Effect of Oral Cimetidine in the Protoporphyrias

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Rare Diseases Clinical Research Network
Porphyrias Consortium

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Protocol Synopsis

Protocol Number	PC7211
Title of Study	Effect of Oral Cimetidine in the Protoporphyrias
Clinical Trial Phase	Phase 2
Indication	Erythropoietic protoporphyria (EPP) & X-Linked protoporphyria (XLP)
Study Objectives	Primary Efficacy Objective: -To investigate the effect of cimetidine in decreasing erythrocyte total protoporphyrin level in patients with protoporphyria Secondary Efficacy Objectives: -To investigate the effect of cimetidine on the time to onset of prodromal symptoms associated with sunlight exposure in patients with protoporphyria -To investigate the effect of cimetidine on patient-reported quality of life in patients with protoporphyria -To investigate the effect of cimetidine on the number and severity of sunlight-induced pain events in patients with protoporphyria -To investigate the effect of cimetidine in increasing the light dose required for onset of prodromal symptoms associated with sunlight exposure in patients with protoporphyria Exploratory Efficacy Objectives: -To investigate the effect of cimetidine on the rate of prodromal symptom onset in patients with protoporphyria -To investigate the effect of cimetidine in decreasing plasma total porphyrins level in patients with protoporphyria -To investigate the ability of cimetidine to decrease the ratio of metal-free to zinc protoporphyrins Safety Objective: -To determine the safety of cimetidine administration in the protoporphyrias
Study Design	Multicenter, prospective, randomized, double-blind, placebo-controlled, crossover trial evaluating the efficacy of cimetidine in patients with EPP or XLP
Duration	9 months consisting of 3 months of treatment followed by 3 months of washout and another 3 months of treatment.
Treatment Arms	Patients randomized to sequence 1 will receive placebo during period 1 and cimetidine (800mg po bid) during period 2. Patients randomized to sequence 2 will receive cimetidine (800mg po bid) during period 1 and placebo during period 2. Between periods, a wash-out period of 3 months will occur in which all patients receive neither cimetidine nor placebo.
Study Center(s)	Porphyrias Consortium (PC) study sites
Study Endpoint(s)	Primary Endpoint: -Percent change in erythrocyte total protoporphyrin level post-treatment relative to pre-treatment

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	Secondary Endpoints: -Time to prodrome measured as prodrome-free outdoor exposure time -Patient-reported quality of life as measured by PROMIS-57 scale -The number and severity of sunlight-induced pain events (phototoxic episodes) -Light dose required for time to prodrome Exploratory Endpoints: -Rate of prodrome events per prodrome-free outdoor exposure time -Percent change in plasma total porphyrin levels post-treatment relative to pre-treatment -Percent change in the ratio of metal-free to zinc protoporphyrin Safety Endpoint: - Rates of adverse events will be computed and reported descriptively
Inclusion/Exclusion Criteria	Inclusion Criteria: 1. Prior enrollment or co-enrollment in the Longitudinal Study of the Porphyrias (PC Study 7201) with a confirmed diagnosis of EPP or XLP 2. Male or female age ≥15 years at screening 3. Characteristic history of non-blistering cutaneous photosensitivity 4. Willing and capable of giving informed consent and following procedures described in the protocol Exclusion Criteria: 1. Participants not willing to expose themselves to light to the point of prodromal symptoms at least weekly 2. History of liver or bone marrow transplant or clinically significant liver dysfunction as determined by the Investigator 3. Known or suspected allergy or intolerance to cimetidine 4. Use of any other experimental therapy in the past 3 months at screening 5. Use of cimetidine within the past 3 months at screening 6. Individuals with elevations of porphyrins in plasma or erythrocytes due to other diseases (i.e., secondary porphyrinemia) such as liver and bone marrow diseases 7. Patients with any clinically significant comorbid conditions, which in the opinion of the Investigator, precludes participation

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	8. Treatment with any drugs or supplements (Appendix 1) that in the opinion of the Investigator can interfere with subject safety or the objectives of the study 9. The participant either does not have a smartphone or is not willing to use his/her smartphone for the study 10. Women who are pregnant, breastfeeding, or actively planning to become pregnant 11. Individuals with moderate to severe renal insufficiency
Sample Size	20 patients provide 80% power to detect that the mean difference in the percent change in total erythrocyte protoporphyrin between cimetidine and placebo is not 0 assuming the actual mean difference is 31, the square root of the within mean square error is 33, using a two-sided, 0.05 level test.
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Investigator Agreement and Signature Page

Protocol Title: Effects of Oral Cimetidine in the Protoporphyrias
Protocol Number: PC7211
Version Number: 11

Investigator Agreement:
I have read the protocol, appendices and procedure manual of the study indicated above. I agree that these documents contain all of the details necessary for me and my staff to conduct this study as described. I agree to conduct the study as described in this protocol and comply with all principles of Good Clinical Practice (GCP), as described in the United States Code of Federal Regulation (CFR) 21 Parts 11, 50, 54, 56, and 312 and the appropriate International Conference on Harmonization guidance documents.

I will provide to all study personnel under my supervision copies of the study protocol, appendices and all other relevant information regarding the study provided to me by the Principal Investigator. I will discuss this material with them to ensure they are fully informed about the study.

_____ _____
Investigator (Printed) Date

_____
Investigator Name (Signature)

_____
Institution (Clinical Site)

_____ _____
Sponsor Representative (Printed) Date

_____
Sponsor Representative Name (Signature)

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Abbreviation List

Abbreviation	Definition
AE	Adverse Event
AIA	Allyl-isopropylacetamide
AIP	Acute Intermittent Porphyria
ALAS	5-Aminolevulinate Synthase
ALT	Alanine Aminotransferase
ANOVA	Analysis of Variance
APF	American Porphyria Foundation
AST	Aspartate Aminotransferase
CFR	Code of Federal Regulations
CLPX	Caseinolytic Mitochondrial Matrix Peptidase Chaperone Subunit X
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Event
CYP	Cytochrome P450
DMCC	Data Management and Coordinating Center
DNA	Deoxyribonucleic Acid
DSMB	Data Safety Monitoring Board
EC	Ethics Committee
EOS	End of Study
EPP	Erythropoietic Protoporphyria
ETV	Early Termination Visit
FDA	Food and Drug Administration
FECH	Ferrochelatase
GCP	Good Clinical Practice
GGT	Gamma-glutamyl Transferase
GINA	Genetic Information Non-Discrimination Act
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
GUID	Global Unique Identifier
HHS	Health and Human Services
HIPAA	Heath Insurance Portability and Accountability Act
ICH	International Council for Harmonization
IDS	Investigational Drug Service
LS	Longitudinal Study of the Porphyrias
IRB	Institutional Review Board
MOP	Manual of Procedures
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NIH	National Institutes of Health
PBG	Porphobilinogen

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PC	Porphyrias Consortium
PCSC	Porphyrias Consortium Steering Committee
PCT	Porphyria Cutanea Tarda
QOL	Quality of Life
RDCRN	Rare Diseases Clinical Research Network
SAE	Serious Adverse Event
TIBC	Total Iron Binding Capacity
UE	Unanticipated Event
UTMB	University of Texas Medical Branch
UV	Ultraviolet
WOCBP	Woman of Childbearing Potential
XLP	X-Linked Protoporphyria

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Section 1: Key Roles

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This is a study of the Porphyrias Consortium (PC). The PC is a network of leading porphyria centers in the United States and The American Porphyria Foundation. The PC is part of the National Institutes of Health's Rare Diseases Clinical Research Network. The mission of the PC is to expand knowledge of the porphyrias and thereby benefit patients and their families. For more information about the PC, visit its website at https://www.rarediseasesnetwork.org/cms/porphyrias/.

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Section 2: Background

Section 2.1: Summary of Disease/Condition

The porphyrias are a diverse group of diseases due to abnormalities in enzymes of the heme biosynthetic pathway.[1] The protoporphyrias are distinct disorders that cause painful, non-blistering cutaneous photosensitivity usually starting in early childhood. EPP is the most common porphyria in children and the third most common in adults (after porphyria cutanea tarda and acute intermittent porphyria). Reports of EPP prevalence vary between 5 and 15 cases per million population.[2–5] However, EPP is suspected to be underdiagnosed. Recent data evaluating the prevalence of pathogenic variants known to cause EPP suggest that EPP prevalence may be 1:16,700. XLP is approximately ten-fold less common than EPP.[2,3] Protoporphyria caused by a mutation in the Caseinolytic Mitochondrial Matrix Peptidase Chaperone Subunit X (CLPX) gene has been described in only one family.[6]

Molecular and biochemical abnormalities. Inheritance of EPP results from loss-of-function mutations of both copies of the ferrochelatase (*FECH*) gene, thereby reducing ferrochelatase activity to ~10-30% of normal. The disease in most EPP families results from a pathogenic *FECH* mutation that is in *trans* to a low-expression *FECH* allele, which is common in the population and causes no disease by itself.[7] The low expression variant alters splicing through use of an alternative splice site, producing approximately 60% of normal FECH activity. For this reason, it is termed hypomorphic. This hypomorphic *FECH* allele in *trans* with a mutation results in the reduction of FECH activity to ~30% or less of normal FECH. This subnormal FECH activity becomes rate-limiting, resulting in accumulation of intracellular protoporphyrin. Most of this is metal-free, because the FECH-catalyzed formation of zinc protoporphyrin is also impaired. Much less commonly (<5% of cases), EPP results from two severe loss of function *FECH* variants.[8]

Reduced FECH activity in EPP causes the accumulation of the photosensitizing molecule protoporphyrin in erythrocyte precursor cells (e.g., reticulocytes) during the late stages of hemoglobin synthesis. Because FECH can accept metals other than iron, in normal individuals and in diseases other than the protoporphyrias any excess protoporphyrin that remains after completion of heme and hemoglobin synthesis is chelated with zinc by FECH to form zinc protoporphyrin. In EPP, as a consequence of deficient FECH activity in the marrow, the excess protoporphyrin found in erythrocytes is predominantly (usually >85%) metal-free. This is a distinctive and diagnostic feature of EPP.

