common or rare factors are detected with 80% power for 90 subjects (and some dropout) when the hazard ratios are 2.3 (alcohol consumption and UROD deficiency), 2.2 (smoking history), 3.2 (estrogen usage), 2.0 (hepatitis C), and 1.9 (HFE mutations). In summary, this approach for times to remission leads to a sample size of approximately 50 subjects per group. All analyses were conducted in nQuery Advisor Version 5.0 (Janet Elashoff (2002); nQuery Advisor Version 5.0 User's Guide. Los Angeles, CA).

5. PROTECTION OF HUMAN SUBJECTS

This clinical trial will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with Good Clinical Practice and all applicable regulatory requirements.

5.A. RISKS TO THE SUBJECTS

Human Subjects Involvement and Characteristics. The study will enroll up to 100 subjects age 18 or greater, including nonpregnant females, with well-documented PCT selected as described above. Criteria for inclusion and exclusion are listed above in Research Design and Methods.

Sources of Materials. Research material will be laboratory information collected before and during treatment, as detailed under Methods. While samples of blood, urine and feces are ordinarily collected for clinical purposes in PCT, the number and frequency of urine and blood samples in this study will exceed somewhat those usually obtained.

Potential Risks. The study involves physical risks. *Risks from phlebotomy* and venipuncture include pain, bruising, infection, and syncope. There is a risk of iron deficiency, which may cause mild symptoms in the absence of anemia. *Risks from hydroxychloroquine*, which are generally seen with use of higher doses than in this study, include side effects such as nervousness, nightmares and other psychiatric changes, headaches, dizziness, muscle weakness, blurred vision, other visual disturbances, nausea, vomiting, diarrhea, abdominal pain and hematological changes. Chloroquine (and presumably hydroxychloroquine) overdose is especially dangerous in children. Hydroxychloroquine poses a risk of retinal damage to the fetus, and phlebotomy might impair iron status in both the fetus and the mother, so pregnancy is a contraindication for entry into the study. Specifically in PCT patients, low-dose hydroxychloroquine can result in transient increases in photosensitivity and slight increases in liver function tests and porphyrin levels. These are normal and expected in PCT patients being treated with hydroxychloroquine.

There is also risk of loss of confidentiality. These risks are not greatly increased compared to those involved in standard treatment of PCT.

5.B. ADEQUACY OF PROTECTION AGAINST RISKS

Recruitment and Informed Consent. Participants with PCT will be recruited for these studies through referrals from their physicians and by notices sent to physicians in appropriate specialties, as described in Research Design and Methods. Some participants may be self-referred or be referred through agencies such as the American Porphyria Foundation and the Porphyrias Consortium. Written informed consent will be obtained using a consent form approved by each sites' Institutional Review Board.

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 investigator or delegate will explain all aspect of the study to the participant and the participant will be given adequate time to read the consent form. The participant will have an opportunity to have all their questions answered before signing the consent form. The participant's willingness to participate in the study will be documented in writing on the 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. An additional copy will be placed in the participant's medical record. 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.

Protection Against Risk. Potential risks will be minimized by close monitoring by the investigators and other study personnel. Venipuncture and phlebotomy will be carried out only by trained personnel. Participants treated with hydroxychloroquine will be instructed in detail about keeping the drug away from children. Confidentially will be maintained by identifying data by Patient Number rather than by participants' names, and by storing study records and any copies of medical reports in locked cabinets. Participants will not be identified by name in published reports or presentations. Any adverse effects that do occur will be managed by a study investigator or the patient's physician. A Data and Safety Monitoring Board will also monitor progress of the study and review all adverse events, as described below.

The PI has obtained an IND (66,042) for use of hydroxychloroquine in the study described in this proposal.

5.C. POTENTIAL BENEFITS OF THE PROPOSED RESEARCH TO THE SUBJECTS AND OTHERS

Benefits to study subjects. No benefit for the research subjects in this study can be assured, because both treatments offered are available as standard of care treatments without enrolling in the study. It is possible that a better understanding of treatment of PCT will benefit the subjects should their disease recur in the future. The frequent study visits could also provide some clinical benefit.

Potential benefit to society. The study is likely to benefit future participants and society by establishing better guidance for choosing either phlebotomy of low-dose hydroxychloroquine for treating PCT, which will enhance health care delivery and lower health care costs.

5.D. IMPORTANCE OF THE KNOWLEDGE TO BE GAINED

This study involves a comparison of two therapies already used for clinical management of PCT, and the risks of the two therapies, while different, are generally equivalent, based on present knowledge. Therefore, the study does not involve a degree of risk that is greater than standard clinical care. Moreover, these risks are small relative to the potential benefits to participants with PCT individually and as a group and to society.

The study will lead to improved guidelines for the use of 4-aminoquinolines in treating PCT and hopefully in the future product labeling for this indication.

5.E. INCLUSION OF WOMEN

The study will not exclude participants based on gender or ethnicity. PCT is more common in men than women, and in women is almost always associated with estrogen use. Because we are studying a rare condition, we will have little influence over the gender composition of the participants who participate, but will endeavor to include all women with PCT who are eligible, including women with child-bearing potential. Pregnant women will be excluded, because there is potential risk to the fetus with either of the treatments under study, and we generally recommend delaying treatment until after delivery. There is no evidence to our knowledge that treatment responses are different in women and men with PCT, and we expect that the results of this study will be relevant to women even if the subjects are mostly men.

5.F. INCLUSION OF MINORITIES

PCT occurs in all races, but in a multiracial population such as ours may be more common in light-skinned individuals, who are less protected because they have less melanin pigment in the skin. Blacks may develop manifest PCT less frequently because their greater skin pigmentation provides considerable protection against photosensitivity. Racial or ethnic background may also influence the many risk factors, such as ethanol use and hepatitis C, that contribute to development of PCT. Because recruitment for a rare condition such as PCT is difficult, we will endeavor to recruit all available participants without regard to race and ethnic background. Our clinical experience indicates that there will be fewer Blacks recognized with PCT and entered into the study, but Hispanics will be well represented. Previous experience and published information indicates that race and ethnicity are unlikely to influence responsiveness to treatment of PCT, and results in the subjects enrolled in this study will be applicable to participants with PCT from any ethnic background.

The anticipated gender, racial and ethnic composition of the 100 study subjects is shown in the table below.

Total Planned Enrollment: 100

TARGETED/PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	**Sex/Gender**		
	Females	**Males**	**Total**
Hispanic or Latino	13	27	40
Not Hispanic or Latino	20	40	60
Ethnic Category Total of All Subjects*	33	67	100
Racial Categories			
American Indian/Alaska Native	0-1	0-1	0-1
Asian	0-1	0-1	0-1
Native Hawaiian or Other Pacific Islander	0-1	0-1	0-1
Black or African American	3	7	10
White	30	60	90
Racial Categories: Total of All Subjects *	33	67	100

*The "Ethnic Category Total of All Subjects" must be equal to the "Racial Categories Total of All Subjects."

