The Thesis Committee for Dung Mac Certifies that this is the approved version of the following dissertation:

Erythropoietin for the Treatment of Porphyria Cutanea Tarda in End Stage Renal Disease

Committee:

Karl Anderson, MD Supervisor

Susan C Weller, PhD

Michael Wilkerson, MD

Dean, Graduate School

Erythropoietin for the Treatment of Porphyria Cutanea Tarda in End Stage Renal Disease

by

Dung Mac, MD, MS, BS

Thesis

Presented to the Faculty of the Graduate School of

The University of Texas Medical Branch

in Partial Fulfillment

of the Requirements

for the Degree of

Master of Science

The University of Texas Medical Branch

May, 2019

Erythropoietin for the Treatment of Porphyria Cutanea Tarda in End Stage Renal Disease

Publication No._____

Dung Mac, MS

The University of Texas Medical Branch, 2019

Supervisor: Karl Anderson

Abstract: Porphyria Cutanea Tarda (PCT) is an iron-related disease that results from acquired inhibition of hepatic uroporphyrinogen decarboxylase (UROD), the fifth enzyme in the heme biosynthetic pathway. Clinically, PCT presents with chronic photosensitive vesiculo-bullous skin lesions that are painful and prone to infection and scarring. Renal disease is often associated with iron overload, and is a risk factor for the development of PCT. It is difficult to treat PCT associated with renal disease due to the presence of anemia, which prevents treatment of these patients by phlebotomy. Anemia in these patients results largely from decreased production of erythropoietin by the diseased kidneys. Erythropoietin administration can mobilize excess iron, correct anemia and support phlebotomy in these patients. This study will analyze data from an unblinded trial of 6 patients who were treated with recombinant human erythropoietin for their renal disease-associated PCT. The aims are to determine the safety and efficacy of erythropoietin treatment of PCT in patients with end stage renal disease as assessed by improvements in plasma porphyrins, measures of iron status, clinical improvement and side effects, and to contribute to developing clear guidelines for erythropoietin treatment of these patients.

TABLE OF CONTENTS

List of Tables

List of Figures

List of Abbreviations

UTMB	University of Texas Medical Branch
GSBS	Graduate School of Biomedical Science
TDC	Thesis and Dissertation Coordinator
CKD	Chronic kidney disease
Epo	Erythropoietin
ESRD	End stage renal disease
Fe	Serum Iron
HD	Hemodialysis
PCT	Porphyria Cutanea Tarda
PD	Peritoneal dialysis
Phleb	phlebotomy
PP	Plasma porphyrin
UROD	uroporphyrinogen decarboxylase

Chapter 1 Introduction

Porphyria Cutanea Tarda (PCT) is an iron-related disease that results from decreased activity of hepatic uroporphyrinogen decarboxylase (UROD), the fifth enzyme in the heme biosynthetic pathway. The decrease in hepatic UROD activity is primarily due to acquired enzyme inhibition, although genetic factors can play a role. Clinically, PCT presents with chronic photosensitive vesiculo-bullous skin lesions that are painful and prone to infection and scarring. End stage renal disease (ESRD) is often associated with iron overload, and is therefore a risk factor for the development of PCT. It is difficult to treat PCT when associated with ESRD because anemia is usually present and prevents treatment by phlebotomy. Anemia in these patients results largely from decreased production of erythropoietin by the diseased kidneys. Administration of human recombinant erythropoietin, which were introduced starting in 1983[1] can stimulate erythropoiesis, mobilize excess iron, correct anemia and support phlebotomy in these patients. The first generation EPO (trade names Epogen, Eprex, Procrit) were short acting drugs requiring frequent dosing, newer second generation (trade names Aranesp, NeoRecormon) and third generation (trade names Dynepo, Mircera, etc) agents were subsequently developed with longer half-life requiring lower administration doses/frequency of dosing.[2] We analyzed data from an unblinded trial of 6 patients who have been treated with recombinant erythropoietin for their renal disease-associated PCT. The aims were to determine the safety and efficacy of erythropoietin treatment for decreasing plasma porphyrins and measures of iron status, and contribute to providing clear guidelines for treatment of these patients with erythropoietin.

Chapter 2 Pathogenesis and Therapy for Porphyria Cutanea Tarda

PCT is the most common type of porphyria (prevalence 1 in 10,000), a group of metabolic disorders caused by altered activities of enzymes in the heme biosynthetic pathway that lead to accumulation of pathway intermediates. PCT is an iron-related disease that results from acquired inhibition of hepatic uroporphyrinogen decarboxylase (UROD), the fifth enzyme in the pathway, in the liver. Uroporphomethene has been isolated as the UROD inhibitor in mice with PCT.[3] Clinically, PCT presents with chronic photosensitive vesiculo-bullous skin lesions, increased skin fragility, scarring, dyspigmentation of affected skin, facial hypertrichosis, and milia.[4] Increased mechanical fragility of the skin leads to blistering or superficial erosions from trivial trauma. Skin lesions are painful and may become infected. Scarring can sometimes be severe and disfiguring.

Factors known to trigger or exacerbate PCT include alcohol abuse, chronic hepatitis C, HIV infection, estrogen use and smoking. How these acquired factors contribute to generation of the hepatic UROD inhibitor, a uroporphomethene, is not well understood. But at least some of these, such as alcohol and hepatitis C, may do so by reducing production of hepcidin by the liver, and thereby increasing iron absorption. Other susceptibility factors include contributors to iron overload, such as HFE

(hemochromatosis) mutations, multiple blood transfusions (as in bone marrow disorders such as myelofibrosis) and end stage renal disease (ESRD). Heterozygous UROD mutations are susceptibility factors found in ~20% of patients. Individual patients with PCT have two or more of these susceptibility factors in various combinations. Rare outbreaks of PCT have occurred after exposure to polyhalogenated aromatic hydrocarbons that were then shown to cause the biochemical features of PCT in rodents.[4,5] Patients with PCT have abnormal liver function tests, possibly resulting from marked porphyrin accumulation in hepatocytes, and also related to liver-damaging susceptibility factors. Therefore, they are at risk of developing cirrhosis and hepatocellular carcinoma.[4]

UROD catalyzes the 4-step decarboxylation of uroporphyrinogen to coproporphyrinogen. PCT manifests clinically when hepatic UROD activity is reduced to less than approximately 20% of normal. Reduced hepatic UROD activity leads to hepatic accumulation of highly carboxylated porphyrinogens; these are oxidized to the corresponding porphyrins (mostly uroporphyrin and heptacarboxyl porphyrin), which appear in plasma, are transported to the skin and are excreted mainly in urine.[5] Coproporphyrin is excreted in both urine and bile. Protoporphyrin, which has only 2 carboxyl groups, is insoluble in water and is excreted in bile and stool. Cutaneous phototoxicity results from photoactivation of porphyrins as they are deposited or circulate through the skin.[4] The diagnosis of PCT is considered in patients with blistering skin lesions on sun-exposed areas such as the dorsal hands, face, etc. It is confirmed by finding elevated plasma and urine porphyrins (predominantly uroporphyrin and heptacarboxyl porphyrins and less elevation of hexa- and pentacarboxyl porphyrins and coproporphyrin), and elevated fecal isocoproporphyrins.[4]

Several forms of PCT, termed Types I-III have been identified, but are not fundamentally different from each other.[4] In the sporadic form (Type I), which includes the majority (~80%) of adult cases of PCT, there are no UROD mutations, and the enzyme is reduced to less than 20% of normal in the liver but is normal in erythrocytes and other tissues. Heterozygous UROD mutations are present in the familial form (Type II), and UROD is again less than 20% of normal in the liver and ~50% of normal in erythrocytes and other tissues. This is an autosomal dominant disorder with low penetrance, and often no family history of photosensitivity. Type II becomes manifest only when other susceptibility factors are present to reduce hepatic UROD activity to less than ~20% of normal. In Type III, which is rare, there are no UROD mutations but more than one family member is affected, presumably due to other inherited or acquired susceptibility factors. Types I-III are clinically similar and difficult to distinguish. Response to treatment for each type is essentially the same.