XLP results from gain-of-function mutations of the erythroid-specific 5-aminolevulinate synthase (ALAS2) gene.[9] ALAS is the first enzyme of the heme biosynthetic pathway, with the ALAS1 isoform being the housekeeping enzyme and the ALAS2 isoform being the erythroid-specific form. *ALAS2* is the only heme biosynthetic pathway gene found on the X chromosome, which accounts for the X-linked pattern of inheritance. The increase in ALAS2 activity results from short C-terminal deletions and leads to overproduction of protoporphyrin in amounts that exceed what is required for heme and hemoglobin synthesis in the marrow. Because the capacity of normal FECH activity to chelate protoporphyrin with zinc is exceeded, both zinc and metal-free protoporphyrin are produced. The ratio of metal-free protoporphyrin in erythrocytes in XLP patients is usually 50-85%, but is sometimes <50%.[10] In CLPX porphyria, ALAS2 function is increased due to mutation of mitochondrial CLPX, which normally degrades ALAS2.[6]

Clinical manifestations. Patients with protoporphyria experience painful cutaneous photosensitivity beginning in early childhood, with pain and/or tingling starting after sunlight exposure as short as a few minutes in typical cases. If they are unable to remove themselves

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from light exposure immediately, progression to severe pain can be expected, followed by redness and swelling of the sun-exposed skin and systemic symptoms that may last for several days. The symptoms of these untoward reactions are poorly responsive to analgesics (including opioids), antihistamines, and other medications. Patients learn to modify their lifestyles, including work and recreational activities, in order to avoid exposure to the sun and other strong light sources. Thus, these protoporphyrias are disabling and greatly impair quality of life.[2,11,12]

Protoporphyrin is taken up by the liver from the plasma and excreted in bile, as protoporphyrin is not water soluble. It can be hepatotoxic and lead to a condition called protoporphyric hepatopathy that in some protoporphyria patients (<5%) becomes severe and may require liver transplantation.[13,14] Iron deficiency is another common feature of these disorders and is poorly understood.[15–18] Although XLP patients generally benefit from iron supplementation, guidance on the management of iron deficiency in EPP is currently unclear, as some patients have reported worsening photosensitivity with iron treatment.[15,16,19–21] Studies on the effect of iron on porphyrin levels in EPP are currently lacking.

Treatment. Afamelanotide, a potent, long-acting analog of naturally occurring alpha-melanocyte-stimulating hormone, darkens the skin sufficiently to protect significantly against the light rays that are harmful to patients with protoporphyria, namely visible light in the blue range and not ultraviolet (UV) light.[22–24] This medication provides no protection for the more serious complications of EPP, such as liver disease. Because most sunscreens block only UV and not visible light, they provide no benefit in EPP.[30] Beta-carotene was originally developed for treatment of EPP, but is now an over-the-counter nutritional product.[25] It may quench activated oxygen species in the skin and may provide some increase in sunlight tolerance. However, most patients find minimal benefit, even when dosage amounts are sufficient to cause skin discoloration. Afamelanotide only provides symptomatic relief for photosensitivity in EPP but is not a disease-modifying agent. Currently, no therapy is proven to be disease-modifying for EPP.[26]

Section 2.2: Summary of the Investigational Agent

Treatment of porphyrias with cimetidine. Cimetidine, the first H2 receptor blocker developed for treating peptic ulcer disease, has gained attention as a possible treatment for human porphyrias because of a potential off-target effect of ALAS inhibition. Later, other H2 receptor blockers, such as ranitidine, and famotidine, came generally to be preferred over cimetidine in clinical practice for peptic ulcer disease because cimetidine is a well-known inhibitor of hepatic cytochrome P450 enzymes (CYPs), increasing the likelihood of drug-drug interactions.[27] Nonetheless, this drug is an attractive prospect for the treatment of protoporphyria because cimetidine is an approved drug with few side effects over a wide dose range.

It initially gained attention when proposed as a less costly alternative to hemin in the treatment of acute hepatic porphyrias.[28,29] When given to several patients with acute hepatic porphyrias, cimetidine was reported to result in biochemical and clinical improvement.28,30 Subsequently, intravenous administration of 900-1200 mg cimetidine daily resulted in clinical and biochemical improvement in 4 patients.[31] Therefore, cimetidine was recommended as an alternative to hemin treatment based on cost and availability, and physicians caring for patients with porphyria often inquire about its use after finding reports in the literature. However, current guidelines do not recommend the use of cimetidine for treatment of acute hepatic porphyrias due to lack of evidence.[32–34]

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Cimetidine has also been reported to be beneficial in porphyria cutanea tarda (PCT), which is a primarily acquired form of hepatic porphyria that causes chronic blistering photosensitivity.[35] However, PCT is readily treated with other therapies. Complete remission is almost always achieved by phlebotomy or low dose hydroxychloroquine, so cimetidine usage for PCT treatment has little scope in clinical practice.[36]

More recently, cimetidine was proposed for the amelioration of EPP.[35,37–40] However, these reports are anecdotal and uncontrolled. Some patients also had pre-existing hepatic damage from EPP, which complicates the observations of drug effects.[37,38] Nonetheless, cimetidine has been proposed for use in children due to (1) lack of an effective treatment, (2) a very small number of case reports suggesting benefit in EPP, and (3) a few case reports describing possible responses to cimetidine in hepatic porphyrias.[35,37–40] Communications in the porphyria community indicate that a large number of patients, including children, have used cimetidine without oversight and without a systematic record of dosage or symptomatic improvement. The considerable interest in the larger porphyria community to assess with care its possible benefit as a treatment approach has therefore neither been addressed nor satisfied.

Possible limitations of this treatment: In EPP, the site of protoporphyrin overproduction is the bone marrow, where heme production is dependent on X-linked ALAS2. Cimetidine, on the other hand, affects the housekeeping ALAS1 found in the liver and other tissues. Furthermore, the effect of cimetidine on erythrocyte protoporphyrin levels in EPP is unconfirmed, although three reported EPP cases showed both a decline in protoporphyrin level and improvement in clinical status. Since two of these patients had advanced liver disease, which impairs protoporphyrin clearance in EPP, improvements might have been spontaneous or secondary to other interventions.[37,40] Nonetheless, these case reports, additional anecdotal accounts, and a plausible mechanism of drug action support further investigation of the use of cimetidine in the treatment of EPP. Widespread off-label use of the drug by EPP patients is an additional incentive to obtain high quality data on efficacy and safety.

Section 2.3: Summary of Nonclinical Data

Cimetidine is a well-known inhibitor of CYPs, and in 1984 was found to also decrease the activity of hepatic ALAS after IP administration to rats.[41] In this study, hepatic ALAS activity was reduced to 56% of controls thirty minutes after the administration of cimetidine.[41] The same group later demonstrated that after the induction of acute porphyria in rats by the administration of allylisopropylacetamide (AIA), cimetidine treatment reduced markedly elevated hepatic ALAS activity from >400% to ~250% of normal.[42] Based on these observations, they suggested that cimetidine might be useful for the treatment of human porphyrias.

In these studies, ALAS activity was measured in the liver, and hepatic ALAS is the ubiquitous ALAS1 isoform expressed in all tissues. Effects of cimetidine on ALAS2, the predominant isoform in the bone marrow, have not been studied to our knowledge. However, the enzyme active sites are similar for these isoforms, so it is likely that cimetidine will inhibit both, as is assumed in case reports of its beneficial effects in EPP. Cimetidine has become widely used by EPP patients even though studies in animal models of this disease have not been reported and would be desirable. However, species differences between humans and mice with EPP might significantly affect the response to cimetidine, and the ongoing widespread interest and use of the drug in the EPP community warrants an emphasis on studies of the drug in humans.

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Section 2.4: Summary of Previous Human Subject Data

Cimetidine had been attempted for the treatment of AIP as early as 1987, when it was administered for two weeks in a 43-year-old man with AIP with chronically elevated ALA and porphobilinogen (PBG). This therapy strongly decreased urinary ALA levels to normal (from >10mg/g creatinine to <3mg/g creatinine), but urinary PBG levels did not change.[29] A feeling of abdominal fullness also resolved with treatment.[29] Later, a 38-year-old female with AIP reported improvement in symptoms after cimetidine administration, with decrease in ALA and PBG to 38% and 51% of previous levels respectively.[28] These all rebounded after stopping cimetidine two weeks later.[28] In another study, cimetidine was administered to a 40-year old man in an acute porphyria attack who presented to the hospital with abdominal pain, constipation, vomiting, and elevated urinary ALA and PBG.[30] The patient was first treated unsuccessfully with glucose. The day after administration of cimetidine, urinary ALA decreased from >150 mg/24h to <50 mg/24h and urinary PBG decreased from >150 mg/24h to <100 mg/ 24h. Urinary PBG slowly decreased to <50 mg/24h over several days. With this therapy, the patient's symptoms improved. A 2005 study reported the use of IV cimetidine in acute porphyria attacks in 4 patients.[31] Urinary PBG ranged from 75 µmol/24h to 225 µmol/24h prior to treatment. Symptoms improved in all patients following cimetidine. Follow up urinary PBG decreased in 2 patients to 13 and 23 umol/24h, respectively, but could not be obtained in one patient. In the fourth patient, PBG was checked over the next 3 years and found to be 13-26 umol/24h.[31] Two out of four patients continued oral cimetidine, resulting in good control of symptoms.[31]

Cimetidine has also been demonstrated to result in control of PCT symptoms. In one 61-year-old male with porphyria cutanea tarda (PCT), cimetidine was administered for 2 weeks, with marked reductions in urinary uroporphyrin, from 4564 µg/L to <500 µg/L.[35] Skin lesions, alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyl transferase (GGT), and serum ferritin also all markedly improved.[35] However, because of the known effectiveness of hydroxychloroquine and phlebotomy for PCT, this therapy has not been commonly employed as a treatment for PCT.

In addition to AIP and PCT, cimetidine therapy has been reported in EPP in a few case studies. In 1993, the reduction in erythrocyte protoporphyrin with cimetidine treatment was reported in a 74-year-old woman with EPP who presented with jaundice and abdominal pain.[37] Following treatment with cimetidine for 2 weeks, her protoporphyrin levels in erythrocytes decreased from 16,227ug/dl to 11,602ug/dl, and her serum bilirubin decreased from 13.9 mg/dl to 6.7 mg/dl. Both of these levels rebounded after stopping cimetidine. Subsequently, a 2006 study reported improvement in photosensitivity in a retrospective report of three children with EPP who had tried cimetidine.[38] In one of these children with normal liver function, erythrocyte protoporphyrin decreased from 8.10umol/L to 5.10umol/L in one year. In the other two patients, although elevated serum aminotransferases decreased to normal during treatment, protoporphyrin changes were unfortunately not recorded. In one of these patients with liver disease, sunlight tolerance improved from <30min to >7 hours. All three patients were newly able to participate in outdoor activities to a similar degree as compared to their peers, improvements which were noted in 2 weeks.[38] The most recent study evaluating cimetidine in EPP is a report from Japan that described cimetidine and lactulose therapy in a patient with protoporphyrin-induced cholestatic liver failure. This patient experienced improvement in both liver function (serum total bilirubin 10mg/dl to <2mg/dl) and erythrocyte protoporphyrin levels (from 11,247ug/dl to 5,678ug/dl) following treatment.[40]

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Section 2.5: Summary of Dose Rationale

In the 74-year-old women with EPP presenting with jaundice and abdominal pain mentioned above, a dose of 800mg daily was used, and the same dose was administered in the individual with liver failure who was treated with cimetidine and lactulose.[37,40] For acute intermittent porphyria, cimetidine has been tried at doses of 800mg /day or 400mg bid.[28,30] In the study of three children with EPP treated with cimetidine, a total daily dose of 30-40mg/kg divided into two equal doses was used, which for a 60 kg individual would be 1800-2400mg daily. However, for gastroesophageal reflux disease in adults and adolescents, cimetidine is recommended to be given in doses up to 800mg po bid.[41] Children < 15 years old will not be included in the study. The elimination half-life is approximately 2 hours in adults, and the time to peak serum concentration is 0.75 hours to 1.5 hours.[41] Consequently, twice daily dosing allows for more stable serum concentrations as compared to daily dosing. Hence, considering it as the highest safe dose, 800mg po bid has been selected for this study.