5.G. INCLUSION OF CHILDREN

No children with PCT who are less than age 18 will be enrolled into this study. Younger children are expected to be less tolerant of repeated phlebotomies. Published information indicates that treatment methods used in adults with PCT are applicable to children, and there is no indication that treatment responses are different. Therefore, even though the study population is mostly adult, the results will be of benefit to children with the disease under study.

5.H. DATA AND SAFETY MONITORING PLAN

The study protocol including amendments will be reviewed and approved by the National Institutes of Health (NIH) and by individual center IRB's. Participant enrollment may only begin with IRB approved consent forms.

This is an interventional phase II study that meets the federal definition of low risk.

The PI and the investigative team will be primarily responsible for day-to-day data and safety monitoring. Adverse events will be reported to the IRB in accordance with the regulations of the IRB at each participating institution, the Data Management and Coordinating Center, the DSMB, NIDDK/NCATS and also to the FDA in accordance with IND regulations.

Observations on participants in the study will be made through scheduled visits, and study results and reports of symptoms reviewed as soon as the information is available by the PI and research team. The safety observations are described in detail under Research Design and Methods. The study involves two treatment regimens that are commonly used in clinical practice for PCT and already considered safe and effective. The major emphasis is on comparing time to remission rather than overall safety and efficacy. The primary experimental intervention in the study is randomization of participants who are eligible for both treatments. The frequency and methods of monitoring are adequate in terms of the risks and complexity of the study, which are both relatively low.

A Data and Safety Monitoring Board (DSMB) is organized by the NIDDK and the Data Management and Coordinating Center (DMCC) for the Rare Diseases Clinical Research Network. The DSMB will review information on study progress and safety information at 6-month intervals, and may elect to do so more frequently. Any Significant Adverse Events or other information that is reported to the IRB will also be reported to members of the DSMB. The DSMB review every 6 months will include information from the CRFs that are compiled for review:

- Number of enrolled subjects and the number providing evaluable data for specific study outcomes.
- Efficacy of treatment as recorded on CRFs in all participants to date, to assure that participants in the study are making expected progress toward remission of PCT
- Safety information recorded on CRFs, including all adverse events (whether considered significant or not), side effects, and laboratory safety information
- Any additional information provided by the investigators, the GCRC Research Subjects Advocate and others related to the study

Members of the DSMB will have the opportunity to ask questions of the PI and other members of the study team and raise any additional concerns. The Board deliberations at 6 month intervals will include presentations by the investigators regarding study progress and compiled information, and also discussions that exclude the investigators before final recommendations are made. The Board will be asked to specifically approve continuation of the study with or without changes, and whether alternative monitoring plans should be instituted. Minutes and other records of the DSMB deliberations will be recorded.

5.H.1. Study Oversight

The Study Chair has primary oversight responsibility of this clinical trial. The NIH appointed Data (Observational) and 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 reports - 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 6 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.

5.H.2. Definitions and Standards

The Rare Diseases Clinical Research Network defines an adverse event as: "…an unfavorable and unintended sign, symptom or disease associated with a participant's participation in a Rare Diseases Clinical Research Network study."

Serious adverse events include those events that: "result in death; are life-threatening; require inpatient hospitalization or prolongation of existing hospitalization; create persistent or significant disability/incapacity, or a congenital anomaly/birth defects."

An unexpected adverse event is defined as any adverse experience…the specificity or severity of which is not consistent with the risks of information described in the protocol. 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

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

5.H.3. Expected/Known Risks/Discomforts/Adverse Events Associated with Study Intervention and Procedures: Definition of Expected Adverse Events

Hydroxychloroquine. In PCT, a low-dose regimen of hydroxychloroquine may cause the following transient side effects before remission occurs:

- worsening of photosensitivity
- slight increases in liver function tests and porphyrin levels

Other reported side effects include those listed below, which are generally seen with use of higher doses than in this study in patients without PCT.

- headache
- dizziness
- loss of appetite
- nausea
- diarrhea
- abdominal pain
- vomiting
- skin rash
- reading or seeing difficulties (words, letters, or parts of objects missing)
- sensitivity to light
- blurred distance vision
- seeing light flashes or streaks
- difficulty hearing
- ringing in ears
- muscle weakness
- bleeding or bruising of the skin
- bleaching or loss of hair
- mood or mental changes
- irregular heartbeat
- drowsiness
- convulsions
- nervousness
- nightmares

- other psychiatric symptoms
- muscle weakness
- hematological changes
- overdose is especially dangerous in children
- retinal damage to the fetus if taken during pregnancy

Other Study Procedures:
Venipuncture for therapeutic phlebotomies and for obtaining samples for testing: The vein in which the needle has been inserted to draw blood may become sore and red. A temporary "black and blue mark" may develop, and rarely fainting may occur.

Therapeutic phlebotomy might impair iron status, but iron status will be monitored to avoid iron deficiency anemia.

Both hydroxychloroquine and iron deficiency entail risks for the fetus, and iron deficiency is a risk to the mother, so pregnancy is a contraindication for entry into the study.

5.H.4. 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 CRC oversight committee and the FDA, if appropriate, remain the responsibility of the treating physician and the Study Chair.

5.H.5. 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, site PIs, the Medical Review Officer, and any additional agencies of any reported adverse events via email.

Serious adverse events: There will be an SAE adjudication committee comprised of the site investigator where the SAE occurred, the study chair, and the Consortium PI. They will review any SAEs that are deemed at least *possibly related* (possibly related, probably related, definitely related) to the study by the site investigator, and will present recommendations for protocol changes or ICF changes to NIDDK and the DSMB for consideration if necessary. The SAE adjudication committee policy will be reviewed and approved by NIDDK and the DSMB prior to initiation. Any changes or modifications to the policy will be submitted for review and approval by NIDDK and the DSMB.

The NIH appointed Medical Review Officer (MRO) reviews the site investigator's assessment of causality of the serious adverse event. The MRO and the sponsor 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. 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.

5.H.6. Study Discontinuation

The NIH, RDCRN 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:

- 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 RDCRN DSMB suspends or closes the trial
- The NIH suspends or closes the trial
- The FDA suspends or closes the trial

5.H.7. 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.

5.H.8. Data Management

The results of the history, physical examination and screening laboratory tests will be recorded on Case Report Forms (CRFs) prepared for this study. Information obtained at each study visit and from telephone will also be recorded on the CRFs. The CRFs will incorporate all the data needed for final analysis of study results.