Standard treatment for PCT is repeated phlebotomy to remove excess iron, until serum ferritin is <25ng/ml. Iron does not inhibit or inactivate hepatic UROD, but contributes to an oxidative state that leads to generation of the uroporphomethene UROD inhibitor.[3] After repeated phlebotomy hepatic iron is reduced and formation of the inhibitor ceases, and hepatic enzyme activity gradually increases as new enzyme protein is synthesized. Low dose hydroxychloroquine or chloroquine, which mobilizes excess porphyrins from the liver, is also effective. Hydroxychloroquine has a somewhat better safety profile than chloroquine, at least when used for other more common diseases. Iron

chelating agents, such as deferoxamine and deferasirox, are less efficient in removing iron than phlebotomy but may be used in patients with PCT and substantial iron overload who have poor venous access or cannot tolerate phlebotomies or hydroxychloroquine.[4]

Chapter 3 Anemia of Renal Disease

Anemia of chronic kidney disease (CKD) usually develops as chronic renal disease progresses and becomes more severe as creatinine clearance progressively decreases. The anemia is characterized by inadequate erythrocyte production in the setting of low serum iron and low iron-binding capacity despite preserved or even increased macrophage iron stores in the marrow. The erythrocytes are usually normocytic and normochromic. The primary cause is a deficiency of erythropoietin, whose renal production is reduced due to progressive destruction and fibrosis.

Anemia of CKD became a common problem in the 1960s when hemodialysis became widely available and allowed prolonged survival of patients with end-stage renal failure. Anemia of CKD was usually severe enough to limit activities of daily living and was treated by blood transfusions until the late 1980s when human recombinant erythropoietin (EPO) became widely available. Maintenance treatment with human recombinant erythropoietin generally alleviates the anemia in patients with CKD.

In the kidney, interstitial fibroblasts of neural crest origin are probably the main source of EPO, but the identity of EPO producing cells in the kidney remains controversial, mostly because the basal production of EPO is very low and ultrasensitive methods are required to detect the source of the hormone. In advanced CKD, the kidneys undergo end stage fibrosis, during which these fibroblasts may transdifferentiate into myofibroblasts and lose their ability to produce appropriate amounts of EPO in response to hypoxia.

Inflammation is also a strong contributor to the pathogenesis of anemia of CKD. An inflammatory state in CKD leads to hepatic overproduction of the hormone hepcidin, which is the major regulator of iron status.[6] Inappropriately high levels of hepcidin also result from its reduced renal excretion in CKD. Hepcidin down-regulates ferroportin, the iron transporter that exports cellular iron and is found in cell membranes especially on the non-luminal surface of small intestinal enterocytes and on the surface of macrophages. Thus, increased levels of hepcidin inhibit both intestinal iron absorption and mobilization of iron that is stored in macrophages, thereby limiting iron available to the bone marrow for hemoglobin synthesis. This contributes further to anemia in patients with advanced renal disease. Inflammation also results in elevations in C-reactive protein (CRP), an acute-phase protein generated by the liver. Hemodialysis patients with CRP levels greater than 20 mg/L have higher levels of hepcidin and require on average 80 percent higher doses of EPO to maintain hemoglobin level at 12 g/dL compared to those with CRP levels less than 20 mg/L. EPO resistance in such cases is thought to be mediated via relative iron deficiency as patients with CRP levels greater than 20 mg/L also exhibit significantly lower levels of serum iron compared to those with CRP less than 20 mg/L. [7]

Patients with PCT and CKD often have much greater retention of excess porphyrins, much higher porphyrin levels and more severe photosensitivity than PCT patients who have normal kidneys.[8] This is probably because the urinary route for excretion has been lost and because porphyrins are poorly dialyzable. Plasma porphyrin levels are somewhat elevated in many chronic renal failure patients before and during treatment with hemodialysis, so the normal range is said to be higher in CKD than in normal individuals.[9,10] A condition termed pseudoporphyria, characterized by PCT-like skin lesions without significant elevation of porphyrins, occurs with or without CKD, and can sometimes be attributed to an antibiotic or nonsteroidal anti-inflammatory drug. It can be difficult to differentiate PCT and pseudoporphyria in CKD patients with modestly elevated plasma porphyrin levels.

Hyperaluminemia was also reported as a precipitating factor for PCT in renal disease patients. For example, one patient was reported to develop PCT after being placed on home dialysis with softened water.[5] He had also developed osteomalacia (implying hyperaluminemia). He was subsequently placed on deionized water dialysis, which resolved his bone and porphyria disease. Aluminum is reported to be an inhibitor of heme synthesis and a suppressor of erythroid production,[11] but has not been studied in terms of inhibiting hepatic UROD. Currently, aluminum toxicity is rare in dialysis due to the use of aluminum-free dialysis water. Therefore, it is no longer considered an important contributor to developing PCT in the setting of CKD.

As already notes, anemia is a common problem in patients undergoing chronic dialysis for end stage renal disease.[12] Many such patients required periodic transfusions in the past. Mechanisms responsible for anemia in end stage renal disease patients include inadequate production of erythropoietin by the diseased kidneys, iron deficiency or reduced iron availability due to elevated levels of hepcidin, blood loss, and hemolysis due to dialysis. Iron deficiency is prevented in dialysis patients by iron administration, so iron overload is now more common than iron deficiency in these patients.

It is recognized that erythropoietin deficiency is the primary cause of anemia in end stage renal disease.[11] Erythropoietin is a glycoprotein hormone containing 165 amino acids with a molecular weight of 31,000 Daltons, originates primarily from the kidney, and is the primary regulator of red blood cell formation (erythropoiesis). The main action of erythropoietin is to stimulate the differentiation of erythroid progenitor cells in the bone marrow into erythroblasts that are functional in terms of synthesizing hemoglobin. Subsequent maturation of erythroblasts into circulating erythrocytes normally occurs in 5-9 days. Synthesis of heme for incorporation into hemoglobin also depends on the availability of iron and the eight enzymes of the heme biosynthetic pathway. As already noted, elevated levels of the recently discovered hormone hepcidin, which is produced by the liver and excreted in the urine, can also contribute to anemia in patients with renal disease by reducing iron availability to the bone marrow.

PCT in the setting of renal disease has been difficult to treat because phlebotomies were almost always contraindicated by the presence of anemia. Hydroxychloroquine and chloroquine are not effective because the excess porphyrins removed from the liver by these drugs are not well excreted by diseased kidneys and are not well dialyzed.[13] The iron chelator deferoxamine may be effective in some cases, but

is an inefficient means of iron removal and might limit endogenous iron availability for erythropoiesis.[8]

Chapter 4 Erythropoietin

Human erythropoietin produced by recombinant technology is approved for the treatment of anemia of end stage renal disease. It was first shown to be effective in supporting phlebotomies and achieving remission of PCT in one case with ESRD before the drug was approved for treatment of anemia in CKD, and this has been verified by subsequent experience.[25] The rational for using erythropoietin in patients with PCT and chronic renal diseases is that it stimulates erythropoiesis and enables phlebotomies to be performed, so that excess iron stores and hepatic iron content can be reduced. This interrupts the production of the uroporphomethene inhibitor of UROD in the liver, enabling hepatic UROD to be replenished by new synthesis. It should be noted again that UROD is inhibited only in the liver, so UROD activity and heme synthesis in the marrow are normal.

Prior to the introduction of erythropoietin, it is estimated that between 1.2-30.2% of patients with ESRD (and without previous history of PCT) develop a dermatosis attributable to PCT,[14] often within 18 months of starting dialysis.[5] After erythropoietin became a treatment for anemia, the prevalence of PCT in ESRD patients decreased dramatically, although, no large-scale observational studies have been done to confirm this. A single center study on 70 hemodialysis patients examined found no occurrence of PCT, in terms of cutaneous disease, despite these patients having up to 4-fold higher plasma porphyrin levels compared to normal controls.[10] However, it remains uncertain whether or not these elevated levels might represent subclinical PCT, i.e. porphyrin elevations in the absence of skin lesions.

Chapter 5 Review of the Literature

A review of the literature for all reported cases of erythropoietin therapy in patients with PCT and renal disease was performed using Medline Ovid, Pubmed, Web of Science, and the gray literature with Mednar. Figure 1 showed the search terms, strategy and results. Our search revealed 12 publications (Table 1) [8,15-25], all of which are individual case reports. The majority of interventions are erythropoietin with phlebotomies (7 out of 12). The range of erythropoietin therapy is wide, from 20 U/kg to >200 U/kg 3 times a week. Total phlebotomy volumes also varies from 250 ml to 900 ml and as high as 1475 ml. Of the cases involving phlebotomies, the majority were small volume phlebotomies of 50 ml (5 out of 7 cases).

Figure 1: Literature Review Search Strategy

Search Terms and Strategy:

- MESH terms for **erythropoietin** (erythropoietin, epoetin, erythropoie*, epogen, eprex, glycoform, epoetin alfa, hematinics) AND
- **porphyria cutanea tarda** (porphyria, porphyria cutanea tarda, porphyrin) AND
- **renal insufficiency** (renal failure, kidney failure, kidney insufficiency, kidney insufficiencies)
- OR **porphyria** AND (**renal insufficiency** OR **renal dialysis**)
- Limited to **English** language and from **1989-2016**.