Section 2.5.1: Known Potential Risks

This is an interventional pilot study that meets the federal definition of moderate risk.

The potential risks of this study are:

A. Drug-related Side Effects: Reported side effects of cimetidine including headache, diarrhea, dizziness, drowsiness and breast enlargement, and some less common but serious side effects like confusion, excitement, depression, nervousness and visual or auditory hallucinations. In addition, cimetidine could have side effects in EPP patients that may not be present in the general population. For instance, an inhibition of ALAS in patients with EPP who have ferrochelatase deficiency could result in impairments of heme synthesis and erythrocyte production that are impossible to predict, as there may be unidentified negative and positive feedback mechanisms. However, many EPP patients have already taken cimetidine without any reported significant side effects, decreasing the likelihood of any EPP-specific side effects from this medication.
B. Drug Interactions: Cimetidine can interfere with the metabolism of concomitant drugs that are substrates for CYPs, which may increase the potency and side effects of these drugs. Those taking drugs known to interact with cimetidine will not be enrolled in this study, minimizing this risk.
C. Blood Draw: Needle insertion can cause pain and bruising at the site of insertion. Rarely fainting caused by a vasovagal response to the phlebotomy procedure may occur. Because the skin is penetrated by the needle, there is a low risk for infection at the venipuncture site. The amount of blood needed for each study visit (three 8mL tubes) is within approved safety limits. For any participant who has a medical condition that restricts their capacity to provide adequate sample volume, the number of tubes of blood obtained will be modified on an individual basis.
D. Photosensitivity and light exposure assessment: Participants will be asked to go outside at least once daily and expose themselves to sunlight up to the point of first or prodromal symptom only and record their experience. Sun exposures may cause pain and skin related side effects from the disease
E. Loss of confidentiality: The risk of loss of confidentiality is low because there are many safeguards in place. Conceivably, data including clinical and diagnostic information could be accidentally divulged. If such an event occurred, subjects might be at risk for discrimination by life or health insurance companies, by employers, and by adoption agencies. The Genetic Information Non-Discrimination Act (GINA) is a federal law that protects Americans from being treated unfairly because of differences in their deoxyribonucleic acid (DNA) that may affect their health. This law prohibits genetic

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discrimination in the workplace and by health insurers. However, it provides no such protection for life insurance and disability insurance.

Section 2.5.2: Known Potential Benefits

Participants may derive no direct benefit from being in this study. The potential benefits of this study are a decrease in erythrocyte protoporphyrin levels and a subsequent improvement of light sensitivity symptoms. Participants and others with protoporphyrias should benefit from new high-quality evidence on the efficacy and safety of this treatment.

Section 2.6: Summary of Clinical Trial Readiness

This initial double-blind controlled study will provide clinical trial experience in EPP and establish dosage forms for cimetidine and placebo that can be used in additional studies, if indicated. Positive results will lay the groundwork for further studies, perhaps a larger phase 3 trial in EPP, probably using the same dosage form and placebo. On the other hand, clearly negative results from this high-quality study will indicate that cimetidine is very likely not effective in EPP and that further trials are not warranted.

Section 3: Study Objectives

Objective: The primary objective of this study is to determine the efficacy and safety of cimetidine administration in the protoporphyrias. Efficacy will be based on protoporphyrin levels, photosensitivity, and quality of life in subjects.

Section 3.1: Primary Efficacy Objectives

- To investigate the effect of cimetidine in decreasing erythrocyte total protoporphyrin level in patients with protoporphyria

Section 3.2: Secondary Efficacy Objectives

- To investigate the effect of cimetidine on the time to onset of prodromal symptoms associated with sunlight exposure in patients with protoporphyria
- To investigate the effect of cimetidine on patient-reported quality of life in patients with protoporphyria
- To investigate the effect of cimetidine on the number and severity of sunlight-induced pain events in patients with protoporphyria
- To investigate the effect of cimetidine in increasing the light dose required for onset of prodromal symptoms associated with sunlight exposure in patients with protoporphyria

Section 3.3: Exploratory Efficacy Objectives

- To investigate the effect of cimetidine on the rate of prodromal symptom onset in patients with protoporphyria
- To investigate the effect of cimetidine in decreasing plasma total porphyrins level in patients with protoporphyria

-Percent change in the ratio of metal-free to zinc protoporphyrin

Section 3.4: Safety Objectives

- To determine the safety of cimetidine administration in the protoporphyrias

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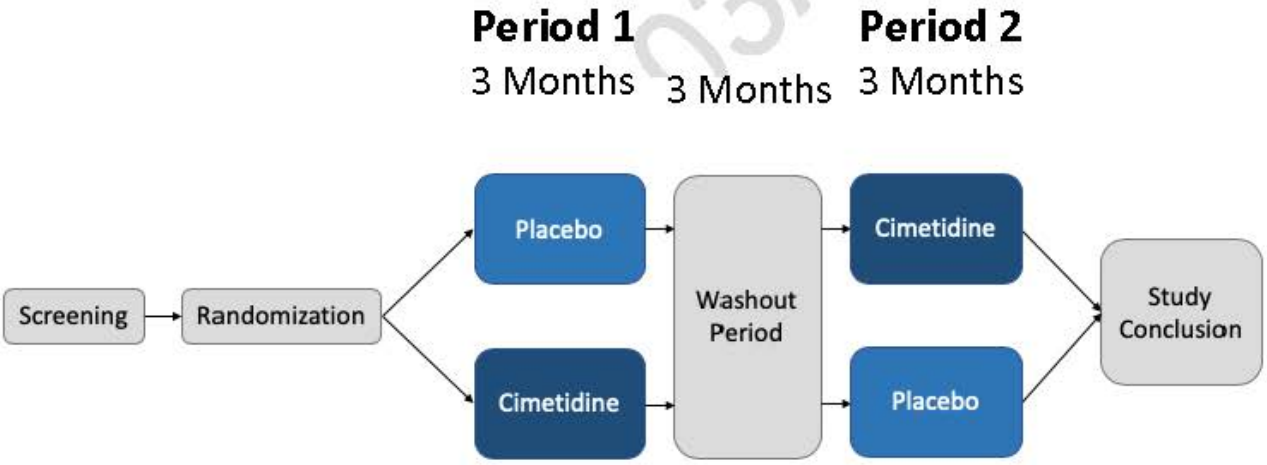
Section 4: Study Design

Section 4.1: Description of Study Design

This is a prospective, blinded, randomized, 2x2 cross-over design comparing cimetidine to placebo in patients with protoporphyria. Eligible protoporphyria patients will be randomized with equal allocation to one of two treatment sequences that will be administered over two 3-month study periods. The DMCC will randomize participants. Randomization will be stratified by site and permuted block randomization will be used to prevent chronological bias. Patients randomized to sequence 1 will receive placebo during period 1 and cimetidine during period 2. Patients randomized to sequence 2 will receive cimetidine during period 1 and placebo during period 2. Between periods, to eliminate any carry-over effects from the treatment administered in period 1, a wash-out period of 3 months will occur in which all patients receive neither cimetidine nor placebo (Figure 1). Three months was selected for each study period and for the wash-out period because of the rapid decline in protoporphyrin in red cells over the lifespan of the red cell (120 days), as well as to account for the time frame needed to measure light sensitivity in EPP. Erythrocyte life span might be shortened somewhat in protoporphyrias, but data is lacking.

Section 4.2: Study Schematic

Figure 1. Study Flow



Section 4.3: Study Endpoints

Section 4.3.1: Primary Endpoint

- Percent change in erythrocyte total protoporphyrin level post-treatment relative to pre-treatment

Description: Before and after each of the 2 study periods, both erythrocyte total protoporphyrin and plasma total porphyrin will be measured. Erythrocyte total protoporphyrin was selected as a primary endpoint in this study because improvement in photosensitivity would be expected to due to protoporphyrin reduction, and protoporphyrin is more easily quantified than light sensitivity.[2,10] Moreover, the hypothesis underlying the use of cimetidine for treating protoporphyrias is that it may lower protoporphyrin levels, and secondarily improve photosensitivity.

Three months was selected for each study period and for the wash-out period because of the rapid decline in protoporphyrin in red cells over the lifespan of the red cell (120 days), as well as to account for the time frame needed to measure light sensitivity in EPP. Erythrocytes are made in the marrow and are in circulation for an about 120 days in healthy adults. Exposure to high concentrations of protoporphyrin causes increased erythrocyte fragility, so it is expected that the erythrocytes of protoporphyria patients may have a shorter lifespan. Cimetidine is suspected to act on erythrocyte precursors in the marrow (not on circulating denucleated erythrocytes) to decrease ALAS2 activity and thereby decrease the erythrocyte total protoporphyrin concentration. Additional protoporphyrin is not made in mature circulating erythrocytes. While the erythrocytes in EPP could be circulating up to 120 days, protoporphyrin is known to rapidly diffuse out of the red cells, with the majority of protoporphyrin lost in less than 3 days.[42,43] This parallels the decline in fluorescent erythrocytes.[42,43] Consequently, the protoporphyrin in only the very young erythrocytes is expected to contribute significantly to the erythrocyte protoporphyrin level and to light sensitivity.[42] Within 1 month, the erythrocyte protoporphyrin level will likely have reached a new steady state. Subsequently, during months 2 and 3 of each study period we will measure light sensitivity. Two months is required for the light sensitivity evaluation because of the need for participants to have sufficient symptomatic events to measure light sensitivity. At the end of 3 months, erythrocyte protoporphyrin will be re-checked. This timing will additionally allow the majority of the circulating erythrocytes (>75%) to have encountered cimetidine during their development in the bone marrow. Because it is thought that only the very young erythrocytes contribute to the erythrocyte protoporphyrin pool, it is not necessary for 100% of the erythrocytes to have received cimetidine treatment during marrow development.