As much as possible data quality is assessed at the data entry point using intelligent on-line 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. In addition to those described above, we propose to build these checks into the initial tables and cross tabulations that should reveal any remaining data quality issues.

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

5.H.9. 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.

5.H.10. 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.

5.H.11. Data Quality Control

As much as possible data quality is assessed at the data entry point. 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. In addition to those described above, we propose

to build these checks into the initial tables and cross tabulations that should reveal any remaining data quality issues.

6. 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. New York: McGraw-Hill; 2001:2991-3062.
2. Elder GH**.** Porphyria cutanea tarda and related disorders (Chapter 88). In: Kadish KM, Smith K, Guilard R, eds. Porphyrin Handbook, Part II. San Diego: Academic Press; 2003:67-92.
3. Singal AK, Phillips JD**.** Porphyria cutanea tarda. In: Ferreira GC, Kadish KM, Smith K, Guilard R, eds. Handbook of Porphyrin Science. Singapore: World Scientific Publishing Co.; 2013:219-69.
4. Smith AG, Clothier B, Carthew P, Childs NL, Sinclair PR, Nebert DW, et al. Protection of the Cyp1A2(-/-) null mouse against uroporphyria and hepatic injury following exposure to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Toxicol Appl Pharmacol. 2001;173(2):89-98.
5. Phillips JD, Bergonia HA, Reilly CA, Franklin MR, Kushner JP**.** A porphomethene inhibitor of uroporphyrinogen decarboxylase causes porphyria cutanea tarda. Proc Natl Acad Sci U S A. 2007;104(12):5079-84.
6. Roberts AG, Whatley SD, Morgan RR, Worwood M, Elder GH**.** Increased frequency of haemochromatosis Cys282Tyr mutation in sporadic porphyria cutanea tarda. Lancet. 1997;349:321-3.
7. Jackson H, Whatley SD, Morgan RR, Roberts AG, Worwood M, Elder GH**.** The haemochromatosis C282Y mutation in sporadic and familial porphyria cutanea tarda (Abstract). Acta Haematological. 1997;98 S1:114.
8. Roberts AG, Whatley SD, Nicklin S, Worwood M, Pointon JJ, Stone C, et al. The frequency of hemochromatosis-associated alleles is increased in British patients with sporadic porphyria cutanea tarda. Hepatology. 1997;25(1):159-61.
9. Bonkovsky HL, Poh-Fitzpatrick M, Pimstone N, Obando J, Di Bisceglie A, Tattrie C, et al. Porphyria cutanea tarda, hepatitis C, and HFE gene mutations in North America. Hepatology. 1998;27:1661-9.
10. Stuart KA, Busfield F, Jazwinska EC, Gibson P, Butterworth LA, Cooksley WG, et al. The C282Y mutation in the haemochromatosis gene (HFE) and hepatitis C virus infection are independent cofactors for porphyria cutanea tarda in Australian patients. J Hepatol. 1998;28(3):404-9.
11. Sampietro M, Piperno A, Lupica L, Arosio C, Vergani A, Corbetta N, et al. High prevalence of the His63Asp HFE mutation in Italian patients with porphyria cutanea tarda. Hepatology. 1998;27(1):181-4.
12. Bulaj ZJ, Phillips JD, Ajioka RS, Franklin MR, Griffen LM, Guinee DJ, et al. Hemochromatosis genes and other factors contributing to the pathogenesis of porphyria cutanea tarda. Blood. 2000;95(5):1565-71.
13. Egger NG, Goeger DE, Payne DA, Miskovsky EP, Weinman SA, Anderson KE**.** Porphyria cutanea tarda: multiplicity of risk factors including HFE mutations, hepatitis C, and inherited uroporphyrinogen decarboxylase deficiency. Dig Dis Sci. 2002;47(2):419-26.
14. Jalil S, Grady JJ, Lee C, Anderson KE**.** Associations among behavior-related susceptibility factors in porphyria cutanea tarda. Clin Gastroenterol Hepatol. 2010;8(3):297-302.
15. Fujita N, Sugimoto R, Motonishi S, Tomosugi N, Tanaka H, Takeo M, et al. Patients with chronic hepatitis C achieving a sustained virological response to peginterferon and

ribavirin therapy recover from impaired hepcidin secretion. J Hepatol. 2008;49(5):702-10.
16. Ajioka RS, Phillips JD, Weiss RB, Dunn DM, Smit MW, Proll SC, et al. Down-regulation of hepcidin in porphyria cutanea tarda. Blood. 2008;112(12):4723-8.
17. Wickliffe JK, Abdel-Rahman SZ, Lee C, Kormos-Hallberg C, Sood G, Rondelli CM, et al. CYP1A2*1F and GSTM1 alleles are associated with susceptibility to porphyria cutanea tarda. Mol Med. 2011.
18. Sinclair PR, Gorman G, Shedlofsky SI, Honsinger C, Sinclair JF, Karagas MR, et al. Ascorbic acid deficiency in porphyria cutanea tarda. J Lab Clin Med. 1997;130:197-201.
19. Anderson KE. The porphyrias (Chapter 11). In: Zakim D, Boyer T, eds. Hepatology. 3rd ed. Philadelphia: W.B. Saunders Co.; 2003:291-346.
20. Munoz-Santos C, Guilabert A, Moreno N, Gimenez M, Darwich E, To-Figueras J, et al. The association between porphyria cutanea tarda and diabetes mellitus: analysis of a long-term follow-up cohort. Br J Dermatol. 2011;165(3):486-91.
21. Rossmann-Ringdahl I, Olsson R. Porphyria cutanea tarda in a Swedish population: risk factors and complications. Acta Derm Venereol. 2005;85(4):337-41.
22. Harber LC, Bickers DR. In: Photosensitivity diseases: principles of diagnosis and treatment. Philadelphia: Saunders; 1981:189-223.
23. Martasek P, Kordac V, Zonbek V, al. e. Epidemiology of porphyria cutanea tarda in Czechoslavakia and Mongolia. Bolletino dell'Instituto Dermatologica S Gallicano. 1987;8:330-1.
24. Barnes HD. Porphyria in the Bantu races on the Witwaterrand. South African Med J. 1955;29:781-4.
25. 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;87:362-70.
26. Poh-Fitzpatrick MB. A plasma porphyrin fluorescence marker for variegate porphyria. Arch Dermatol. 1980;116:543-7.
27. Timonen K, Niemi KM, Mustajoki P. Skin morphology in porphyria cutanea tarda does not improve despite clinical remission. Clin Exp Dermatol. 1991;16(5):355-8.
28. Poh-Fitzpatrick MB. Childhood onset familial porphyria cutanea tarda: effects of therapeutic phlebotomy. J Am Academy Dermatol. 1992;27:896-900.
29. Bruce AJ, Ahmed I. Childhood-onset porphyria cutanea tarda: successful therapy with low-dose hydroxychloroquine (Plaquenil). J Am Acad Dermatol. 1998;38(5 Pt 2):810-4.
30. Bulaj ZJ, Franklin MR, Phillips JD, Miller KL, Bergonia HA, Ajioka RS, et al. Transdermal estrogen replacement therapy in postmenopausal women previously treated for porphyria cutanea tarda. J Lab Clin Med. 2000;136(6):482-8.
31. Ramsay CA, Magnus IA, Turnbull A, Barker H. The treatment or porphyria cutanea tarda by venesection. Q J Med. 1974;43:1-24.
32. Topi GC, Amantea A, Griso D. Recovery from porphyria cutanea tarda with no specific therapy other than avoidance of hepatic toxins. Br J Dermatol. 1984;3:75-82.
33. Siegel LB, Eber BB. Porphyria cutanea tarda remission. Ann Int Med. 1994;121:308-9.
34. Sheikh MY, Wright RA, Burruss JB. Dramatic resolution of skin lesions associated with porphyria cutanea tarda after interferon-alpha therapy in a case of chronic hepatitis C. Dig Dis Sci. 1998;43(3):529-33.
35. Ippen H. Treatment of porphyria cutanea tarda by phlebotomy. Semin Hematol. 1977;14:253-9.
36. Grossman ME, Bickers DR, Poh-Fitzpatrick MB, DeLeo VA, Harber LC. Porphyria cutanea tarda. Clinical features and laboratory findings in 40 patients. Am J Med. 1979;67:277-86.