Search Results:

- Ovid (83)
- Pubmed (17)
- Web of Science (27)
- Mednar (0)
- Combined with duplicates removed (95)
  - Exclusion
    - Not PCT (43)
    - No treatment with Erythropoietin (30)
    - Animal (1)
    - Letter to Editor (3)
    - Review Article (2)
    - No details on Erythropoietin dosage and/or post treatment outcomes (4)
- **Total Publications Included: 12**

Table 1 lists treatment outcomes and adverse events reported. Anemia was reported as an adverse event in 2 cases, and pseudo-seizure in 1 case. PCT improvement was reported in 10 cases, no improvement in 1 case, and not reported in 1 case. Duration of therapy before remission range from 2 weeks to 11 months, with the most reported around 4 months. Remission was reported at follow up visits at 7, 9 and 18 months. The dose of erythropoietin ranged from 20 to 150 IU/kg.

Table 1: List of Publications, Interventions, Adverse Events, and Outcomes

	Author/ year	Design	Intervention	Adverse events	Improvement of PCT	Duration of therapy before remission	Remission duration
1	Anderson 1990	Case Report	Epo 150 U/kg 3x/week, small vol phleb (7x120-180ml, total 900ml)	None	Yes	4 months	NR
2	Yaqoob 1992	Case Report	Epo 50 U/kg 3x/week, iron 100mg/week	Anemia (Hgb 5.6)	Yes	NR	18 months
3	Piazza 1992	Case Report	Epo 40 U/kg	None	Yes	2 weeks	NR
4	Stevens 1993	Case Report	Epo 50 U/kg 3x/week, phlebx7= 1475 ml total, Renal Transplant	Pseudoseizure post initial phlebotomy	No (Renal transplantation improved)	11 months	NA
5	Sarkell 1993	Case Report	Epo 50 U/kg 3x/week	None	Yes	NR	NR
6	Peces 1994	Case Report	Epo 20 U/kg	None	Yes	1 month	9 months
7	Poux 1997	Case Report	Epo 200 U/kg 3x/week, phleb 50 ml/2 wks= 900 ml total	NR	Yes	4 months	NR
8	Lee 1999	Case Report	10,000 U 3x/week, small repeated phlebotomies of 50 ml	None	NR	NR	NR
9	Shieh 2000	Case Report	200 U/kg 3x/week, phleb 50ml/2wksx9 months= 900 ml, increased to 400 U/kg and 100ml phleb weekly	Anemia (2 units of blood transfusion)	Yes	4 months	7 months
10	Kelly 2001	Case Report	Was on Epo, given 200 mg hydroxychloroquine 2x/week, d/c and started phleb monthly	None	Yes	4.6 months	NR
11	Albalate 2001	Case Report	Epo, IFN-alpha therapy, stopped iron, dialysis membrane replaced by a high flux polysulfone membrane	NR	Yes	NR	NR
12	Ryali 2010	Case Report	Epo maximized and small vol phleb (total 250 ml)	None	Yes	4 months	NR

Table 2 lists the social and demographic factors of the 12 cases. Their ages range from 21 to 65 years, with the majority in their mid 40s and 50s. 66% of cases were female. There were limited data on race/ethnicity, with 2 reported as Black, 2 White, 1 Hispanic and 7 Not Reported. Causes of renal disease were focal segmental glomerular sclerosis, malignant hypertension, Bright's disease, chronic pyelonephritis, systemic lupus erythematosus (SLE), renal tuberculosis, membranoproliferative glomerulonephritis, and unknown or not reported. The majority of cases were on hemodialysis (11 out of 12), with 1 case on peritoneal dialysis. The length of time on dialysis before onset of PCT ranged from 3 months to 15 years, with the majority of cases developing PCT within 2-5 years of dialysis use. None reported tobacco use. 1 out of 12 reported alcohol use. 1 out of 12 reported estrogen use. 6 out of 12 were reported as positive for HCV infection, and 1 was positive for HBV infection. None reported a family history of PCT.

Table 2: Social Demographic Factors of Cases

	Author/ year	Age	Gender	Race/ Ethnicity	Cause of renal dx	Length of time on dialysis before onset of PCT	Tobacco	Alcohol	Estrogen	HCV	HBV	Family hx PCT
1	Anderson 1990	57	F	NR	Unknown	4 years (HD)	NR	No	No	NR	NR	No
2	Yaqoob 1992	58	F	NR	Chronic pyelonephritis	5 years (HD)	NR	No	No	NR	NR	No
3	Piazza 1992	65	M	NR	MPGN	5 years (HD)	NR	Yes	N/A	Yes	Yes	No
4	Stevens 1993	21	F	White	Systemic lupus erythematosus	2 years (PD)	NR	NR	NR	NR	NR	NR
5	Sarkell 1993	40	M	Black	NR	NR (HD)	NR	No	N/A	NR	NR	NR
6	Peces 1994	49	F	NR	Renal tuberculosis	8 years (HD)	NR	No	NR	Yes	NR	No
7	Poux 1997	37	F	NR	Spinio bifida	3 Months (HD)	NR	No	No	Yes	No	No
8	Lee 1999	35	F	NR	SLE	14 years (HD)	NR	NR	NR	Yes	NR	NR
9	Shieh 2000	38	F	Hispanic (Mexican)	Chronic pyelonephritis	15 years (HD; 2 of those years was PD)	NR	NR	NR	yes	NR	No
10	Kelly 2001	60	F	White	Bright's disease	NR (PD)	NR	No	Yes	No	No	NR
11	Albalate 2001	48	M	NR	Malignant hypertension	3 years (HD)	NR	No (Hx of Alcohol Abuse)	N/A	Yes	NR	No
12	Ryali 2010	39	M	Black	FSGS	4 years (HD)	NR	NR	N/A	NR	NR	NR

The target for phlebotomy treatment is to reduce the serum ferritin to near the lower limit of normal (15-20 ng/mL). From Table 3, in all successful cases (defined by remission of skin lesions) of erythropoietin therapy with reported ferritin levels, there is a dramatic reduction of ferritin; notice that plasma porphyrin in these cases achieved significant reduction, however they did not reach below normal limits or it was unclear if they did normalize due to non reporting. The majority of these cases achieved post treatment ferritin levels of approximately 10% of pre treatment levels (6 out of 9) corresponding to ferritin levels in the low normal range (~40-86 ng/mL). A few cases achieved PCT remission with higher post treatment ferritin levels (144-658 ng/mL, 3 out of 9, porphyrin levels were not reported in these cases, making it difficult to determine if they were normalized or not).

Table 3: Pre and Post Labs of Cases

				Ferritin (NL, 20-500 ng/mL)		Plasma Porphyrin (NL, <2 ug/dL or <21 nmol/L)		Hemoglobin (NL, 12-16 g/dL)	
	Author/Year	Intervention	Success of Epo therapy	Pre	Post	Pre	Post	Pre	Post
1	Anderson 1990	Epo 150 U/kg 3x/week, small vol phleb (7x120-180ml, total 900ml)	Yes	324-501	~40	211 ug/dL	<20	8.6	NR
2	Yaqoob 1992	Epo 50 U/kg 3x/week, iron 100mg/week	Yes	870	86	2800 nmol/L	<100	8	10.5
3	Piazza 1992	Epo 40 U/kg	Yes	388	42	NR (Urine uroporphyrin 143 ug/ 24hrs)	NR (Urine uroporphyrin 69 ug/ 24hrs)	6.6	11
4	Stevens 1993	Epo 50 U/kg 3x/week, phlebx7= 1475 ml total, Renal Transplant	No	NR	NR	1850 nmol/L	NR	7.3	NR
5	Sarkell 1993	Epo 50 U/kg 3x/week	Yes	505	144	NR (Urine uroporphyrin 2475 ug/ 24hrs)	NR	NR	NR
6	Peces 1994	Epo 20 U/kg	Yes	831	63	86.9 ug/dL	22.9	NR	10
7	Poux 1997	Epo 200 U/kg 3x/week, phleb 50 ml/2 wks= 900 ml total	Yes	2500	<25	2490 nmol/L	<50	7	11
8	Lee 1999	10,000 U 3x/week, small repeated phlebotomies of 50 ml	NR	701	NR	NR (Serum uroporphyrin 1766 nmol/L)	NR	11	NR
9	Shieh 2000	200 U/kg 3x/week, phleb 50ml/2wksx9 months= 900 ml, increased to 400 U/kg and 100ml phleb weekly	Yes	441	39	260	23.9	7.9	5.8
10	Kelly 2001	Was on Epo, given 200 mg hydroxychloroquine 2x/week, d/c and started phleb monthly	Yes	1213	658	NR (Serum uroporphyrin 53.4 mg/dL)	NR	11.2	11-11.5
11	Albalate 2001	Epo, IFN-alpha therapy, stopped iron, dialysis membrane replaced by a high flux polysulfone membrane	Yes	NR	NR	56.9 ug/dL	NR	NR	NR
12	Ryali 2010	Epo maximized and small vol phleb (total 250 ml)	Yes	2640	409	53.5 ug/dL	NR	10.8	12.5