In view of the published observed timeframes of cimetidine-induced protoporphyrin decrease in protoporphyria and cimetidine-induced ALA/PBG decrease in acute intermittent porphyria (AIP), a timeframe even shorter than 90 days would likely be sufficient to evaluate the measurable effect of cimetidine on heme and protoporphyrin biosynthesis. In two studies evaluating cimetidine for protoporphyria in individuals with liver impairment, protoporphyrin decreased by 30% in one week in one study and by ~50% in 21 days in the other study.[37,40] In one AIP patient, PBG and ALA levels were each reduced by ~50% after 8 days of therapy.[32] Similarly, in another AIP patient, ALA levels were reduced by >50% within 7 days on 2 occasions after the administration of cimetidine.[29] However, turnover of protoporphyrin in erythrocytes is likely to be more prolonged than ALA turnover in the setting of AIP. In the study evaluating cimetidine therapy in 3 children with protoporphyria, the authors report symptomatic improvement after 2 weeks. Unfortunately, interval protoporphyrin levels were not drawn on any of these 3 patients at 2 weeks, and only one patient had a follow-up protoporphyrin drawn one year later demonstrating a 37% decrease.[38] Collectively, this data suggests that cimetidine may have a measurable effect on erythrocyte protoporphyrin within a timeframe of less than one month. Nonetheless, in order to ensure that the majority of the participants' circulating erythrocytes encountered cimetidine during early development in the bone marrow, the primary endpoint will rely on measurement of total erythrocyte protoporphyrin after 3 months of therapy.

Section 4.3.2: Secondary Endpoints

-Time to prodrome measured as prodrome-free outdoor exposure time

Description: The secondary endpoint will be to investigate the efficacy of cimetidine on the time taken for the onset of prodromal symptoms, defined as the initial symptom experienced (generally burning, tingling, itching, or stinging) in patients with protoporphyria following sunlight exposure. Because of the severity and duration of pain associated with full phototoxic reactions, it will be unethical and also unnecessary to subject participants to sun exposure past the prodromal period. It is well established that prodromal symptoms, which are early warning symptoms of progression

to painful phototoxic episodes, with continued light exposure progress rapidly to a severe and prolonged reaction. Participants will report their outdoor exposure and symptoms via a daily text survey during the last 2 months of each treatment period. The mean of patient-reported daily prodrome-free outdoor exposure time will be compared between the treatment and placebo period for each participant.

- Patient-reported quality of life as measured by PROMIS-57 scale
Description: Patient-reported quality of life is an important endpoint to measure. Our study will use the PROMIS-57 survey which is a validated quality of life (QOL) tool and has been previously used in PC studies and assessed in Protoporphyria patients.[44] QOL will be assessed at enrollment and after each study period.

-The number and severity of sunlight-induced pain events (phototoxic episodes)
Description: In patients with protoporphyria, an effective drug would be expected to decrease the number and severity of phototoxic episodes. However, some may experience more than normal pain events in a clinical trial setting which requires them to test new light exposure limits. Furthermore, because many protoporphyria patients protect themselves from sunlight exposure due to the severity of pain associated with phototoxic reactions, the number and severity of phototoxic episodes have not been consistently reliable as endpoints in prior trials.[23] Nevertheless, this will be recorded as a secondary endpoint in this study because of its clinical relevance. This information will be collected via a daily text survey and severity will be patient-reported on a scale of 1-10.

-Light dose required for time to prodrome
Description: A small, wearable light dosimeter that continuously measures UV and both indoor and outdoor blue/red light will be used to provide a secondary endpoint in this study, specifically, the amount of light participants have been exposed to and find tolerable during treatment with cimetidine vs. placebo.

This is an innovative approach, as no prior clinical trial has incorporated light intensity measurements as a clinical trial endpoint in protoporphyria, although patients report a quicker onset of symptoms in direct sunlight. Unpublished data by the study PI support the use of light dosimetry as a secondary endpoint. Because protoporphyrin is primarily activated by light within the blue range of the visible spectrum (peak at the Soret band, ~411nm) as well as the red spectrum (635nm), the intensity of light exposure in the blue range and the red range will be measured by the light dosimeter in addition to UV.[45] Importantly, the blue and red light measurement will also help to capture the contribution of indoor artificial light exposure, to which many patients report sensitivity. The endpoint will be a comparison of the average light dose without symptoms during the treatment period vs. the placebo period. The measurement of light sensitivity will be captured during the last 2 months of each study period.

Section 4.3.3: Exploratory Endpoints

-Rate of prodrome events per prodrome-free outdoor exposure time
Description: Participants will report their time spent outdoors via a daily text survey during the last 2 months of each treatment period. The total number of prodromal symptom episodes divided by the total amount of sunlight exposure without prodromal symptoms will be compared between the treatment and placebo period for each participant.

-Percent change in plasma total porphyrin levels post-treatment relative to pre-treatment
Description: Erythrocyte protoporphyrin is typically measured for protoporphyria diagnosis. While plasma total porphyrins is also known to be elevated in protoporphyria, it is considerably lower

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and more variable over time in patients than erythrocyte protoporphyrin, suggesting more rapid turnover.[46] Some have speculated that plasma porphyrins may relate more closely with symptoms because of its more intimate contact with tissues and greater ability to more readily accumulate in subcutaneous tissues and be taken up and excreted by hepatocytes. Therefore, plasma total porphyrins will be an exploratory endpoint in this study.

-Percent change in the ratio of metal-free to zinc protoporphyrin
Description: The ratio of metal-free to zinc protoporphyrin is less variable in patients over time in protoporphyria than total erythrocyte protoporphyrin and plasma porphyrins.[10] The proportion of zinc protoporphyrin seems to be greater in EPP patients with milder disease, but almost never exceeds 15% of the total in EPP. However, it may be up to 50% or occasionally even higher in XLP. One might expect that if cimetidine lowers total protoporphyrin in red cells that it might change the ratio of metal-free and zinc proto, at least in EPP.

Section 4.3.4: Safety Endpoints

-Rates of adverse events will be computed and reported descriptively
Description: Rates of adverse events will be computed and safety variables such as laboratory findings will be described.

Section 5: Study Enrollment

Section 5.1: Inclusion Criteria

1. Prior enrollment or co-enrollment in the Longitudinal Study of the Porphyrias (PC Study 7201) with a confirmed diagnosis of EPP or XLP
2. Male or female age ≥15 years
3. Characteristic history of non-blistering cutaneous photosensitivity
4. Willing and capable of giving informed consent and following procedures described in the protocol

Section 5.2: Exclusion Criteria

1 Participants not willing to expose themselves to light to the point of prodromal symptoms at least weekly

2 History of liver or bone marrow transplant or clinically significant liver dysfunction as determined by the Investigator

3 Known or suspected allergy or intolerance to cimetidine

4 Use of any other experimental therapy in the past 3 months at screening

5 Use of cimetidine within the past 3 months at screening

6 Individuals with elevations of porphyrins in plasma or erythrocytes due to other diseases (i.e., secondary porphyrinemia) such as liver and bone marrow diseases

7 Patients with any clinically significant comorbid conditions, which in the opinion of the Investigator, precludes participation

8 Treatment with any drugs or supplements (Appendix 1) that in the opinion of the Investigator can interfere with subject safety or the objectives of the study.

9 The participant either does not have a smartphone or is not willing to use his/her smartphone for the study

10 Women who are pregnant, breastfeeding, or actively planning to become pregnant

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11 Individuals with moderate to severe renal insufficiency

Section 5.3: Study Recruitment and Retention

Recruitment efforts by the investigators in the PC will target the following:

A. Patients followed by any of the Investigators in the PC: Each of the investigators is a porphyria specialist in a Porphyria Center, providing clinical care to individuals suspected of and diagnosed with porphyria, along with diagnostic and follow-up evaluations, consultations, and/or treatment. For such patients who are likely to be eligible for the study, the investigators will either discuss the study with the patient during a clinical or study visit or will contact the patient by telephone, email, or mail. Any efforts to contact and recruit patients and families will be with Institutional Review Board (IRB) approval and adhere to standards for ethical conduct of research and be fully Heath Insurance Portability and Accountability Act (HIPAA) compliant. Individuals who were not patients of the PC investigators but have given them consent for being contacted for studies may also be contacted.

B. Referrals from patient advocacy groups to the PC centers: Patient advocacy organizationsprovide education about porphyria to patients, their families, healthcare professionals, and the public, as well as supporting porphyria research. Their outreach programs include websites and active disease-specific Facebook groups, as well as periodic newsletters and special announcements emailed and/or mailed to individuals who have registered with information about new developments in treatment, porphyria-related education opportunities, and research studies available through the PC. Information about research studies includes contact information for the studies, if patients are interested either in obtaining additional information or in participating.

C. Facebook advertisements: The study staff can directly advertise for the study on rare disease or porphyria social media group pages, such as porphyria or EPP pages on Facebook or Twitter.

D. Non-study physician referrals: Physicians providing clinical care to individuals suspected of or diagnosed with EPP who are not investigators in this study may refer patients who may be eligible for and express interest in the study to a PC center. Co-enrollment into the Longitudinal Study of the Porphyrias (LS) would be required.

E. Self-referrals, including family members of individuals diagnosed with EPP, as well as other individuals who may have heard about the study from other subjects or prospective subjects: In the case of family members of individuals diagnosed with EPP, initial contact with the family member will not be made by the study team. These family members will be asked to contact the patient, requesting that they contact the study coordinator or to register through the Rare Diseases Clinical Research Network (RDCRN) PC if interested in obtaining additional information or participating. These individuals will also be asked to enroll in the LS, which is the principal study of the PC, and will assure documentation of the diagnosis of EPP or XLP.

In the case of patients followed by one of the consortium investigators, the physician/investigator or study coordinator will either: (a) contact the patient by telephone, email, or mail informing him/her of the study and requesting that he/she contact the study coordinator if interested in obtaining additional information or participating, or (b) discuss the study in-person during a clinical visit. Informed consent may be obtained at that time. In all other cases, prospective subjects are instructed to contact the study coordinator, either the Project Manager or a site-specific study

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coordinator, by telephone, email, or mail if interested in obtaining additional information or in participating.

Recruitment considerations:
The study requires subjects old enough to understand how to participate in the assessment of photosensitivity. Therefore, participants need to be at least 15 years of age. This will allow for inclusion of adolescents, ages 15-18 years, which is important because EPP is the most common porphyria in children.

Participants with protoporphyria (EPP, XLP, or CLPX porphyria) 15 years of age or older, including both genders and all ethnic groups can be enrolled in this early interventional study, should they meet inclusion/exclusion criteria. The REDCap/Twilio text surveys to assess light exposure and symptoms are suitable for adults and adolescents, but not for children younger than age 15. Participants potentially eligible will include those known to the investigators who can be contacted and informed of the study, and participants who hear about the study through the APF, their physicians, or other sources and contact one of the centers to express interest in the study.

Because XLP is an X-linked disorder, it does not affect both genders equally, but overall, because EPP is more common that XLP, enrollment of an approximately equal number of males and females is expected. EPP is uncommon in Africa and in African Americans because the low-expression *FECH* allele, which is associated with >90% of EPP cases, is rare in these groups. Therefore, few if any African Americans are expected to be enrolled.