37. Rocchi E, Gibertini P, Cassanelli M, Pietrangelo A, Borghi A, Ventura E. Serum ferritin in the assessment of liver iron overload and iron removal therapy in porphyria cutanea tarda. J Lab Clin Med. 1986;107:36-42.
38. Ratnaike S, Blake D, Campbell D, Cowen P, Varigos G. Plasma ferritin levels as a guide to the treatment of porphyria cutanea tarda by venesection. Australas J Dermatol. 1988;29:3-7.
39. Grossman ME, Poh-Fitzpatrick MB. Porphyria cutanea tarda: diagnosis and management. Med Clin North America. 1980;64:807-27.
40. Adjarov D, Ivanov E. Clinical value of serum g-glutamyl transferase estimation in porphyria cutanea tarda. Brit J Dermatol. 1980;102:541-3.
41. Cortés JM, Oliva H, Paradinas FJ, Hernandez-Guío C. The pathology of the liver in porphyria cutanea tarda. Histopathology. 1980;4:471-85.
42. Chlumsky J, Malina L, Chlumská A. The effect of venesection therapy on liver tissue in porphyria cutanea tarda. Acta Hepato-Gastroenterol. 1973;20:124-30.
43. Seubert S, Seubert A, Stella AM, Guzman H, Batlle A. [Results of treatment of porphyria cutanea tarda with bloodletting and chloroquine]. Z Hautkr. 1990;65:223-5.
44. Cainelli T, Padova CD, Marchesi L, Gori G, Rovagnati P, Podenzani SA, et al. Hydroxychloroquine versus phlebotomy in the treatment of porphyria cutanea tarda. Br J Dermat. 1983;108:593-600.
45. Singal AK, Kormos-Hallberg C, Lee C, Sadagoparamanujam VM, Grady JJ, Freeman DH, Jr., et al. Low-dose hydroxychloroquine is as effective as phlebotomy in treatment of patients with porphyria cutanea tarda. Clin Gastroenterol Hepatol. 2012;10(12):1402-9.
46. Rocchi E, Gibertini P, Cassanelli M, Pietrangelo A, Borghi A, Pantaleoni M, et al. Iron removal therapy in porphyria cutanea tarda: phlebotomy versus slow subcutaneous desferrioxamine infusion. Br J Dermatol. 1986;114:621-9.
47. Rocchi E, Cassanelli M, Ventura E. High weekly intravenous doses of desferrioxamine in porphyria cutanea tarda. Br J Dermatol. 1987;117:393-6.
48. Stolzel U, Kostler E, Schuppan D, Richter M, Wollina U, Doss MO, et al. Hemochromatosis (HFE) gene mutations and response to chloroquine in porphyria cutanea tarda. Arch Dermatol. 2003;139(3):309-13.
49. Valls V, Ena J, Enriquez-De-Salamanca R. Low-dose oral chloroquine in patients with porphyria cutanea tarda and low-moderate iron overload. J Dermatol Sci. 1994;7(3):169-75.
50. Easterbrook M. An ophthalmological view on the efficacy and safety of chloroquine versus hydroxychloroquine. J Rheumatol. 1999;26(9):1866-8.
51. Thornsvard MAJCT, Guider BA, Kimball DB. An unusual reaction to chloroquine-primaquine. JAMA. 1976;235:1719-20.
52. Liu AC. Hepatotoxic reaction to chloroquine phosphate in a patient with previously unrecognized porphyria cutanea tarda. West J Med. 1995;162(6):548-51.
53. Sweeney GD, Jones KG. Porphyria cutanea tarda: clinical and laboratory features. Canad Med Assn J. 1979;120:803-7.
54. Felsher BF, Redeker AG. Effect of chloroquine on hepatic uroporphyrin metabolism in patients with porphyria cutanea tarda. Medicine. 1966;45:575-83.
55. Taljaard JJF, Shanley BC, Stewart-Wynne EG, Deppe WM, Joubert SM. Studies on low dose chloroquine therapy and the action of chloroquine in symptomatic porphyria. Br J Derm. 1972;87:261-9.
56. Kordac V, Semradova M. Treatment of porphyria cutanea tarda with chloroquine. Br J Derm. 1974;90:95-100.
57. Ashton RE, Hawk JLM, Magnus IA. Low-dose oral chloroquine in the treatment of porphyria cutanea tarda. Br J Dermatol. 1984;3:609-13.