The only reported case of treatment failure was that by Stevens and coworkers in 1993, in which the patient received a small dose of erythropoietin at 50 U/kg 3 times a week, and a large total volume of phlebotomies (1475 ml).[22] This patient subsequently underwent renal transplantation, which resolved her PCT symptoms. The low dose of erythropoietin used in this case was apparently sufficient to support the large total

volume of phlebotomies. But it was not documented whether or not serum ferritin was lowered significantly, so it is not known if adequate iron reduction was achieved. Other reasons for lack of response to treatment with EPO and phlebotomy, such as elevations in hepcidin levels, which might be more prominent in some patients than others, have not been studied. Regardless, this case illustrates that renal transplantation, which restores both erythropoietin production and hepcidin excretion to normal, can lead to resolution of PCT. There have also been several reported cases in which small volume phlebotomies alone are successful in ESRD patients who were not severely anemic and did not require transfusions or erythropoietin.[26-28]

Other than these isolated case reports, there have been no larger studies to evaluate the safety and efficacy of erythropoietin for the treatment PCT patients with ESRD. Although the available case reports suggest that erythropoietin is beneficial in this condition, the package insert states that the drug should be used with caution in patients with porphyria, implying that there may be an adverse effect in porphyria. Apparently, this cautionary statement is included because an attack of acute intermittent porphyria occurred in one patient who also had renal disease and was part of a pre-marketing study in Europe. But it is unclear that the study drug precipitated the attack in this patient. In any case, acute intermittent porphyria and PCT differ substantially in pathogenesis and clinical features. Therefore, further studies of erythropoietin in PCT associated with end stage renal disease were justified and needed for developing guidelines on the use of this drug in a safe and efficacious manner for the management of this condition when associated with ESRD.

Chapter 6 Purpose of Study and Specific Aims

The purpose of our study was to determine the safety and efficacy of erythropoietin in conjunction with phlebotomy for the treatment of PCT associated with ESRD. We hypothesized that PCT associated with ESRD has a similar pathogenesis to PCT occurring in the setting of normal renal function, with excess hepatic iron playing a major role in the generation of the uroporphomethene inhibitor of hepatic UROD. Because treatment of PCT with phlebotomy in the setting of renal disease is usually contraindicated by the presence of anemia related to erythropoietin deficiency, we hypothesized that the administration of human recombinant erythropoietin in this setting should mobilize excess hepatic iron, eliminate transfusion requirements, support phlebotomy, and lead to remission of PCT as manifest by a decreases in plasma porphyrins and cessation of new skin lesions. Based on these hypotheses the specific aims were as follows:

1. To evaluate the efficacy and safety of recombinant human erythropoietin compared to a pretreatment control period in the treatment of PCT in patients with ESRD and to determine whether erythropoietin corrects iron overload and anemia and eliminates transfusion requirements in PCT associated with ESRD, and to characterize time courses of these effects. Measures of efficacy are effects on levels of serum ferritin and plasma porphyrins and on cutaneous lesions. Additional measures of efficacy include levels of hemoglobin, hematocrit, ferritin, serum iron, transferrin (iron-binding capacity) and transferrin saturation.

2. To develop clear clinical guidelines for erythropoietin treatment of patients with PCT and ESRD. These guidelines include the (a) frequency of monitoring of plasma porphyrin and ferritin levels during treatment, (b) indications for erythropoietin dose adjustments, (c) indications for therapeutic phlebotomy and (d) indications for iron supplementation to prevent or treat an absolute or relative iron deficiency that may develop with erythropoietin treatment.

Chapter 7 Study Design and Methods

This was an unblinded proof of concept study of 6 patients in which each patient served as his or her own control. Patients were followed at a dialysis center capable of providing the samples and assessment needed for completion of the study objectives. Study visits were at the UTMB Clinical Research Center (CRC) unless patients resided and are treated at a distance that precluded such visits. Provisions were made for collecting data and obtaining samples from patients unable to come to the CRC. Patients underwent an initial medical history, physical examination, and clinical laboratory evaluations which include hematocrit, hemoglobin, reticulocytes, leukocyte, differential and platelet counts, ferritin, iron, iron binding capacity, percent saturation, prothrombin time, partial thromboplastin time, fibrinogen, sodium, potassium, chloride, blood urea nitrogen, creatinine, total protein, total bilirubin, SGOT (AST), SGPT (ALT), glucose, uric acid, calcium, phosphorous. PCT was documented by biochemical measurements that also excluded other vesico-bulous porphyrias and provided baseline porphyrin levels before starting treatment.

Patients in this study were 18 years of age or older and were receiving chronic hemodialysis or peritoneal dialysis at or under the supervision of an approved dialysis center that agreed to assist in sample and data collection, had well documented PCT (as defined above), and in the absence of treatment with erythropoietin, had anemia (or a transfusion requirement) sufficient to contraindicate treatment by repeated phlebotomy. Anemia sufficient to contraindicate phlebotomies was defined either as hemoglobin below 9.5 mg/dl and not due to an identifiable or treatable cause other than end stage renal disease, or a higher hemoglobin level maintained by blood transfusions.

Exclusion criteria include known hypersensitivity to erythropoietin, human albumin or mammalian cell-derived products, uncontrolled hypertension, uncontrolled seizure disorder, significant hematologic disease other than anemia due to end stage renal disease, or any other condition that might increase the risk to the patient or decrease the chances of obtaining satisfactory data to achieve the objectives of the study.

Patients received a standard dose of erythropoietin three times weekly for at least a 36-week period. It was anticipated that prior to treatment with erythropoietin, patients will have been anemic or will have required transfusions to maintain acceptable hemoglobin levels. Therefore, repeated phlebotomy alone was not a suitable treatment. The dose of erythropoietin could be adjusted upward or downward as needed according to the usual clinical indications for the use of this drug. If therapeutic phlebotomy was indicated to reduce excess iron, the dose of erythropoietin could be maintained at a level

that would support phlebotomy, or could be increased to such a level. Erythropoietin was administered under the supervision of the nephrologist who was primarily responsible for the patient's care in an approved dialysis program.

Plasma porphyrins and serum ferritin, iron and iron binding capacity were measured weekly whenever possible. The erythropoietic response to erythropoietin was assessed by weekly hemoglobin, hematocrit and reticulocyte count determinations. Plasma was shipped to UTMB on dry ice for plasma porphyrin measurements and for iron studies. Ferritin, iron and iron binding capacity can decrease rapidly when erythropoietin treatment is initiated or when phlebotomy is instituted. While such decreases are potentially beneficial for the treatment of PCT, it is also important to maintain sufficient endogenous iron stores to allow an erythropoietic response to erythropoietin. Frequent monitoring of indices of iron status and plasma porphyrin levels permitted adjustments in erythropoietin dosage and therapeutic phlebotomies if needed, in a manner to reduce excess iron while avoiding a need for iron supplementation or red blood cell transfusion. Therefore, if the serum ferritin or iron binding capacity were to fall below normal, these could then be measured at twice-weekly intervals until these values have returned to normal. After a remission of PCT was achieved, plasma porphyrins and assessments of iron status could be repeated at less frequent (monthly) intervals. Blood chemistry tests were carried out at 3-month intervals to assess safety of the regimen.

The number of units of packed red blood cells received during the 12-weeks period prior to study entry, and the number received during the study were recorded. The number of therapeutic phlebotomies and the volumes of blood removed were also recorded.

Concomitant therapy that might relate to the treatment of PCT or to management of end stage renal disease, such as transfusion, phlebotomy and iron supplementation were recorded. It was anticipated that erythropoietin would eliminate transfusion requirements. Any transfusions of packed red blood cells were recorded and the requirements before and during treatment compared. Other causes of anemia were sought when anemia remained unresponsive or was less than fully responsive to erythropoietin. Hepcidin was unknown at the time of the study, so the contribution of high levels of that recently described hormone could not be assessed.