Inclusion of vulnerable groups: Children less than 15 years of age are excluded because the text surveys to assess light exposure and symptoms in this initial study are designed for adults and adolescents. Pregnant women are excluded because pregnancy is reported to lower erythrocyte protoporphyrin levels and improve photosensitivity in EPP, and this would interfere with observations on the effects of cimetidine treatment. Any subjects who are not able to give informed consent will also be excluded.

Afamelanotide is now available and effective for treatment of EPP-related photosensitivity, and to require participants to stop this therapy might raise ethical issues and also limit the number of patients available for this trial. Participants taking afamelanotide must still meet all the inclusion and exclusion criteria.

The following criteria apply to primary and secondary study endpoints for patients who are treated with afamelanotide during this trial. It is recognized that variations in afamelanotide treatment during the trial may be beyond the control of study subjects.

> Primary endpoint of a change in erythrocyte protoporphyrin: All enrolled subjects will contribute to this primary endpoint without regard to any concurrent treatment with afamelanotide or changes in that treatment during the trial.
>
> Secondary endpoints of change in light sensitivity and quality of life:
>
> Subjects may contribute to these secondary endpoints if, for at least one of the last two months of both treatment periods, they are on afamelanotide treatment or off afamelanotide treatment (but not on afamelanotide for one treatment period and off for the other), as defined as the following:
>
> > "On afamelanotide" treatment is defined as having received an afamelanotide implant within the prior 1-9 weeks.

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"Off afamelanotide" treatment is defined as not having received an afamelanotide implant within the past 12 weeks.

Subjects may contribute to the secondary endpoint for quality of life and fill out the after-Treatment Period quality of life surveys if they are on afamelanotide treatment or off afamelanotide treatment, as defined above, for both blinded cimetidine vs placebo treatment periods (but not on afamelanotide for one treatment period and off for the other). When filling out the after-Treatment Period quality of life surveys, subjects will be asked to answer the questions to best represent their quality of life experiences when they were either on or off afamelanotide. For example, if they were on afamelanotide for all of Treatment Period 1 and the first month of Treatment Period 2, but not on afamelanotide for the last month of a Treatment Period, when filling out the after-Treatment Period quality of life surveys, they will be asked to remember and answer questions about their quality of life from 1 month ago when they were still on afamelanotide.

Washout extension: The 3-month washout period can be extended in order to allow time to address issues related to Scenesse access during that period. This allows for problems related to delays in access to afamelanotide that may be beyond a subject's control.

No enrollment procedures will occur until the subject has provided informed consent/assent.

Retention strategies:
EPP and the other protoporphyrias are rare diseases, and only a small number of specialists have the expertise to evaluate and treat affected individuals. Clinical relationships established by the investigators with patients who enroll in this study, as well as other studies, will provide a context for increased communication with patients and their primary physicians that can facilitate retention. Furthermore, this study will provide a number of beneficial services to the participants and their primary physicians that will enhance retention. These include periodic evaluations, repeat laboratory testing, and information on new research developments in the field. Coordinators and other personnel at each site will also work to maintain close contact with participants, including regular clinic visits and communication by phone and email. There will be no charge for the physician services or laboratory tests done as part of this pilot study. A high level of interest in cimetidine is already present in the community of protoporphyria patients, and the investigators and coordinators will continue to emphasize the importance of ascertaining whether this drug is effective in the treatment of protoporphyrias.

Section 5.4: Screen Failures

Screen failures are defined as participants who consent to participate in the human subject research but are not subsequently eligible for the study. A minimal set of screen failure information is required to ensure transparent reporting of screen failure participants, in case the investigator must respond to queries from regulatory authorities. Minimal information includes demographic data, screen failure details, eligibility criteria, and any serious adverse event.

Subjects who do not meet the inclusion/exclusion criteria can be rescreened after at least 2 weeks if there is reason to believe that they will meet inclusion/exclusion criteria at the second screening. An individual may be rescreened up to 5 times. A participant who was on an investigational drug for EPP at the initial screening can be rescreened 3 months after cessation of drug administration. Subjects who are taking cimetidine, afamelanotide, or other non-experimental phototherapy at screening must be willing to stop for at least 4 weeks before being rescreened. The screening procedures for rescreening will remain the same as in the initial screening. Rescreened participants should be assigned the same participant number as in the initial screening.

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Section 5.5: Subject Withdrawal

All data as collected prior to termination for the reasons outlined below will be included in the primary analysis unless the patient withdraws consent. If participants withdraw, every effort will be made to conduct a final study visit with the participant, and they will be followed clinically until, if applicable, all adverse events resolve. Reasons for withdrawal may include the following:

- Withdrawal of consent. Subjects are free to withdraw on their own accord at any time.
- Withdrawal by the investigator
- Intercurrent illness or event that precludes further visits to the study site or ability to evaluate disease (e.g., mental status change, inpatient admission).
- The subject is unable to tolerate the study drug in the opinion of the investigator

Subjects who withdraw from the study, can reconsent and restart the study from the beginning, using the new data for study results. Alternatively, if withdrawal happened during the study washout period, participants can re-consent and restart the study for Treatment Period 2. This situation may arise, for instance, if a participant decides to withdraw from the study due to concerns over access to afamelanotide but this concern later resolves.

Section 6: Study Drug/Device

Section 6.1: Acquisition

Cimetidine 800 mg capsules and placebo capsules will be obtained from Greenpark Compounding Pharmacy. They will be supplied to each site either directly from Greenpark Compounding Pharmacy or from another pharmacy that will be serving as the coordinating pharmacy for this study.

Section 6.2: Formulation, Appearance, Packaging, and Labeling

Cimetidine 800mg capsules are size 00 with a light blue opaque color and contain 800mg of active drug product. The matching placebo capsules of spray dried lactose powder will be manufactured to be physically identical to cimetidine 800mg tablets.

A bulk supply of Cimetidine 800-mg and matching placebo capsules will be packaged into prescription medication bottles.

All labeling will comply with applicable regulatory requirements.

Section 6.3: Storage and Stability

Both cimetidine and placebo capsules will be stored in tightly closed containers at room temperature between 59-86 degrees F (15-30 degrees C) away from moisture and light.

Section 6.4: Investigational Agent/Device Preparation

Cimetidine 800mg capsules and placebo capsules will not require further preparation before dispensation once received by the coordinating pharmacy.

Section 6.5: Dosing and Administration

All participants will take one capsule of cimetidine or placebo once in the morning and once in the evening. Investigational product will be dispensed twice in the study, once prior to each treatment period. In some cases, it may be dispensed once at the beginning of the study to minimize the number of in-person visits. At each dispensation, 12 weeks (3 months) of investigational product,

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plus extra in case of damage, loss, or blood draw delays will be provided to participants. In the case of blood draw delays or errors, participants can continue the study drug until the porphyrin tests has been completed by UTMB. Capsules should not be broken or chewed. Participants will be on investigational product treatment for a total of 24 weeks (6 months). There will be no dose escalations, de-escalations, or dose adjustments. If a subject is unable to tolerate investigational product, they will be withdrawn from the study.

Section 6.6: Subject Compliance Monitoring

After each Treatment Period, the subject will return all previous study drug bottles. The site coordinator or pharmacy will count and reconcile all study medication at each visit.

Section 6.7: Return or Destruction of Investigational Agent/Device

Medication returned by subjects will be destroyed per institutional policies in accordance with all applicable regulations after monitoring and reconciliation has been completed.

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Section 7: Study Procedures

Section 7.1 Schedule of Events

	Screening[6]	Treatment Period 1 (TP1)				Washout		Treatment Period 2 (TP2)				EOS[3]
	Screening[3]	Day 1[2]	1M[2]	2M[2]	3M[3]	Start	End[3]	Day 1[2]	1M[2]	2M[2]	3M[3]	
Study Procedure	D-60 to -1 TP1	D1	D30 (+/- 7 days)	D60 (+/- 7 days)	D90 (+/- 7 days)	D91	D180 (+2 weeks)	D181 (+/- 7 days)	D210 (+/- 7 days)	D240 (+/- 7 days)	D270 (+/- 7 days)	D270 (+/- 7 days)
Informed Consent	X											
Eligibility Confirmation	X											
Medical History & EPP Symptoms	X				X		X				X	X
Erythrocyte Protoporphyrin & Plasma Porphyrins	X				X		X				X	X
Hepatic Function Panel & Iron Studies	X				X		X				X	X
Hemoglobin or hematocrit or CBC	X				X		X				X	X
Pregnancy Test (Urine)[5]	X		X	X	X		X	X	X	X	X	X
Daily text survey (time outdoors, time-to-prodrome, phototoxic episodes, pain severity)	X			X	X		X			X	X	X
Light dosimeter	X[4]			X	X					X	X	X
PROMIS-57	X				X		X				X	X
Drug Start		X						X				
Drug Return & Compliance					X						X	

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AE Review		X	X
Concomitant Medication Review	X	X	X
Randomization[7]	X		
[1]The study medications can be mailed to participants [2]Telephone calls/telehealth visits [3]These visits can occur at the study site or at the participants home via telehealth and with a visit to a local lab for sample collection [4]Training of how to wear the light dosimeter will occur at the Screening visit [5]Women of child-bearing potential [6]Pre-screening phone call to occur prior to screening visit [7]Only once eligibility is confirmed			

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Section 7.2: Pre-Screening Call or Visit

At each of the PC sites, the investigative team will identify subjects with protoporphyria who are eligible for the study. Patients will be already enrolled in the LS with documentation that will enable them to be eligible for this study or will be co-enrolled into the LS and this trial. The team will then approach the subject, either via a phone call or at a clinic visit. They will fully describe the intent of the study and ask screening questions consisting of the inclusion and exclusion criteria. Subjects will also be given a copy of the consent form and study instructions to take home with them.

For participants that are <18 years old, verbal consent for the REDCap survey will be obtained by the parent and verbal assent will be obtained by the child. Both one parent and child will be present on the pre-screening call.

If the subject satisfies the inclusion and exclusion criteria of the study and if the subject is still interested in participating in the study, a screening visit can be scheduled. If the pre-screening visit is a telephone call, the patient will be emailed/mailed the copy of the consent form, study instructions, and the light dosimeter, and a screening visit will be scheduled.