58. Malkinson FD, Levitt L**.** Hydroxychloroquine treatment of porphyria cutanea tarda. Arch Dermat. 1980;116:1147-50.
59. Vizethum W, Dahlmann D, Bolsen K, Goerz G**.** Influence of chloroquine (Resochin) on hexachlorobenzene (HCB) induced porphyria of the rat. Arch Dermat Res. 1979;264:125.
60. Goerz G, Bolsen K, Merk H**.** Influence of chloroquine on the porphyrin metabolism. Arch Dermatol Res. 1985;277:114-7.
61. Goerz G, Bolsen K, Seuwen P, Schmitter H, Merk H, Lissner R**.** Effects of chloroquine and hydroxychloroquine on hexachlorobenzene-induced porphyria in rats. IARC Sci Publ. 1986;77:513-5.
62. Cohen SN, Phifer KO, Yielding KL**.** Complex formation between chloroquine and ferrihaemic acid *in vitro*, and its effect on the antimalarial action of chloroquine. Nature. 1964;202:805-6.
63. Scholnick PL, Epstein J, Marver HS**.** The molecular basis of the action of chloroquine in porphyria cutanea tarda. J Invest Dermatol. 1973;61:226-32.
64. Egger NG, Goeger DE, Anderson KE**.** Effects of chloroquine in hematoporphyrin-treated animals. Chemico-Biological Interactions. 1996;102:69-78.
65. Egger NG, Pow-Sang M, Johnson SA, Motamedi M, Anderson KE, Orihuela E**.** Accumulation of porphyrins in plasma and tissues of dogs after ∂-aminolevulinic acid administration: implications for photodynamic therapy. Pharmacology. 1996;52:362-70.
66. Pimstone NR, Mukerji SK, Saicheur T, Lee J**.** Utility of chloroquine therapy in two patients with congenital erythropoietic porphyria (CEP). In: Blake D, McManus J, Ratnaike S, Hill J, Poulos V, Rossi E, eds. Porphyrins, Porphyrias and Photodynamic Therapy (Conference Proceedings), 1993. Melbourne, Australia, November 22-26.
67. Elder GH**.** Porphyria cutanea tarda. Semin Liver Dis. 1998;18(1):67-75.
68. Kordac V, Jirsa M, Kotal P, Kalab M, Cervinka J, Kotyk A, et al. Agents affecting porphyrin formation and secretion: Implications for porphyria cutanea treatment. Semin Hematol. 1989;26:16-23.
69. Chlumska A, Chlumsky J, Malina L**.** Liver changes in porphyria cutanea tarda patients treated with chloroquine. Br J Dermatol. 1980;102:261-6.
70. Daniell WE, Stockbridge HL, Labbe RF, Woods JS, Anderson KE, Bissell DM, et al. Environmental chemical exposures and disturbances of heme synthesis. Environmental Health Perspectives. 1997;105, Suppl 1:37-53.
71. Bonkovsky HL, Barnard GF**.** Diagnosis of porphyric syndromes: a practical approach in the era of molecular biology. Semin Liver Dis. 1998;18(1):57-65.
72. Hahn M, Bonkovsky HL**.** Multiple chemical sensitivity syndrome and porphyria. A note of caution and concern. Arch Intern Med. 1997;157:281-5.
73. Mauzerall D, Granick S**.** The occurrence and determination of δ-aminolevulinic acid and porphobilinogen in urine. J Biol Chem. 1956;219:435-46.
74. Schwartz S, Edmondson P, Stephenson B, Sarkar D, Freyholtz H**.** Direct spectrofluorophotometric determination of porphyrin in diluted urine. Ann Clin Res. 1976;8:156-61.
75. Sassa S, Granick JL, Granick S, Kappas A, Levere RD**.** Studies in lead poisoning. I. Microanalysis of erythrocyte protoporphyrin levels by spectrofluorometry in the detection of chronic lead intoxication in the subclinical range. Biochem Med. 1973;8:135-48.
76. McManus J, Blake D, Ratnaike S**.** An assay of uroporphyrinogen decarboxylase in erythrocytes. Clin Chem. 1988;34:2355-7.
77. Lockwood WH, Poulos V, Rossi E, Curnow DH**.** Rapid procedure for fecal porphyrin assay. Clin Chem. 1985;31:1163-7.

78. Marmor, M. F., U. Kellner, T. Y. Lai, J. S. Lyons, W. F. Mieler and O. American Academy of (2011). "Revised recommendations on screening for chloroquine and hydroxychloroquine retinopathy." Ophthalmology **118**(2): 415-422
79. Kellner U, Kraus H, Foerster MH**.** Multifocal ERG in chloroquine retinopathy: regional variance of retinal dysfunction. Graefes Arch Clin Exp Ophthalmol. 2000;238(1):94-7.
80. Alcock NW**.** A hydrogen peroxide digestion system for tissue trace metal analysis. Biol Trace Elem Res. 1987;13:363-70.
81. Liang L, D'Haese PC, Lamberts LV, De Broe ME**.** Direct determination of iron in urine and serum using graphite furnace atomic absorption spectrometry. Analyst. 1989;114(2):143-7.

7. Appendices

A. Informed Consent Template:

You are being asked to participate as a subject in this research project because you have porphyria cutanea tarda (PCT).

The title of the project is **"Therapeutic Studies in Porphyria Cutanea Tarda."** The study is directed by Karl E. Anderson, M.D. The Sponsor of the study is the National Institutes of Health (NIH). Portions of Dr. Anderson's and his research team's salaries may be paid by this grant. This study is a multi-center study directed by the Rare Diseases Clinical Research Network, Porphyria Consortium. UTMB is the lead center and there are 5 other porphyria centers participating: 1. The University of California, San Francisco; 2. The University of Alabama, Birmingham; 3. Carolinas Medical Center, Charlotte, NC; 4. Mount Sinai School of Medicine, New York; 5. The University of Utah, Salt Lake City.

1. PURPOSE OF STUDY

The purpose of this study is to compare two treatments for PCT. Phlebotomy (removal of blood) is considered the preferred treatment at most centers. Treatment with hydroxychloroquine is an alternative that might be used more widely if more information were available comparing the two treatments. The study aims to compare how rapidly PCT improves with the two treatments, and determine how frequently PCT recurs after each.

2. PROCEDURES

The study will be carried out at UTMB in the outpatient part of the Institute for Translational Sciences- Clinical Research Center (ITS-CRC) or in another UTMB clinic, such as the GI Clinic. At the beginning of the study, there will be a screening visit to be sure you are eligible. You can enter the study if you are eligible for either treatments. The screening visit will include recording your medical history, a physical examination and blood, urine and stool tests that are standard for documenting a diagnosis of PCT and evaluating the stage of the condition.