Therapeutic phlebotomy in addition to erythropoietin was anticipated to be required in some patients in this study to treat moderate or marked iron overload. Indications for phlebotomy in this study (for patients responsive to erythropoietin administration) were as follows. (a) An initial ferritin in excess of 1000 ng/ml in a patient with anemia not previously treated with erythropoietin. Clinical evaluation should suggest strongly that the anemia was due to erythropoietin deficiency and not to another definable hematological condition. (b) A normal or increased ferritin during treatment with erythropoietin in a patient in whom anemia has responded to erythropoietin but in whom plasma porphyrins have not decreased or have leveled off after an initial decrease. When one of these indications was met, repeated phlebotomy (60-180 ml) was permitted at the beginning of each dialysis. Volumes removed did not exceed 540 ml per week. The volume of a phlebotomy was reduced or omitted if the patient was hypotensive, if the

hemoglobin was below 9.5 g/dl on the current or previous dialysis day, or if another clinical contraindication exists.

An iron supplement was permitted if iron saturation and ferritin were below normal or there was evidence of a poor response to erythropoietin and if there was evidence of a clear decrease in plasma porphyrins. Oral iron (e.g. ferrous sulfate 325 mg three times daily) was preferred. Parenteral iron was not recommended because there is no experience with its use in PCT associated with end stage renal disease. However, because it is now known that ESRD patients may have high levels of hepcidin, which impairs iron absorption, parenteral iron should probably be given at least if there is a poor response to oral iron.

Patients could continue to receive other medications for the treatment of end stage renal disease, its complications, or any other associated medical conditions. Dosage, frequency and reason for administration of any concomitant medication were recorded. Estrogens, which are known to exacerbate PCT in some patients, were avoided. No patients in this study were on drugs such as barbiturates, sulfonamides, etc., which are contraindicated in acute porphyrias and may be relatively contraindicated in PCT. Patients were to continue their usual diets during the study.

Diagnosis of PCT: In this study the diagnosis of PCT was well documented and other conditions that can produce similar skin lesions were excluded. PCT was documented by finding increased plasma porphyrins, with a typical neutral fluorescence spectrum, and increased isocoproporphyrin in feces. Patients found to have PCT were further characterized by measuring uroporphyrinogen decarboxylase (UROD) activity in erythrocytes. See Table 5 for summary of PCT diagnosis lab findings for our study cases.

Some hemodialysis patients develop “pseudoporphyria”, a condition of unknown cause characterized by a bullous dermatosis resembling PCT but without increased porphyrins. Because “pseudoporphyria” may be due to exposure to or retention of an unknown photosensitizing chemical unrelated to porphyrins, there is no reason to think that it will improve with erythropoietin treatment. Skin lesions identical to those found in PCT also occur in two much less common forms of porphyria – hereditary coproporphyria and variegate porphyria, which are unresponsive to treatments that decrease iron and are effective in PCT. To our knowledge these porphyrias have not yet reported in association with end stage renal disease. Although acute intermittent porphyria (AIP), the most common acute hepatic porphyria, does not ordinarily cause skin manifestions, it can cause vesiculo-bullous lesions when associated with ESRD (a known and increasingly recognized complication of AIP). In this study, it was important to exclude these conditions by biochemical testing because they are not expected to respond to erythropoietin administration.

Assessment of iron status was performed before and during treatment with erythropoietin in this study. The objective of treatment was to reduce iron stores in the liver and stop the iron-dependent production of the uroporphomethene inhibitor of UROD and permit regeneration of new enzyme protein. On the other hand sufficient iron must be present in bone marrow to support hemoglobin formation needed for effective

erythropoiesis. Erythropoietin-stimulated erythropoiesis can utilize a considerable amount of storage iron.

Chapter 8 Cases Summary

In this study a total of 6 cases of PCT (5 on hemodialysis and 1 on peritoneal dialysis) were treated with recombinant erythropoietin for up to 390 days. Because this is a rare condition the study included several centers. Results of each case are summarized below. Case 1 was previously reported[25] and provided pilot observations that justified a larger proof of concept study, as reported here.

Case 1

This is a 57-years-old female with severe PCT (as documented by marked increases in plasma porphyrins and fecal isocoproporphyrin), end stage renal disease secondary to chronic pyelonephritis, and iron overload was treated with erythropoietin and judicious phlebotomy for 6 months. The patient received erythropoietin therapy under this protocol, and transfusion requirements for anemia of end stage renal disease were eliminated, phlebotomies were supported and excess iron was mobilized, as indicated by decreases in ferritin and transferrin saturation. Plasma porphyrins decreased substantially and new skin lesions and secondary infections ceased. No untoward side effects were noted. Frequent monitoring was necessary to prevent iron deficiency from limiting the response to erythropoietin. After plasma porphyrins began to decrease the patient became able to tolerate standard oral doses of iron and thereby maintain an erythropoietic response to erythropoietin and without developing an increase in plasma porphyrins.

Her plasma porphyrin levels before treatment were 166-211 ug/dL (reference <0.9), with a predominance of uroporphyrin and heptacarboxyl porphyrin. These levels are ~10-fold greater than levels seen in patients with PCT and normal renal function, which accounts for her unusually severe skin lesions especially on her hands, with complicating osteomyelitis and loss of the end of one finger. Fecal porphyrins were modestly elevated with an increase in isocoproporphyrin. After treatment initiation with erythropoietin 150 U with each dialysis, a reticulocytosis (5.4%) was observed and no further transfusions were required. A total of 900 ml of whole blood (done in small volumes of 120-180 ml at dialysis) was removed over a 3-week period beginning 3.5 weeks after therapy. Serum ferritin decreased gradually to 38 ng/mL, and plasma porphyrins to 9.4 ug/dl at the end of therapy (10.4 months). Two unexplained spikes in PP levels occurred at 1.3 and 2.5 months of therapy. Her skin symptoms continued to improve, and no new lesions developed after PP decreased to less than 10% of pretreatment levels.

Case 2

A 74-year-old male with ESRD secondary to chronic hypertension was on peritoneal dialysis for 5 years before developing blistering skin lesions. He consumed alcohol occasionally and was a former smoker (65 pack years until 1 year ago). Medications included clonidine, Ca acetate, ferrous sulfate 325 mg po TID, phenergan 25 mg po prn nausea, phytonadione (vit K1) 10 mg po qd, and aluminum hydroxide 2 gm TID. His initial ferritin level was 372 ug/L, plasma porphyrins 30 ug/dL, hemoglobin 13.9 g/dL and hematocrit 42.9%. He was initially started on small volume phlebotomies (6 sessions of 120-270 mL phlebotomies, total = 1,290 mL) over the course of 14 days. His ferritin decreased to 280, hemoglobin to 11.2, hematocrit to 35.7% and plasma porphyrins remained at 29, iron at 110, TIBC 343, and Sat 32%. Because his erythropoietic response to phlebotomies was inadequate, he was started on erythropoietin 100 U/kg subcutaneously 3 times a week (at 1 month into the study). He continued to develop new skin lesions on the face. Over the next 2 weeks he underwent 3 phlebotomies of 240 mL each (total 720 mL). With 5 doses of erythropoietin his hemoglobin stabilized at ~12.4. Serum ferritin then decreased further to 108 and plasma porphyrins to 28, and hemoglobin/hematocrit remained stable at 11.3/35.6%. He reported feeling better with only a few new blisters at 2.5 months, however his skin worsened subjectively a week later and he complained of occasional angina pain treated with nitroglycerin. He was subsequently hospitalized for peritonitis with pseudomonas and was treated successfully with IV antibiotics (Vanc and Tobrax).

At 3.5 months into the study, he complained of itching of the skin on hands and face. He underwent phlebotomy of 240 mL x3 (720 mL total) from over the next 2 months. After the first phlebotomy, his hemoglobin decreased to 11.1, so he was again started on erythropoietin 50 U/kg for two doses and then continued at a lower dose of 25 U/kg for 2 months, when his skin symptoms improved. During this time he was hospitalized and successfully treated for another episode of pseudomonas peritonitis. His PP levels remained at 12-14 ug/dl despite phlebotomy. He was started judiciously on iron replacement therapy with FeSO4 because of iron deficiency (serum iron at 29 ug/dL), which was discontinued after 2 months when he developed itching without active lesions. Afterwards (at 7-8 months into the study) his Ferritin stabilized at 69-88, PP at 11-12, and hct at 39.2-41.7. At this point his PCT had improved, as plasma porphyrins had decreased from 30-35 to ~11 mcg/dl, and ferritin, which had decreased to borderline iron deficient levels had now increased without a further increase in plasma porphyrins. Skin symptoms had also improved. However, he still complained of itching, and noted intermittent bumps under the skin that he associated with pct. He was subsequently phlebotomized for four months (at 9-12 months into the study) with 600 ml, 400 ml, 600 ml, and 300 ml. Ferritin decreased from 92 to 8.5, hct 44.2 to 35.4 and pp 15 to 8.