Section 7.2: Screening Visit

- The screening visit, including consenting, can be done in-person or remotely, via telehealth. The screening visit can be split into multiple visits, as long as these occur within the required time frame. In most cases, the informed consent will need to occur during a virtual visit prior to receiving the study medication and device.
- All patients will be enrolled in the LS either previously or at the screening visit for this study.
- The study staff will describe the intent of the study and what the potential risks and benefits of participation will be. The subject will have an opportunity to review the consent form and to ask any questions of the study team, prior to signing the consent form.
- For participants that are <18 years old, written consent for the study will be obtained by the parent and written assent will be obtained by the child. Both one parent and child will be present on the screening call.
- For remote screening, the remote consent process will follow all applicable single IRB and local IRB guidelines.
- REDCap screening survey asking for information such as medical history, EPP symptoms, light sensitivity, and concomitant medications, which will be completed by the subject before or during the screening visit. This screening survey will contain consent language, as it will be filled out by the participant before formal consent for the study. If participants do no end up enrolling in the study, the subjects' responses from this survey will still be stored and can be analyzed as needed.
- Electronic Case Report Form (CRF) surveys completed by the subject in the REDCap EDC directly will serve as source documents (Direct Data Entry). Alternatively, paper surveys can be completed if preferred by the participant. Information will also be recorded in the subject's medical record at the study sites if allowable per local guidelines, which will serve as backup source documentation.
- All concomitant medications taken beginning at screening will be recorded
    - If the subject is currently using a tanning agent such as phototherapy or afamelanotide, they will be instructed to continue this therapy throughout both study periods, or they will be instructed to stop the therapy prior to initiation of the study drug, 4 weeks for phototherapy and afamelanotide. The subject can return at a later date for rescreening. If a subject is using cimetidine at the time of

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screening, the subject may stop therapy for at least 3 months and return at a later date for rescreening.

- Overnight fasting is not needed. Laboratory studies will include the following:
  - A complete blood count with differential or hemoglobin or hematocrit
  - Hepatic function panel (ALT, albumin, alkaline phosphatase, AST, bilirubin total, bilirubin direct, protein total)
  - Iron Studies (serum iron, serum ferratin, total iron binding capacity (TIBC), transferrin saturation)
  - Total erythrocyte protoporphyrin level with ratios of metal-free protoporphyrin and zinc protoporphyrin
  - Plasma total porphyrins
  - A urine pregnancy test will be completed on women of child-bearing potential (WOCBP) defined as women who are menstruating at the time of screening. If a participant has a positive pregnancy test, they will be withdrawn from the study.
  - Laboratory assessments will be done at local laboratories or UTMB, and protoporphyrin samples will be shipped to University of Texas Medical Branch (UTMB).
- The diagnosis of EPP, XLP, or CLPX porphyria and inclusion/exclusion criteria will be confirmed prior to Day 1 of the study.
- Patients will be instructed on the reporting of light exposure and symptoms (time to prodrome) using daily REDCap/Twilio text surveys.
- Patients will be instructed on the use of the light dosimeter.
- Patients will complete a quality-of-life questionnaire (PROMIS-57) at the screening visit and before day 1 using a survey link sent through email
- Subjects will be randomized to one of the two treatment sequences. Subjects will be provided with a 3-month (12 week) supply of study drug capsules with a surplus of 14 capsules to account for dropped/lost capsules. This can be mailed to study subjects if the visit is not in person.

Section 7.3: Study Day 1

- Participants will begin treatment period 1 on a pre-defined date, which will be within 60 days of the screening visit. If 60 days is exceeded, the screening visit procedures must be repeated prior to day 1.

Section 7.4: Follow-up Phone Calls at 1 Month and 2 Months

- Coordinators will contact subjects by phone to monitor study compliance with the REDCap/Twilio text surveys and study drug, as well as review adverse events and concomitant medications. Participants will also receive automated text and email reminders. If there is evidence of non-compliance with these surveys, the study coordinator will reach out additionally to the participant by phone or by email for follow-up.
- At the beginning of the second month, the participant will begin daily text surveys reporting their daily outdoor exposure and time-to-prodrome and location, to be continued through the end of month 3. Repeat instructions for use of the light dosimeter and for the completion of text surveys will be provided at the 1 month phone calls in each study period (i.e., after the completion of 1 month of that study period and before beginning use of the light dosimeter). Participants may be asked to complete additional days/weeks of light dosimetry and text surveys, most likely prior to the 2 months of data collection for each period, in order to ensure proper device connection and functioning.
- A urine pregnancy test will be completed on WOCBP. If a participant has a positive pregnancy test, they will be withdrawn from the study

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- At each study visit symptoms, participants will be asked whether or not they have symptoms that have been reported to be associated with cimetidine, using a checklist of cimetidine side effects.
- We expect that participants will fill out >80% of the daily surveys and wear the dosimeter device for >80% of the required days. We expect >80% compliance with taking the study medication.

Section 7.5: Follow-up Visits at 3 months and End of Study (EOS)

- The follow up visits can be done remotely via telemedicine or on site, at the discretion of the PI after consideration of circumstances related to the subject's availability.
- Adverse events and medical history will be reviewed and recorded
- Changes in concomitant medication will be recorded
- Patients will complete a quality-of-life questionnaire (PROMIS-57) after the visit using a survey link sent by email or via telephone call with the coordinator. At EOS visit, the quality-of-life survey will be part of the study conclusion survey.
- At month 3 their self-perceived time-to-prodrome over the prior 2 months will be assessed. This will be in addition to the daily text surveys reporting their daily outdoor exposure and symptoms and location.
- Laboratory studies will include the following:
    - A complete blood count with differential or hemoglobin or hematocrit
    - Hepatic function panel (ALT, albumin, alkaline phosphatase, AST, bilirubin total, bilirubin direct, protein total)
    - Iron Studies (serum iron, serum ferratin, TIBC, transferrin saturation)
    - Erythrocyte and plasma protoporphyrin levels with ratios of metal-free protoporphyrin and zinc protoporphyrin
    - Plasma total porphyrins
    - Protoporphyrin samples will be shipped to UTMB. Other laboratory assessments will be performed at local laboratories or UTMB.
    - A urine pregnancy test will be completed on WOCBP. If a participant has a positive pregnancy test, they will be withdrawn from the study
- Study drug counts will be performed and recorded to monitor compliance
- Unused medication will be collected.
- The study medication will be stopped after 3 months of treatment in compliance with the protocol.
- At each study visit symptoms, participants will be asked whether or not they have symptoms that have been reported to be associated with cimetidine, using a checklist of cimetidine side effects.
- We expect that participants will fill out >80% of the daily surveys and wear the dosimeter device for >80% of the required days. We expect >80% compliance with taking the study medication.

Section 7.6: Follow-up Visit After Washout Period

- The follow up visits can be done remotely, at the discretion of the Investigator based on the circumstances of the participant.
- Adverse events (AE) and medical history will be reviewed and recorded
- Changes in concomitant medication will be recorded
- Patients will complete a quality-of-life questionnaire (PROMIS-57) after the visit using a survey link sent through email or via telephone call with the coordinator
- Laboratory studies will include the following:

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- A complete blood count with differential or hemoglobin or hematocrit
- Hepatic function panel (alanine aminotransferase, albumin, alkaline phosphatase, aspartate aminotransferase, bilirubin total, bilirubin direct, protein total)
- Iron Studies (serum iron, serum ferratin, TIBC, transferrin saturation)
- Erythrocyte and plasma protoporphyrin levels with ratios of metal-free protoporphyrin and zinc protoporphyrin
- Plasma total porphyrins
- A urine pregnancy test will be completed. If a participant has a positive pregnancy test, they will be withdrawn from the study.
- Protoporphyrin samples will be shipped to UTMB.
- Other laboratory assessments will be performed at local laboratories or UTMB.

- At end of the wash-out period a 3-month (12 week) supply of study drug capsules with a surplus of 14 capsules to account for dropped/lost capsules will be dispensed. This can be mailed to study subjects if the visit is not in person. Special arrangements will be made if study drug is lost or stolen in order to maintain treatment, and these events will be recorded.
- At each study visit symptoms, participants will be asked whether or not they have symptoms that have been reported to be associated with cimetidine, using a checklist of cimetidine side effects.

Section 7.7: Study Conclusion Survey

- Before completion of the second treatment period, participants will be sent a study conclusion survey that contains the quality-of-life survey (PROMIS-57).
- In the event of AEs, every effort will be made to continue monthly follow-up with subjects until all AEs are resolved.

Section 7.8: Early Termination Visit (ETV) /Unscheduled Visit

If a patient decides to completely withdraw consent/assent from the study, every attempt will be made to have the patient complete an ETV visit which will collect the same information as an EOS Visit (Month 9).

Unscheduled visits may occur at the discretion of the Investigator and is defined as a visit outside of the protocol specified time points for safety or other reasons at the discretion of the investigator. All unscheduled visits and assessments performed during the visits will be recorded in the electronic CRF.

Section 8: Safety

Section 8.1: Safety Parameters

- An adverse event (AE) is defined as any untoward medical occurrence associated with the use of an intervention in humans, whether or not it was considered intervention-related (21 CFR 312.32(a)).
- A serious adverse event (SAE) is defined any untoward medical event that results in death, is life threatening, requires inpatient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, or causes a congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon medical judgement, they

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may jeopardize the subject and may require medical or surgical intervention to prevent on of the outcomes listed in this definition.

- An unanticipated event (UE) is an incident that meets all the following criteria:
  - Unexpected in nature, severity, or frequency: Not described in the study-related documents such as the protocol, consent, or not expected based on the study population.
  - Related or possibly related to participation in the research: Reasonably possible that the incident may have been caused by the procedures involved.
  - Suggest that the research places subjects or others at greater risk of harm that alters previous risk/benefit ratio established.
- Sun sensitivity events *will not* be treated as AEs.

Section 8.2: Adverse Event Classification (Severity, Relatedness, Expectedness)

Common Terminology Criteria for Adverse Events (CTCAE) v5.0

- Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2: Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living
- Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care activities of daily living
- Grade 4: Life-threatening consequences; urgent intervention indicated
- Grade 5: Death related to the adverse event

The clinician's assessment of an adverse event's severity and relationship to the study agent/device or study procedure is part of the documentation process, but it is not a factor in determining what is or is not reported in the study. If there is any doubt as to whether a clinical observation is an AE, the event should be reported. All AEs must have their relationship to the study agent/device or study procedure assessed. In a clinical trial, the study agent/device or study procedure must always be suspect. To help assess, the following guidelines are used.

- **Related** – The AE is known to occur with the study agent/device or study procedure, there is a reasonable possibility that the study agent caused the AE, or there is a temporal relationship between the study agent/device or study procedure and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study agent/device or study procedure and the AE.
- **Possibly Related** – A clinical event with a reasonable time sequence to the study agent/device or study procedure, but which could also be explained by concurrent disease or other drugs or chemicals.
- **Unlikely Related** – A clinical event with little or no temporal relationship to the study agent/device or study procedure and which other drugs, chemicals, or underlying disease provide plausible explanations.
- **Not Related** – There is not a reasonable possibility that the administration of the study agent/device or study procedure caused the event, there is no temporal relationship between the study agent/device or study procedure and event onset, or an alternate etiology has been established.