The following conditions are needed for you to enter the study:

- age 18 or greater
- well documented PCT
- you are willing to consent, after you are provided with the information you need
- if you are a woman capable of becoming pregnant, you are willing to avoid becoming pregnant during treatment and use an effective contraceptive method

The following are reasons for you to be excluded from the study:

- skin problems due to a type of porphyria other than PCT
- pregnancy
- previous treatment as a participant in this protocol.
- prior treatment by phlebotomy, hydroxychloroquine or chloroquine within one month, unless baseline labs are available before treatment was started
- unwilling or unable to comply with the protocol

The following will exclude you from being treated with hydroxychloroquine, but you may still be treated by phlebotomy:

- unwilling to consider this form of treatment
- psoriasis (a skin disease)
- lactation (nursing mothers)
- significant disease of the retina (light-sensitive part of the eye)
- glucose-6-phosphate dehydrogenase deficiency (a genetic condition of the blood that is possibly made worse by hydroxychloroquine)
- advanced liver disease or substantially abnormal liver tests
- advanced kidney disease
- recent and continued heavy use of alcohol
- recent and continued use of drugs toxic to the liver, such as acetaminophen, isoniazid or valproic acid
- marked excess body iron or certain genetic features associated with an iron overload condition called hemochromatosis
- poor tolerance of hydroxychloroquine in the past

The following will exclude you from being treated with phlebotomy, but you may still be treated with hydroxychloroquine:

- unwilling to consider this form of treatment
- bone marrow disease and anemia
- very poor veins
- poor tolerance of phlebotomies in the past

If you have exclusions for both treatments, you may still be treated by the method that your doctors think is most likely to be safe and effective.
Patients who have already started treatment may enter the study if there is sufficient information about the start of treatment and their treatment regimen is consistent with what is provided in this study.

PCT is always associated with some liver abnormalities, which can resemble those due to other liver conditions. Therefore, tests will be done as part of your medical care to rule out other liver diseases, including other types of hepatitis (such as hepatitis B) and diabetes. A liver ultrasound and a liver biopsy are not required as a part of this study, but may be recommended as part of your medical care.

You will also be tested for other conditions that would make treatment of PCT less effective, such as diseases affecting the kidneys or bone marrow.

As part of your treatment for PCT, you have been advised to avoid things that make PCT worse, such as drinking alcohol, smoking, estrogens, iron pills and certain drugs. You should also avoid ultraviolet light (sunlight, tanning studios, etc.) until your condition has been successfully treated. You can avoid sunlight by wearing protective clothing or remaining indoors during the day.

Entering the study. When you enter the study, samples will be obtained to document the diagnosis of PCT. This will require samples of blood, urine (24 hour) and stool for tests that are

standard for diagnosing PCT. Also, a history and physical examination will be done. In addition, follow-up visits will be scheduled every 2 months for an additional 6 months. Information will be collected and laboratory tests done to see which of the following risk factors for PCT are present:

1. Alcohol usage, including amount, duration and how recent
2. Smoking, including amount, duration and how recent
3. Estrogen use
4. Hepatitis C (blood tests)
5. HIV (blood test for the virus that causes AIDS)
6. Genetic test for hemochromatosis (an iron overload condition)
7. Enzyme test for UROD in red blood cells. (UROD, or uroporphyrinogen decarboxylase, may be in part an inherited deficiency in about 20% of PCT patients.)
8. Genetic test for UROD deficiency.

Genetic testing for UROD will be done at Icahn School of Medicine at Mt. Sinai in New York City. The purpose is to look for changes in DNA (mutations in the genetic material) in PCT. You may be asked to sign a separate consent form from that institution.

Your DNA sample:

- will have your name on it
- may be stored indefinitely
- can be used only by the porphyria research team at UTMB or another institution researching porphyria
- may be used in the future to identify other mutations that are not anticipated now, but may lead to a better understanding of porphyria

Any research done on your DNA now or in the future must have the approval of the UTMB Institutional Review Board (IRB), which oversees research on humans. You may request that your DNA sample be withdrawn or destroyed at any time.

You will be tested for HIV as part of the screening process and have appropriate counseling regarding HIV testing. If you unexpectedly test positive, you will be contacted by a qualified medical provider and receive counseling regarding the impact of HIV on your physical, mental, and social well-being and be given help in finding further medical care for HIV infection. The results of your HIV test and other tests will be confidential, but will appear in your UTMB hospital medical records. Positive results will be reported to appropriate state and local agencies as required by law.

Assigning treatment. You will either be treated by low-dose hydroxychloroquine or repeated phlebotomy. The treatments to be compared in this study are both considered effective, but it is not known how quickly each of these treatments works. You will be assigned to one of these treatments based on your preference and whether you are medically eligible for one treatment but not the other. If you are eligible and willing to consider either treatment, you will be assigned by randomization. (Like the flip of a coin.) The purpose of randomization is to avoid introducing unnecessary bias into the study.

If your treatment needs to be changed during the study, you will be assigned the other treatment and continue in the study.

Phlebotomy. "Phlebotomy" means removing blood through a needle that is inserted into a vein. If you are treated by this method, the standard method will be followed. One unit (450 ml, which is approximately one pint) of whole blood will be removed from an arm vein at the Blood Bank or the ITS-CRC at intervals of approximately 2 weeks. This will be like donating blood, except that the blood removed will be discarded. This is the most effective way of removing iron from the body, because the red blood cells contain large amounts of iron. As blood is removed, more red blood cells are made in your bone marrow, which gradually uses up excess iron in your body. A needle will be inserted into an arm vein each time a unit of blood is removed. Phlebotomies will be repeated until a blood test (serum ferritin) shows that enough iron has been removed. This test is a good indicator of how much iron is in your body. The aim is to reduce the ferritin value to about 15 ng/ml. At this point phlebotomies are stopped.

To avoid your becoming anemic during this treatment, a hematocrit or hemoglobin test will be done before each phlebotomy. If these are considered to be too low, removing a unit of blood will be postponed until the next visit in 1-2 weeks.

Hydroxychloroquine. If you are treated with hydroxychloroquine, the standard treatment by this method will be followed. The dose will be 100 mg twice weekly until at least one month after the porphyrins in your blood plasma and urine become normal. You will be provided this medication as part of the study. You will be provided a diary to record when you take each dose. At each study visit, you will bring the medication bottle with you to see if you have missed any doses and to be sure you have an adequate supply to last until the next visit. The maximum duration of treatment with hydroxychloroquine will be 12 months.
You will be supplied with tablets of hydroxychloroquine that have been cut in half. Each half tablet is 100 mg. So the dose will be one of these half-tablets taken twice weekly.
Hydroxychloroquine sometimes causes damage to the retina of the eye, although this is rare and usually only with larger doses for longer periods of time than in this study. As a precaution, your eyes will be examined for retinal damage before starting hydroxychloroquine, and then at least yearly and at the end of treatment.

Hydroxychloroquine is much more toxic in children than in adults. Therefore, you are advised to keep the drug out of the reach of children.

The effect of hydroxychloroquine on the unborn child is not known. But studies in animals suggest that it may be harmful to the eyes. Therefore, you should not take chloroquine if you are pregnant or intend to become pregnant during the study. Women who are capable of becoming pregnant will be tested for pregnancy every month during the study if they are taking chloroquine. The drug will be stopped if you become pregnant.