Three months later (at 15 months) he reported his skin was normal and no lesions were seen on exam; at this time his ferritin was 19, pp 7, and hct 44.4. After an additional 3 months he had no skin lesion, ferritin was 15, pp 5 and hct 42.2. However, three months after this (at 22 months) he reported recurrent lesions on the left upper forearm from sun exposure while driving; at this time his ferritin was 12, pp 3.9 and hct 46.2. Since his plasma porphyrin level had not increased, he was advised to avoid sunlight. Four months

later, his skin manifestations resolved (at 26 months); his pp was 3, ferritin 71 and hct 47.3.

On follow up at 48 months, he reported nodular non-PCT skin lesions on the abdomen, which was not sun exposed. At that time his ferritin was 73 and pp 3.4. He was taking ferrous sulfate 325 TID at this time and another 525 mg ferrous sulfate from a multivitamin, and was advised to discontinue iron supplementation so his ferritin level would remain below 100 ng/ml.

In summary, this patients with porphyria cutanea tarda associated with ESRD was unusual because he was not anemic initially. He responded to phlebotomies which required only brief support from erythropoietin. At last follow up his pp of 3.4 was greatly improved from an initial level of 30 mcg/dL, which was sufficient to prevent skin lesions but was not normal. It is likely but not known whether additional iron reduction would have brought his levels of pp down to normal. All the other cases in this series were anemic and required continuous treatment with epo for anemia and to eliminate the need for erythrocyte transfusions and mobilize excess iron.

Case 3

A 50-year-old Caucasian female with end stage renal disease due to chronic pyelonephritis was on home peritoneal dialysis and developed skin blisters. These were recognized to be due to PCT 7 months later when she developed peritonitis and was transitioned to hemodialysis. At that time her ferritin level was 1036 ng/mL. One month later she was treated as in inpatient for an infected shunt, and plasma porphyrin levels were increased to 105 ug/dL (normal, <2), with a fluorescence scan of diluted plasma at neutral pH showing a peak at 616nm, a finding characteristic of PCT.[29] A month later her ferritin level increased to 4,727 ng/mL with Fe 166 ug/dL, TIBC 237, Hct 37.6, Hgb 12.3, retics 3.8, AP 210, SGOT 125. She was enrolled in this study for treatment with erythropoietin and repeated phlebotomy. At one month into the study, her ferritin decreased to 3343 with Fe 138, TIBC 273, Hct 38.9, Hgb 12.5, Retics 3.9, AP 231, SGOT 55. Except for an admission for treatment of pseudomonas peritonitis one month after that, she did well. At nine months, her ferritin had decreased to 1776 and plasma porphyrins to 32 and skin blistering was improved. Her treatment was interrupted at 11 months when she underwent cadaveric renal transplantation and was placed on immunosuppressive therapy including systemic corticosteroids. Her ferritin decreased to 1254 and plasma porphyrins to 28; other results included Fe or 96, TIBC 323, Hct 24.2, Hbg 7.8, Retics 2.8, AP 94, SGOT 67. She developed osteoporosis and stress fractures from corticosteroid therapy, and one year after transplant had a hip fracture and required 2 units packed RBCs for anemia (Hct 23). Her ferritin decreased to 590 one year later, and increased to 1539 one year after that. The following year she developed a left hip abscess, which was drained and debrided, and required 2 units packed RBCs for blood loss during surgery. Three months later her plasma porphyrins had decreased to 5.3 and had no skin lesions. She was then lost to follow up.

In summary, this patient developed PCT 7 months before transitioning from peritoneal dialysis to hemodialysis. Despite infections and other disease complications, she improved with treatment with erythropoietin and phlebotomy (total amounts were not recorded), with ferritin reduced from 4,727 to 1254 and plasma porphyrin from 105 to 28 ug/dL. She underwent renal transplantation after 11 months, which restored endogenous erythropoietin production and further improvement in PCT.

Case 4

A 41-year-old female with ESRD of unknown cause, renal osteodystrophy and idiopathic peripheral neuropathy developed blistering skin lesions after being on HD for 4 years. PCT was documented by increased plasma porphyrins and isocoproporphyrins in feces. Initial labs included ferritin 2120, Fe 131, TIBC 327, sat 40%, PP 125, Hbg 6.8, Hct 21.6, and aluminum 73. Blood lead and aluminum levels were normal. Patient was treated with epogen 4000 U for 8 months prior to enrollment into our study. She was started on erythropoietin therapy at 100 IU/kg (4,200 U), at that time her ferritin decreased to 197. Her course was complicated by bacterial skin infections, malnutrition, and gastrointestinal bleeding that required RBC transfusions. She then stabilized and did not require transfusions for 6 months. At 1 month into the study, serum ferritin decreased to 204, serum iron 38 and sat 15%. However she continues to experience considerable problems with skin lesions. These are very painful esp. when involving the digits of her left hand (the fistula side). At 2 months, her labs showed ferritin at 75, pp 61, and hct 21.2. However, she had a reported GI bleeding event on at this time and received blood transfusion (unknown amount), her labs subsequently showed ferritin 221, PP 105, and hct 23.7 (with hgb 7.7). She also had increased erythropoietin dose to 150 U/kg, however, it was not clear when this occurred. At 7 months, her lab results suggested that she has developed iron deficiency with ferritin 45 and PP 66-70, Fe at <10, with TIBC 171, hbg 6.4 and hct 20.2. Because her Hct has dropped so low, her nephrologist decided to transfuse her and start on oral iron therapy (unclear amounts). 2 months later (at 9 months) patient had dose adjustments of her erythropoietin from 6300 U to 6000 U. During this time, the patient's skin lesions seem to be improving but not completely resolved. She continued to have low level hematocrit (in the 20s) despite being on high dose erythropoietin at 9-18 months into the study and was subsequently loss to follow up. 8 years later, follow up lab result suggested a recurrence of PCT with PP at 338.

In summary, this patient developed PCT after 4 years on hemodialysis. Despite treatment with erythropoietin, she continued to require periodic transfusions for anemia, with only temporary improvement in her PCT. We suggest that factors other than erythropoietin deficiency (perhaps increased hepcidin) contributed substantially to anemia in this patient, and erythropoietin was therefore not able to correct anemia or contribute to treatment of PCT. This was the only case in this series that did not show improvement with treatment.

Case 5

A 30 years old African American male with PMH of ESRD (possibly 2/2 to FSGS) on HD, accelerated HTN, chronic pancreatitis 2/2 alcohol abuse, syphilis, chronic anemia, IV drug abuse history presented with PCT, was enrolled into our study and started on erythropoietin at 5000 IU (100 IU/kg). His meds included Iberet with Folate 500 mg po qd, Capoten 250 mg po q12h, Catapress 0.3mg po q12h, Lopressor 50 mg po qd, Titrelac 2 po meals TID, Lasix 120 mg po bid, and Cardene 30 mg po bid. Approximately 10 months prior to enrollment into our study, patient was hospitalized for accelerated hypertension with acute renal failure. His social history during this hospitalization is notable for tobacco use (14 pk years), alcohol use (one can of beer daily and one pint of whiskey every two days) and cocaine use (smokes cocaine on a daily basis for several weeks prior to admission and on the day of admission). He also had a history of IV drug abuse which he reported his last exposure was >6 years ago. No blood transfusions and no homosexual or bisexual activity in patient's history elicited. No surgeries elicited. Family history with father with HTN, otherwise mother and siblings are healthy. He was diagnosed with ESRD and started on HD during this hospitalization.

A week prior to enrollment into the study, he was diagnosed with PCT based on his porphyria labs with PP at 200. He was previously on iron replacement with imferon for two weeks, which was discontinued on prior to starting on erythropoietin due to increased serum iron and ferritin levels at 243 and 624 respectively. At 3 months into the study, erythropoietin was on hold for 2 weeks due to high hct (39.3) without any adverse effects noted. At 4 months, PP levels decreased to 147, ferritin peaked at 861, then decreased to 622, hgb increased from 7.8 to 11.1 and hct from 25.8 to 34. He was briefly on iron replacement therapy (iron-containing multivitamin, iberet) for a month for iron deficiency, which improved his iron/TIBC/Sat from 29/400/7% to 134/235/53%, ferritin increased from 496 to 931, while pp remained unchanged from 147 to 143. Despite the unchanged pp level, he continued to improve with his skin symptoms.