The Investigator will report unexpected problems to the reviewing IRB and to the study chair. This report will include the following information:

- Protocol identifying information: protocol title and number, Primary Investigator's name, and the IRB project number;
- A detailed description of the event, incident, experience, or outcome;

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- An explanation of the basis for determining that the event, incident, experience, or outcome represents an unexpected problem;
- A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the unexpected problem.

Section 8.3: Adverse Event Reporting (Timeline and Procedures)

The site must complete the AE form in REDCap EDC within the following timelines:

- All deaths and immediately life-threatening events, whether related or unrelated, will be recorded in the EDC within 24 hours of site awareness and submitted to the National Institutes of Health (NIH), IRB, and Federal Drug Administration (FDA).
  - Notification to NIH and study co-chairs will occur automatically via REDCap EDC
  - Notification to local and central IRB (if required) is the responsibility of the site staff
  - Notification to FDA. Dr. Dickey will send notification by Fax or in the eCTD format within 7 days as per FDA requirements.
- All other (suspected) reportable AEs must be reported in REDCap EDC within **3 working days** of the notification of the event or of the site becoming aware of the event. Follow-up and reporting to the FDA and IRB will occur as follows:
  - As per FDA requirements, Dr. Dickey will report any (1) serious, unexpected or suspected adverse reactions, (2) findings from other clinical, animal, or in-vitro studies that suggest significant human risk, and (3) a clinically important increase in the rate of a serious suspected adverse reaction to the FDA and to all investigators no later than 15 calendar days after determining that the information qualifies for reporting. These reports can be by paper or in the eCTD format.
  - As per the MGH IRB requirements, internal adverse events that are unexpected and related/possibly related to the research and external adverse events that are serious, unexpected, and related/possibly related must be reported to the IRB within 5 working days/7 calendar days of the date the investigator first become aware of them. Adverse events that are expected (i.e., documented in the protocol) are not reported to the IRB. A cumulative report of all adverse events must be submitted at annual IRB continuing reviews.
  - All SAEs will be followed until satisfactory resolution or until the Investigator deems the event to be chronic or the adherence to be stable. Other supporting documentation of the event may be requested by the IRB or NIH and should be provided as soon as possible.

Section 8.4: Study Suspension Criteria

The study may be suspended if it is recommended or required by the Study Co-Chairs, local IRB, data safety monitoring board (DSMB), FDA, or Sponsor, or if a study participant dies. The NIH, RDCRN DSMB, and local IRBs (at their local site) have the authority to stop or suspend this trial at any time. This study may be suspended or closed if:

- The Study Co-Chairs / Study Investigators believe it is not safe for the study to continue because of unexpectedly frequent adverse events
- The RDCRN DSMB suspends or closes the trial
- The NIH suspends or closes the trial
- The FDA suspends or closes the trial

If the study is suspended at any time, every attempt should be made to have the patient complete an ETV as described above.

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Section 8.5: Unblinding Procedures

This is a double-blinded study; neither the subject nor the study site personnel will know which treatment is being taken. Each subject will be assigned a code, and this can be broken only in case of emergency by the local pharmacy. The Investigator should request unblinding approval from the study co-chairs in advance, if possible. If this is not possible, the Investigator can request that the local pharmacy break the code and document the reason for doing so. If the blind is broken for an individual subject, the subject must be withdrawn from the study and complete an early termination visit.

Section 8.6: Study Oversight

The Study Chair has primary oversight responsibility of this clinical trial. Safety oversight will be under the direction of the NIH appointed DSMB. The DSMB will review accrual, patterns and frequencies of all adverse events, and protocol compliance every 6 months. The DSMB will make recommendations to the NIH regarding the continuation status of the protocol and their decisions will be provided by NIDDK.

Each site's Investigator and their research team (co-Investigators, research nurses, clinical trial coordinators, and data managers) are responsible for identifying AEs. AEs will be reviewed at each study visit by the research team. The research team will then evaluate whether the protocol or informed consent document requires modification based on any safety information and will consult with the Study Co-Chairs regarding such concerns.

Section 8.7: Subject Early Stopping Rules

The safety endpoint will be adverse events related to cimetidine as described above. If the Study Co-Chairs / Study Investigators believe it is not safe for the subject to continue in the study because of UEs, the study drug may be discontinued. Discontinuing the study drug will be at the discretion of the site Investigator after discussion with the Study Co-Chairs. All AEs will be recorded.

If study drug is discontinued for a safety endpoint the subject should continue to have follow-up visits per the study schedule after the date of study drug discontinuation to monitor protoporphyrin levels and safety labs.

Additional follow up safety visits can be scheduled at the discretion of the Investigator.

If a safety concern arrises for a participant, then enrollment of new participants in the study will be paused, and the study reviewed with the Porphyrias Consortium Steering Committee (PCSC). If the PCSC determines it is safe to continue, enrollment will resume. The Consent Form will be revised if needed, and currently enrolled subjects re-consented at the discretion of the IRB.

Section 9: Clinical Monitoring

The study will be monitored locally by the Investigator and/or members of the study staff at least twice monthly according to the accepted data and safety monitoring plan and these findings will be recorded. This clinical trial will also follow the Monitoring Plan developed by the PC. The Investigator will ensure the monitor provided by the PC has the necessary access and time to complete all required activities, and will ensure that the monitor is provided all study-related documents and facilities. The Investigator will provide appropriate space to conduct the monitoring visits.

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Section 10: Statistical Considerations:

Only individuals who begin Treatment Period 1 will be included in the analysis.

Section 10.1 Primary Efficacy Analysis:

Section 10.1.1 Analysis of the Primary Efficacy Endpoint

The primary outcome is the percent change in erythrocyte protoporphyrin at post-treatment relative to pre-treatment. For each period the percent change in erythrocyte protoporphyrin will be calculated as follows:

Let x_{10} = erythrocyte protoporphyrin at randomization
x_{11} = erythrocyte protoporphyrin at the end of period 1 (month 3)
x_{20} = erythrocyte protoporphyrin at the end of the washout period (month 6)
x_{21} = erythrocyte protoporphyrin at the end of period 2 (month 9)

For period 1, the percent change is $100*(x_{11} - x_{10})/x_{10}$ and for period 2, the percent change is $100*(x_{21} - x_{20})/x_{20}$.

The null hypothesis is that the mean percent change on cimetidine is equivalent to the mean percent change on placebo (H_0: $\mu_{Cimetidine} - \mu_{Placebo} = 0$). Assuming no or equal carryover effects, this will be assessed using a two-sided, 0.05 level T-test.

Section 10.1.2 Power and Sample Size

Power calculations are based on data from the PC's study of Iron. In this study, all patients were given iron and erythrocyte protoporphyrin was collected in 3-month intervals for one year. No evidence of an iron effect was observed and therefore these patients likely serve as a reasonable sample to estimate expected variability in the percent change overtime. Ten patients from this study had percent change values available at the 3-month visit versus baseline and the 9-month visit versus the 6-month visit. A repeated measures analysis of variance (ANOVA) of these values yielded an estimate of 33 for the square root of the mean square error. A two-sided T-test achieves 80% power to infer that the mean difference between cimetidine and placebo is not 0 when the total sample size of a 2x2 cross-over design is 20 (with an equal number in each sequence), the actual mean difference is 31, the square root of the within mean square error is 33, and the significance level is 0.05. Power for varying effect sizes is shown in table 1. Power was estimated using PASS 2019 [47].

Table 1. Power Under Varying Differences of Means

N	Alpha	Sqrt(MSE) from Repeated Measures ANOVA	Difference of Means under Null	Difference of Means under Alternative	Power
20	0.05	33	0	30	77.6%
20	0.05	33	0	32	82.6%
20	0.05	33	0	34	86.9%
20	0.05	33	0	36	90.3%
20	0.05	33	0	38	93.1%

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20	0.05	33	0	40	95.2%

Section 10.2 Secondary Analyses:

Section 10.2.1 Time to prodrome

Time to onset of prodromal symptoms associated with sunlight exposure will be collected daily during the last 2 months of each treatment period. Patients' time to prodrome for each period will be measured by the mean patient-reported daily prodrome-free outdoor exposure time . Days on which the patient does not complete the daily survey or does not spend time outside in sunlight will not be included in patients' mean calculations. Within each patient, the difference in the daily average between treatments will be calculated as the daily average on cimetidine minus the daily average on placebo. The null hypothesis is that the mean daily average on cimetidine is equivalent to the mean daily average on placebo (H_0: $\mu_{Cimetidine} - \mu_{Placebo} = 0$). Assuming no or equal carryover effects, this will be assessed using a two-sided, 0.05 level T-test.

Section 10.2.2 Quality of Life

PROMIS assessments will be scored per the established protocol using the PROMIS Assessment Center's publicly available scoring tool. PROMIS scores at baseline, 3, 6, and 9 months will be summarized by randomization group as the number observed, mean, and standard deviation. Individual trajectories of quality-of-life overtime will be plotted. Treatment effects will be explored using mixed effect linear regression models of quality of life with treatment as a time-varying variable and patient as a random effect.

Section 10.2.3 Number and Severity of Sunlight Induced Pain Events

The number of pain events experienced during the last two months of each treatment period and the rates per patient time will be reported by randomization group. Treatment effects will be explored using a generalized linear mixed model of the number of events per period with a log link, treatment as a time-varying variable, and patient as a random effect.

Severity of events will be reported descriptively as the number and rate of events per patient time in each severity class.

Section 10.3.4 Light Dosimeter Data:

For this endpoint, light sensitivity will be measured as the average daily light dose without symptoms. Individual-level exposure of visible light dose across the sampling period will be reported and the distribution will be described. If participants only complete treatment period 1 or part of treatment period 1 and they were on cimetidine during that treatment period, the 2 weeks of screening light dosimetry before treatment period 1 can be used to calculate the participant's "control" light sensitivity to enable them to contribute to the light sensitivity endpoints of the study.

Section 10.3 Exploratory Analyses:

Section 10.3.1 Rate of Prodrome Events Per Prodrome-free Outdoor Exposure Time

Time spent outside exposed to sunlight and time to prodrome will be collected daily the last 2 months of each treatment period. The total number of prodromal symptom episodes divided by the total amount of outdoor exposure without prodromal symptoms will be compared between the treatment and placebo period for each participant. The rate of prodrome events per prodrome-free outdoor exposure time will be explored using a generalized linear mixed model of the number of events per prodrome-free outdoor exposure time with a log link, treatment as a time-varying variable, and patient as a random effect.

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Section 10.3.2 Plasma Total Porphyrin:

Treatment effects on the percent change in plasma total porphyrin will be assessed in the same manner as described for the percent change erythrocyte protoporphyrin in section 10.2.1 above. Changes in plasma total porphyrin values overtime will be plotted by patient and randomization group and assessed descriptively.