Study visits during treatment. You will be asked to return to the ITS-CRC or to another UTMB clinic for study visits to provide a blood sample and a urine sample and determine the progress of the treatment. Visits will be every 2-4 weeks until the amount of porphyrins in your plasma is normal. After that, visits will be every 4-8 weeks, until the porphyrins in both plasma and urine are completely normal. The number of visits will be approximately the same with either treatment.

At each visit you will complete a questionnaire about your symptoms and progress. The skin will be examined and the nature and severity of any lesions recorded. Photographs of the skin may be taken. Blood and urine samples will be obtained at each visit to determine the course of treatment and look for adverse effects.

Optionally, patients with both PCT and hepatitis C may have another tube of blood drawn on 5 visits to see the effect of treatment for PCT on the amount of hepatitis C virus in the blood. Optionally, urine samples may be collected to measure iron concentration in urine.

End of treatment. Treatment by phlebotomy will end when the target ferritin value is reached. Hydroxychloroquine treatment will end at least one month after the plasma and urine porphyrins are completely normal.

After the end of treatment. After treatment is completed, you will be asked to return for follow-up visits every 3-6 months for at least 5 years. The purpose of these later visits is to see if PCT recurs more or less frequently depending upon which treatment has been given.

No more than 30 mL (one tablespoon) of blood will be taken every 2 weeks during this study. Also, if you are treated by phlebotomy, one unit of blood will be removed at approximately 2-week intervals until that treatment is completed.

3. PROCEDURES RELATED ONLY TO THE RESEARCH

This study compares two accepted treatments for PCT, to see which acts more quickly and how often PCT recurs after each. Because both treatments are accepted, many procedures are considered standard of care, and would be done if you were not in the study. The following is a list of the procedures, which are already described above, that might not be done if you were not in the study.

1. Assignment to treatment at random, if you are eligible for and willing to consider both treatments.
2. More tests may be done to be sure you are suitable for either treatment, rather than just one treatment. However, the same tests might be done outside the study to decide which treatment should be chosen.
3. Visits during and after treatment are considered standard of care, but might be somewhat more frequent than would always be done if you were not in the study.

4. PROCEDURES NOT RELATED TO THIS RESEARCH (i.e., standard of care)

Tests that are important for proper diagnosis and treatment of porphyria are considered standard of care. These tests and procedures that would otherwise be required for evaluation and treatment of your condition will be your responsibility or will need to be covered by your health insurers.

5. RISKS OF PARTICIPATION

The study involves potential risks and discomforts. But these risks will not be very different from standard treatment by either method.

Risks from phlebotomy and venipuncture include pain, bruising, dizziness, fainting and infection. There is a risk of iron deficiency, which may cause mild symptoms even without causing anemia.

Hydroxychloroquine removes porphyrins from the liver into the blood stream, so the porphyrins can then be excreted in the urine. As a result, in the first few weeks of treatment, porphyrin levels and skin symptoms may increase before they start to decrease. Liver function tests may

also worsen somewhat during this time. Therefore, you should avoid sunlight during the first few weeks of treatment.

Side effects from hydroxychloroquine, which are generally seen with use of higher doses than in this study, can include nervousness, nightmares and other psychiatric changes, headaches, dizziness, muscle weakness, blurred vision, other visual disturbances that are sometimes long lasting, nausea, vomiting, diarrhea, abdominal pain and hematological changes. Blurring, trouble reading, eye pain and other disturbances may be due to effects of the drug on the back of the eye (retina). If eye disturbances occur, you should notify the study team immediately. This drug can cause permanent damage to the retina, although this is rare, and unlikely at the dosage used in this study.

Hydroxychloroquine may also be harmful to the unborn child, especially to the eyes, although this risk is not known with certainty. Hydroxychloroquine overdose is very dangerous for young children. Therefore, it is important we discuss with you how to keep it away from children.

The investigative team will make every effort to rapidly identify and treat any undesirable effects. In the event of any unusual reaction you will be medically managed by the investigators and other physicians at UTMB.

There may be risks because of the HIV and hepatitis tests, which could cause mental and social harm. A positive test could have a bad influence on your being able to get insurance or be employed, and could make family relationships difficult. You will be referred for appropriate counseling and medical care for any such condition that is discovered.
There is also risk of loss of confidentiality of sensitive personal, medical and genetic information. However, the investigative team will take precautions to keep all personal information about you confidential and be sensitive to any findings that might cause mental or social discomfort.

6. NUMBER OF SUBJECTS PARTICIPATING AND THE DURATION OF YOUR PARTICIPATION

A total of up to 100 patients with PCT will participate at UTMB and up to 5 other centers in the United States. Both women and men will be included. Pregnant women will not be included. The length of time needed to complete the treatment itself will be the same as if you were not in the study, and should be within 12 months. You will participate in the study with visits to the ITS-CRC or an outpatient clinic every 2-4 weeks until the plasma porphyrin concentration becomes normal, then every 4-8 weeks until plasma and urine porphyrins are completely normal. After that, visits will continue every 3-6 months for the at least 5 years to compare how often PCT recurs after each treatment.

7. BENEFITS TO THE SUBJECT

There may be no direct benefit to you for participating in this research study. Either treatment might be available to you if you are not in the study. The study is likely to lead to a better understanding of the treatment of PCT, which may benefit you in the future.
Some of the laboratory information obtained during these studies may be used to manage your medical condition, and you can give your doctor permission (through a “release of information” form) to request this information.

8. BENEFITS TO SOCIETY

The findings of this study may benefit other patients with PCT and society by providing a better understanding of the treatment of this disease and by determining how quickly these two treatments work and how often PCT recurs after each treatment.

9. OTHER CHOICES (ALTERNATIVE TREATMENT)

You may choose to not participate in this study, and either of these treatments will still be available to you in the usual manner. If you do not participate in the study, this will not affect your medical care at UTMB.

10. REIMBURSEMENT FOR EXPENSES

You will be reimbursed for parking with a parking token, and offered reimbursement for travel costs based on mileage driven at 45¢ per mile up to $90.

11. COMPENSATION FOR RESEARCH RELATED INJURY

If you are physically injured because of any substance given to you or procedure performed on you under the plan for this study, UTMB will provide you with the appropriate medical treatment. Your insurance company will be billed and any charges not covered by your own insurance or health care program will be provided at no cost to you. You will be responsible for paying any costs related to illnesses and medical events not associated with being in this study. There are no plans to provide other forms of compensation. However, you are not waiving any of your legal rights by participating in this study. Questions about compensation may be directed to the study doctor.