He was hospitalized for acute pancreatitis with hepatocellular necrosis at 6 months into the study. His treatment course is further complicated by ongoing alcohol use due to family stressors, which was noted at 7 months into the study. His dose of erythropoietin was decreased to 3000 IU (~50 IU/kg) at 7 months into the study (for 5 months), then increased to 4000 IU (~75 IU/kg). Labs during this time showed that his ferritin decreased to 408- 526 and PP decreased to as low as 18, hgb/hct at 12.2/37.4. His treatment course continued to be complicated with multiple skipped dialysis sessions and admissions to hospital due to pancreatitis related to ethanol abuse. At 14 months, his pp was at 27.4 without evident of PCT skin lesions on exam. He had a recurrence of skin lesions at 21 months when he missed several dialysis sessions and at 22 months when he had recurrence of pancreatitis; his pp was at 65.2 and ferritin at 294. We suggested for patient to have phleb 100 ml blood at the end of each dialysis three times per week to reduce the ferritin to about 20-40 ng/ml, however it is not clear how much phlebotomy has been done as his ferritin continues to be elevated at 300s at 25 months into the study and there were no records of phlebotomy amounts from his dialysis center. Progress to complete remission has been slow in this patient due to poor compliance on his part. At 25 months, his dose of erythropoietin was increased to 110 IU/kg. Hgb at 9-10 during this time with iron at 111-227. Skin symptoms were reported to improved at this time. At 26

months, patient was admitted for missed HD session and transfused 2 units pRBCs for his anemia. He was taken to OR for thrombectomy of his shunt. Patient was loss to follow up afterwards. He was monitored peripherally via labs, at 46 months, which showed PP continues to decreased to 7 and Hct remained low at 24.5.

Case 6

A 51 years old Caucasian female with past medical history of PCT was admitted to Rockefeller University Hospital with chief complaint of skin blisters, ulcers and hirsutism. Patient had a left nephrectomy at age 6, suffered from renal insufficiency secondary to chronic pyelonephritis, and has been on hemodialysis for 10 years. Patient also had iron overload secondary to multiple blood transfusions for anemia. About 18 months prior to enrollment into our study, she developed painful bullae on the dorsal surfaces of her hands. She was treated with Desferal for iron chelation. However, she suffered an edematous reaction after the 10th treatment. Desferal was discontinued because of possible allergic reaction. Patient was followed at Massachusetts General Hospital Dermatology and plasmapheresis was begun. She has received approximately 10-15 of these treatments and stated that she has experienced good relief. She has not had many recurrences of bullae. Her past medical history is significant for hypertension prior to dialysis. She smoked cigarette (31 pack-years), doesn't drink alcohol or use estrogen, and has no family history of PCT. She was diagnosed with PCT based on labs which included elevated total stool porphyrin (418) and plasmas porphyrin (269), and TLC pattern of stool porphyrins with increased isocoproporphyrins consistent with the diagnosis of PCT.

Patient was enrolled into our study and started on erythropoietin 100 IU/kg 3x weekly. From 1-3 months into the study, she was hospitalized at Brigham and Women's for CABG (double) and received 3 units transfusion while hospitalized. During the 3rd month, her hematocrit level at increased from 32 to 34. Plasma porphyrins were undetectable throughout the study. There were no reports of skin lesions during study. At 4 months, patient's ferritin level at was 490 and hct 34.5. At 5 months, erythropoietin was reduced to 2,500 units for elevated hct at 37-42; ferritin at this time was 361. Patient was loss to follow up afterwards.

Case Summary

Characteristics summary of study cases are seen in Table 4. The age range of our cases is 30-74 yrs. with average of 50.4 yrs. and female predominance at 67%. Pyelonephritis is a common renal disorder with a prevalence of 50%, other causes of renal disorder include chronic HTN, FSGS, and unknown. Majority of cases, 4 out of 6, are hemodialysis (HD); 2 out of 6 are peritoneal dialysis (PD); one case has received PD and HD, before receiving transplant. Duration of dialysis prior to onset of PCT varies from 7 months to 5 years with average of 2.6 years. 4 out of 6 cases achieve remission of symptoms after treatment, of which one case was improving with therapy and ultimately received remission with renal transplant. Of the 2 cases that did not received complete remission, Case #5 received partial remission, however his treatment course was complicated by medical non adherence preventing him from achieving complete remission. Case #4 was refractory to treatment.

Table 4: Cases Characteristics

Case #	age	gender	renal dx	HD or PD	duration of dialysis prior to sx	tx start	tx end	duration tx	duration f/u	Remission obtained
1	57	F	chronic pyelonephritis	HD	4 years	1988	1989	10.4 months	10.4 months	Yes
2	74	M	Chronic HTN	PD	5 years	Sep-89	Oct-90	13 months	3 yrs	Yes
3	50	F	chronic pyelonephritis	PD, HD, Transplant	7 months	Sep-89	Aug-90	11 months	5 yrs	Yes (with transplant)
4	41	F	unknown (renal osteodystrophy ?)	HD	4 years	Oct-90	Jul-91	8 months	8 yrs	Yes
5	30	M	focal segmental glomerulosclerosis	HD (began on 05/89)	16 months (ESRD on 06/89, PCT dx labs on 10/90)	Nov-90	Jan-94	3 yrs 10 months	8 months	Partial (nonadherent)
6	51	F	chronic pyelonephritis	HD	10	Apr-90	Jul-90	3 months	Loss to f/u	Yes

Table 5 showed the diagnosis of PCT for study cases. All of the cases have elevated plasma porphyrins with a neutral fluorescence spectrum maximal emission wavelength of 616-617 nm. Other labs present to help confirm diagnosis include elevated urinary porphyrins with TLC showing predominance of uroporphyrin and 7-carboxylate porphyrin, elevated fecal porphyrin with predominance of isocoproporphyrins (although one case has predominance of coproporphyrin instead of isocoproporphyrin).

Table 5: Cases PCT diagnosis labs

Case	labs
1	PP 211 ug/dL (normal <2), neutral fluorescence spectrum maximal emission wavelength 616nm, total urinary porphyrins 800nmol/l (normal <300), TLC predominantly uroporphyrin and 7-carboxylate porphyrin in urine (normal urine contains predominantly coproporphyrin). Fecal porphyrin levels 202 nmol per gram of dry weight (normal 0-200), with predominance of isocoproporphyrins with ratio of isocoproporphyrin to coproporphyrin 35 (normal <0.1)
2	PP 30 ug/dL, neutral fluorescence spectrum maximal emission wavelength 616nm. Unable to locate other porphyrin labs for diagnosis.
3	PP 105 ug/dL, neutral fluorescence spectrum maximal emission wavelength 616nm. Unable to locate other porphyrin labs for diagnosis.
4	PP 125 ug/dL, neutral fluorescence spectrum maximal emission wavelength 617 nm. TLC fecal porphyrins with predominance of coproporphyrin with ratio of isocoprophyrin to coproporphyrin 0.34.
5	PP 200 ug/dL, neutral fluorescence spectrum maximal emission wavelength 617 nm, erythrocyte porphobilinogen deaminase 74 nmol/ml/h (20-40 nl), erythrocyte protoporphyrin 1546 mcg/dl (20-100 nl), serum prophobilinogen 0.148 ug/ml, total fecal porphyrins 249 nmol/g dry weight (0-200 nl)
6	PP 269 ug/dL, neutral fluorescence spectrum maximal emission wavelength 617 nm, total stool porphyrin 418 nmol per gram dry weight, and TLC pattern of stool porphyrins with increased isocoproporphyrins.

Chapter 9 Results and Discussion

DISCUSSION OF SPECIFIC AIM 1

Specific Aim 1 for this study was to evaluate the efficacy and safety of recombinant human erythropoietin in the treatment of PCT in patients with ESRD. A pilot crossover study of 6 treated patients was planned, with baseline values serving as control values for each patient, and repeated observations intended to characterize the time courses of these effects. Aspects of treatment to evaluate were anticipated to include (a) reduction or elimination of transfusion requirements, (b) correction of anemia as measured by levels of hemoglobin and hematocrit, (c) support for phlebotomy to further reduce iron overload, and (d) improvement of PCT, an iron-related diseases, as assessed by reductions of serum ferritin and plasma porphyrin levels and improvements in cutaneous symptoms in this condition. Therapeutic phlebotomies were most feasible in these patients if carried out in small amounts at the time of dialysis, rather than according to the usual regimen for PCT, which is to remove ~450 mL at intervals of ~2 weeks. Additional measures of efficacy include serum iron, transferrin (iron-binding capacity) and transferrin saturation. Safety observations include standard laboratory testing and observation for development of concurrent or complicating medical conditions.