Section 10.3.3 Metal-free to Zinc Protoporphyrin

Treatment effects on the percent change in the ratio of metal-free to zinc protoporphyrin will be assessed in the same manner as described for the percent change erythrocyte protoporphyrin in section 10.2.1 above. Changes in ratio values overtime will be plotted by patient and randomization group and assessed descriptively.

Section 10.3.4 Impacts of Seasonality

Additional exploratory analyses of the primary endpoint, time to prodrome, quality of life, and light dose will be conducted to explore the impacts of season, a potential time-varying confounder, which could affect disease burden. Multivariable mixed effects models will be used to model trajectories adjusted for season (winter, spring, summer or fall). Trajectories by month will also be explored with descriptive by-patient plots of erythrocyte protoporphyrin, mean patient-reported daily prodrome-free outdoor exposure time, and PROMIS scores by month to depict potential seasonal impacts.

Section 11: Source Documents and Access to Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a human subject research study necessary for the reconstruction and evaluation of the study. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the human subject research study.

Section 12: Quality Assurance

Quality control procedures will be implemented beginning with the data entry system and data quality control checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written standard operating procedures, the monitors will verify that the human subject research is conducted, and data are generated, documented (recorded), and reported in compliance with the protocol, Good Clinical Practice (GCP), and the applicable regulatory requirements (e.g., Good Laboratory Practice (GLP), Good Manufacturing Practices(GMP)).

The investigational site will provide direct access to all study related sites, source data/documents, and reports for the purpose of monitoring and auditing by the RDCRN DMCC, and inspection by local and regulatory authorities.

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Section 13: Ethics

Section 13.1: Ethical Standards

This study will be conducted in full conformity with Regulations for the Protection of Human Subjects of Research codified in 45 code of federal regulations (CFR) Part 46, 21 CFR Part 50, 21 CFR Part 56, and/or the International Council for Harmonisation (ICH) E6.

It will also be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki, and that are consistent with GCP and all applicable regulatory requirements.

Section 13.2: Institutional Review Board

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study will be made in writing to the Investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The Investigator should provide a list of EC/IRB members and their affiliate to the sponsor upon request.

Section 13.3: Informed Consent

Consent forms describing in detail the study agent, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study product.

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. IRB approved consent form electronic consent media will be used to provide information to a potential subject or legally authorized representative and to document consent.

Extensive discussion of risks and possible benefits of participation will be provided to the participants. The participant will be asked to read and review the consent form or electronic consent media. The Investigator will explain the research study to the participant and answer any questions that may arise. All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form or electronic consent media and ask questions prior to documenting consent. The participants should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The participant will document consent prior to any procedures being done specifically for the study. The participants may withdraw consent at any time throughout the course of the study. A copy of the informed consent form or electronic consent media with documented consent will be given to the participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study. Any child subjects who are enrolled and turn 18 during the study will be re-consented as an adult.

Section 13.4: Data Confidentiality

Participant confidentiality is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their agents. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

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We 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. The registering site will have access to the linkage between this number and the personal identifier of the subject. In addition to the local identifier, a Global Unique Identifier (GUID) may be assigned to each participant using an NIH GUID system. This GUID will allow for linkage of data across protocols for participants who enroll in multiple protocols without using identifiable information to do so.

To further protect the privacy of study participants, a Certificate of Confidentiality will be obtained from the NIH. This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

Section 13.5: Future Use of Collected Samples

Data collected for this study will be analyzed and stored at the RDCRN funded Data Management and Coordinating Center (DMCC). After the study is completed, the coded, archived data will be transmitted to and stored at the Icahn School of Medicine at Mount Sinai and/or Massachusetts General Hospital, under the supervision of the Investigator, for use by other researchers and institutions, including those outside of the study. Permission to transmit data to other researchers within or outside of the study will be included in the informed consent.

With the participant's approval and as approved by local IRBs, coded biological samples will be stored at the PC sites with the same goal as the sharing of data with appropriate NIH repositories. These samples could be used for research into the causes of protoporphyrias, its complications, and other conditions for which individuals with protoporphyrias are at increased risk. The sites will also maintain a code-link that will allow linking the biological specimens with the phenotypic data from each participant, maintaining the masking of the identity of the participant. Identifiable biological samples can be stored and used for other research at a PC site or a non-PC site if participants separately consent to those studies.

Section 14: Data Handling and Record-Keeping

Section 14.1: Data Collection and Management Responsibilities

Data collection is the responsibility of the study staff at the site under the supervision of the site Primary Investigator. The Investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

The DMCC will develop, test, and maintain the data capture system using a web-based data collection system, REDCap, as the primary source of data entry and storage. REDCap is a software toolset and workflow methodology for electronic collection and management of research and clinical trial data developed by Vanderbilt University, with collaboration from a consortium of institutional partners including the University of Cincinnati Academic Health Center. The REDCap system provides a secure, web-based application that is flexible and provides: 1) an intuitive interface for users to enter data and have real time validation rules (with automated data type and range checks) at the time of entry; 2) HIPAA-compliant and 21 CFR Part 11-ready audit trails for

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tracking page views, data manipulation and export procedures; 3) record locking and electronic signature functions; 4) fine grained control of user rights to view and manipulate data, and tool to sequester data access for multiple sites; 5) a report builder for reporting, monitoring and querying patient records; and 6) automated export procedures for seamless data downloads to common statistical packages (SPSS, SAS, Stata, R/S-Plus).

Study data will be entered into REDCap, supported by the DMCC. The REDCap system complies with all applicable guidelines to ensure patient confidentiality, data integrity, and reliability. All data management best practices including quality controls and data validation procedures will be applied to ensure the validity and accuracy of the clinical database. A data management plan will be developed which will describe the data collection process, database development, quality control processes and reporting.

Section 14.2: Study Records Retention

In compliance with Protection of Human Subjects regulations, (45CFR Part 46), records related to the conduct of this trial, including but not limited to source documentation, case report forms, informed consent forms, essential study documentation, and documentation of IRB activities, will be retained by the Investigator for a period of 7 years following the official close of the study, as per the requirement of Massachusetts General Hospital. Such records may be preserved in hardcopy, electronic or other media form and must be accessible for inspection and copying by authorized representatives of Health and Human Services (HHS), NIH, the study sponsor and/or their representatives at reasonable times and in a reasonable manner. These documents should be retained for a longer period, however, if required by local regulations. No records will be destroyed without the written consent of the sponsor, if applicable. It is the responsibility of the sponsor to inform the Investigator when these documents no longer need to be retained.

Section 14.3: Protocol Deviations

A protocol deviation is any noncompliance with the human subject research protocol, GCP, or manual of procedures (MOP) requirements. The noncompliance may be either on the part of the participant, the Investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly. It is the responsibility of the site to use continuous vigilance to identify and report major deviations within 7 working days of identification of the protocol deviation, or within 7 working days of the scheduled protocol-required activity. All deviations must be addressed in study source documents, reported in the study database. Protocol deviations must be sent to the local IRB per their guidelines. The site Primary Investigator/study staff is responsible for knowing and adhering to their IRB requirements. Further details about the handling of protocol deviations will be included in the MOP.

Section 14.4: Publication Policy

This study will comply with the NIH Public Access Policy, which ensures that the public has access to the published results of NIH funded research. It requires scientists to submit final peer-reviewed journal manuscripts that arise from NIH funds to the digital archive PubMed Central upon acceptance for publication.

In compliance with national regulations, this clinical study will be listed on clinicaltrials.gov.

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Section 15: Study Administration and Conflict of Interest

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the trial. The study leadership in conjunction with the PC has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

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Section 17: Appendices

Appendix 1: Prohibited and Precautionary Medications[41]

Prohibited Concomitant Medications:

If a subject is taking cimetidine at screening, the subject can stop using cimetidine for at least 3 months and then rescreen.

Subjects must not take any medication with known interaction with cimetidine.

- Cefuroxime
- Chloroquine
- Dasatinib
- Delavirdine
- Dofetilide
- EpiRUBicin
- PAZOPanib
- Pimozide
- Risedronate

Subjects must not take any systemic or topical treatment with known potential to affect photosensitivity in EPP or XLP unless they are able to maintain treatment at the same dose throughout both treatment periods. Exceptions will be made in the setting of an inability to access one of these medications. Alternatively, a subject can stop using these therapies for at least 4 weeks and then rescreen. Sunscreen without zinc oxide is allowed.

- Afemelanotide
- Beta-carotene
- Cysteine
- MT-7117
- Pyridoxine
- Cholestyramine
- Zinc oxide

Precautionary Concomitant Medications:

Precautionary medications may be allowed based on medical discretion. Allowance of any precautionary concomitant medication should be based on confirmation that the medication does not represent a safety issue and will not impact study conduct adversely.

Consider therapy modification:

- Acalabrutinib
- Amiodarone
- Atazanavir
- Bosutinib
- Calcium Channel Blockers
- Carmustine
- Cefditoren
- Cisapride
- Citalopram
- CloZAPine
- Dacomitinib
- Erlotinib
- Fosphenytoin-Phenytoin
- Gefitinib
- Itraconazole
- Ketoconazole (Systemic)
- Ledipasvir
- Lemborexant
- Lomitapide
- Mesalamine
- MetFORMIN
- Moclobemide
- Neratinib
- Nilotinib
- Pexidartinib
- Posaconazole
- Procainamide
- QuiNIDine
- QuiNINE
- Rilpivirine
- Secretin
- Selpercatinib
- Tafenoquine
- Thioridazine
- TiZANidine
- Triazolam
- Ubrogepant
- Vitamin K Antagonists
- Zaleplon

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- ZOLMitriptan

Monitor Therapy:

- Alfentanil
- AtorvaSTATin
- Azelastine (Systemic)
- Bromazepam
- CarBAMazepine
- Carvedilol
- Cefpodoxime
- Chlormethiazole
- CloBAZam
- CloZAPine
- CycloSPORINE (Systemic)
- Cysteamine (Systemic)
- Dalfampridine
- Dexmethylphenidate
- Dofetilide
- Doxofylline
- Enoxacin
- Enoxacin
- Erdafitinib
- Escitalopram
- Flibanserin
- Flunitrazepam
- Fluorouracil Products
- FLUoxetine
- Fosamprenavir
- Indinavir
- Iron Preparations
- Mebendazole
- Melatonin
- Meperidine
- Methylphenidate
- Mirtazapine
- Multivitamins/Minerals (with ADEK, Folate, Iron)
- Nelfinavir
- Nicotine
- NiMODipine
- Nitisinone
- Octreotide
- PARoxetine
- Pentoxifylline
- Pilsicainide
- Pramipexole
- Praziquantel
- Pretomanid
- Propafenone
- Roflumilast
- Saquinavir
- Sulfonylureas
- Tacrolimus (Systemic)
- Tamsulosin
- Teriflunomide
- Theophylline Derivatives
- Tricyclic Antidepressants
- Urapidil
- Varenicline
- Velpatasvir

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