12. COSTS OF PARTICIPATION

There will be no charge to you for any tests and procedures directly related to the study. Tests and procedures, including phlebotomies, that would otherwise be required for evaluation and treatment of your condition will be your responsibility or will need to be covered by your health insurers. Additional funds may be available for such costs for patients without insurance. Hydroxychloroquine half tablets will be provided at no cost.

13. USE AND DISCLOSURE OF YOUR HEALTH INFORMATION

Study records that identify you will be kept confidential as required by law. Federal privacy regulations provided under the Health Insurance Portability and Accountability Act (HIPAA) provide safeguards for privacy, security, and authorized access of your records. These regulations require UTMB to obtain an authorization from you for the use and disclosure of your health information. By signing this consent form, you are authorizing the use and disclosure of your health information for the purpose of completing the research study. Except when required by law, you will not be identified by name, social security number, address, telephone number, or any other direct personal identifier in study records disclosed outside of the University of Texas Medical Branch (UTMB). For records disclosed outside of UTMB, you will be assigned a unique code number. The key to the code will be kept in a locked file in the office of Dr. Anderson and his coworkers.

As part of the study, the study team may report the results of your study-related tests to a drug company, regulatory agencies or other recipients. While these recipients may understand the importance of protecting the confidentiality of your health information, UTMB cannot guarantee

the confidentiality of your health information or protect from further disclosures once these recipients receive your health information.

If you sign this form, you are giving us permission to collect, use and share your health information. You do not need to sign this form. If you decide not to sign this form, you cannot be in the research study. We cannot do the research if we cannot collect, use and share your health information. Whether or not you agree to the research project or give us permission to collect, use or share your health information will not affect the care you will be given at UTMB.

Dr. Anderson and his coworkers will use and disclose your study related test results both to treat you and to complete the research study. These would include laboratory tests such as your blood counts and tests to measure the function of your liver and kidneys, for example. Some of these tests would have been done as part of your regular care. These test results will be recorded in your medical record. You may see or receive a copy of any research information that will be included in your medical record. For all other health information we collect on you that will not be included in your medical record, you may not be allowed to access or receive a copy of the information until the conclusion of the study.

Your records may be reviewed in order to meet federal or state regulations. Reviewers may include, for example, representatives of a drug company that might be interested in developing a treatment for your type of porphyria, the Food and Drug Administration and UTMB. This authorization for the use and disclosure of your health information as described above expires upon the conclusion of the research study except for FDA regulated studies. For FDA regulated studies, the study sponsor and government agencies, such as the FDA may review your records after the study ends.

If you change your mind later and do not want us to collect or share your health information, you need to contact the researcher listed on the attached consent form by telephone or by letter. You need to say that you have changed your mind and do not want the researcher to collect and share your health information. You may also need to leave the research study if we cannot collect any more health information. We may still use the information we have already collected. We need to know what happens to everyone who starts a research study, not just those people who stay in it. The results of this study may be published in scientific journals without identifying you by name.

We will keep your study records confidential. However, certain people may need to see your study records. By law, anyone who looks at your records must keep them completely confidential. The only people who will be allowed to see these records are:

- The research team, including the Principal Investigator, study coordinator, research nurses, and all other research staff.
- The UTMB Institutional Review Board (IRB) and the staff that work for the IRB.
- People from the federal Food and Drug Administration (FDA).
- People from the National Institutes of Health (NIH), who sponsored this study.
- People from the Department of Health and Human Services (DHHS).

Transfer of Data to DMCC

The clinical information collected for this study will be stored at the National Institutes of Health Office of Rare Diseases Research designated Rare Diseases Clinical Research Network Data Management Center and also sent to a Federal data repository. The data management center

uses several layers of protection for the clinical data stored there. It meets all of the local and federal security requirements for research datacenters. Your information is stored only using a study ID.

A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This website will not include information that can identify you. At most, the website will include a summary of the results. You can search this website at any time.

14. ADDITIONAL REQUIRED CLAUSES

1. If you have any questions, concerns or complaints before, during or after the research study, or if you need to report a research related injury or adverse reaction (bad side effect), you should immediately contact Dr. Karl E. Anderson or Dr. Csilla Hallberg at 409-772-4661 or, if after normal office hours, at 409-772-1218.

2. Your participation in this study is completely voluntary and you have been told that you may refuse to participate or stop your participation in this project at any time without penalty or loss of benefits and without jeopardizing your medical care at UTMB. If you decide to stop your participation in this project and revoke your authorization for the use and disclosure of your health information, UTMB may continue to use and disclose your health information in some instances. This would include any health information that was used or disclosed prior to your decision to stop participation and needed in order to maintain the integrity of the research study. If there are significant new findings or we get any information that might change your mind about participating, we will give you the information and allow you to reconsider whether or not to continue.

3. If you have any complaints, concerns, input or questions regarding your rights as a subject participating in this research study or you would like more information, you may contact the Institutional Review Board Office , at (409) 266-9475.

The purpose of this study, procedures to be followed, risks and benefits have been explained to you. You have been allowed to ask questions and your questions have been answered to your satisfaction. You have been told who to contact if you have additional questions. You have read this consent form and voluntarily agree to participate as a subject in this study. You are free to withdraw your consent, including your authorization for the use and disclosure of your health information, at any time. You may withdraw your consent by notifying Dr. Anderson at 409-772-4661. You will be given a copy of the consent form you have signed.

Informed consent is required of all persons in this project. Whether or not you provide a signed informed consent for this research study will have no effect on your current or future relationship with UTMB.

Date	Signature of Subject

Using language that is understandable and appropriate, I have discussed this project and the items listed above with the subject and/or his/her authorized representatives.

Date	Signature of Person Obtaining Consent

B. Patient Compliance Chart for Hydroxychloroquine Use

Name_____

UH # _____ **Date of Birth**_____

Hydroxychloroquine Use Record

Month	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31

GCRC Protocol 682

Mark an [X] the day you take your medication IRB # 92-435

Bring this chart with you during each clinic visit to schedule next visit.

C. Report Form for Phlebotomies

Phlebotomy facility: _____ Date of Phlebotomy : _____

Hematocrit or Hemoglobin level documented **prior** to phlebotomy (include reference range):

_____

Volume of blood removed: _____mL

Venous access: _____

Other tests/samples collected: _____

Side effects: _____

Other comments or observations: _____

_____

Signature of medical personnel overseeing phlebotomy: _____

D. Patient Compliance Chart for Phlebotomies

Name_____

UH # _____ **Date of Birth**_____

Phlebotomy Record

Month	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31

Mark an [X] on each day that a phlebotomy is done

Bring this chart with you to each clinic visit.