(a) Effects of erythropoietin on transfusion requirements:

The effects of erythropoietin on transfusion requirements are summarized in Table 6. Data in this pilot study are incomplete because transfusion records before treatment were incomplete. However, when the amounts of transfusion pre and post treatment were known, there was often a significant reduction of transfusion requirements. For example case 1 had 50% reduction of transfusion, from 8 units prior to erythropoietin use to 4 units afterwards. Cases 5 and 6 likely had some reduction in transfusions but data on amounts prior to the study were not known. There was no transfusion data for Case 2, who was the only case that did not require transfusion before or during study, but as was described did show improvement in anemia. Case 4 did not show a response to erythropoietin in terms of decreased transfusions or improvement in anemia, suggesting that additional unidentified factors were important in causing her anemia. From these pilot observations, it seems likely that as in other patients with ESRD, erythropoietin can decrease transfusion requirements in most but not all patients with PCT associated with ESRD. Future studies should include more rigorous collection of transfusion data during both control and treatment periods.

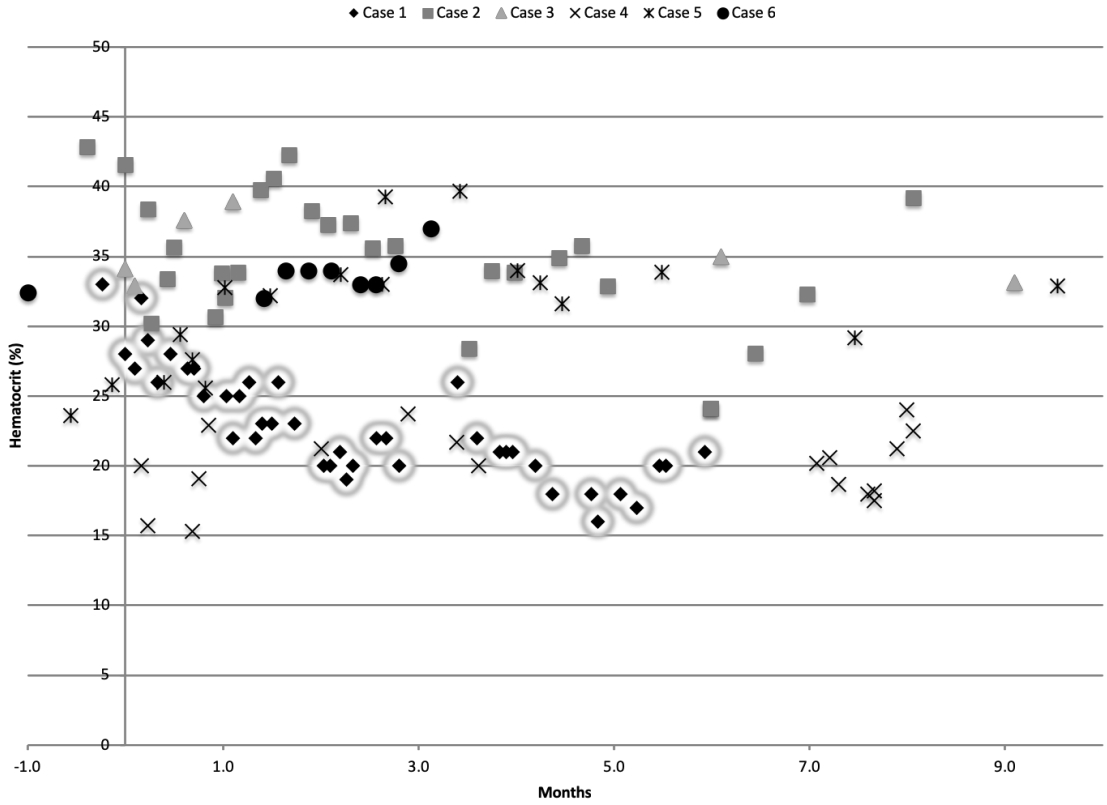
Table 6: Effects of Erythropoietin on Transfusion Requirements

	Remission obtained	Total Phlebotomy	Epo dosing	Iron Dosing	Transfusion
Case 1	Yes	900mL	150	FeSO4 various dosing	8Units before, 4 Units after
Case 2	Yes	4630 mL	100,50,25	FeSO4, then Imferon	No transfusion needed before and after
Case 3	Yes (with transplant)	Unknown	Unknown	None recorded	At least 4 Units after transplant
Case 4	No	None reported	95,100,150,143	Unknown	Unknown amounts transfused during study from GI bleed
Case 5	Partial (nonadherent)	At least 180 mL, unknown how many total	100,50,75,110 (Held once for high hct)	Imferon, multivit	Unknown how many units prior, 2 Units on follow up
Case 6	Yes	None recorded	100,50	Desferal (9 sessions) prior to study, none after study	Multiple transfusions prior to study, 3 Units after study

(b) Effects of erythropoietin on hemoglobin and hematocrit levels:

Hematocrit values at baseline and during treatments are shown for all cases in Figure 2. These levels are affected positively by treatment with erythropoietin and by transfusions, which as described above, were required less frequently at least in some patients during treatment. Hematocrit levels were negatively affected by factors that were largely not measurable, possibly including inadequate dosage levels of erythropoietin, insufficient iron supplies, elevated hepcidin due to impaired renal excretion and inflammation and inapparent blood loss. During the study, the lowest hematocrit observed for case 1 was 17%, corresponding to hemoglobin of 5.2 g/dL; case 2 24.1% and 7.6 g/dL; case 3 24.2% and 7.8 g/dL; case 4 15.3% and 5 g/dL; case 5 18% and 6 g/dL; case 6 32% (no hemoglobin was recorded for this hematocrit level). Erythropoietin is used to maintain hemoglobin at or above 9.5 g/dL (hematocrit at or above 28.5%). Cases 1 and 4 were difficult to achieve this goal, both cases had relative iron deficiency requiring iron supplementation (as discussed in below section). But in all cases except Case 4, hematocrit levels were maintained or increased with lower requirements for transfusions.

Figure 2: Cases Hematocrit Level



(c) Effects of erythropoietin to support for phlebotomy to further reduce iron overload

In all cases that had erythropoietin (case 1, 2, 4, 5, 6), as noted in part b, the hemoglobin and hematocrit values were positively affected. Erythropoietin was used to maintain hemoglobin at or above 9.5 g/dL. In case 1, this hemoglobin level was difficult to maintain, however with the use of erythropoietin, phlebotomy was initiated at 0.6 to 1.4 months, at which times her hemoglobin level was at 9 and 7.1 g/dL respectively. Case 2 was initially not requiring epo with hgb at 13 when he was started on phlebotomy, later required epo at 100 U/kg (when his hgb was at 10.3) with subsequent improvement of hgb to 12.4. Case 4 had epo but not phleb. Case 5 had epo with phleb (at least 180 ml) however unclear total amount, hgb was maintained at 9-10 g/dL however his clinical course was complicated due to medical nonadherent and numerous missed HD sessions and hospitalizations with blood transfusions. Case 6 had epo with hct at 32 initially which improved to 37% (only hct was documented routinely for this patient, hgb was recorded at 11.5 for the hct of 37%). She did not require phleb due to undetectable PP level and no skin symptoms.

(d) Effects of erythropoietin on plasma porphyrins, serum ferritin and clinical symptoms

Figure 3 shows the changes in porphyrin and ferritin for each case with phlebotomies as indicated. These are the key laboratory measures of efficacy based on the pathophysiology of PCT and the disease response to treatment. For each case, the changes in these measurements are described below. It is apparent that even with Case 4, declines in both ferritin and porphyrins occurred consistently, and it was apparent for some cases that ferritin decreased earlier than plasma porphyrins, as occurs in patients with PCT and normal renal function when treated by phlebotomy.

Case 1. This patient was on an erythropoietin dose of 150 U throughout the treatment period and had only 2 series of phlebotomies totaling 900 mL starting 3.5 weeks after initiation of erythropoietin treatment. Ferritin began to decrease promptly after starting erythropoietin and prior to therapeutic phlebotomies, indicating that an erythropoietin-induced increase in erythropoiesis resulted in mobilization and use of excess iron stores for hemoglobin formation. Erythropoietin also supported phlebotomies, since no further transfusions were needed even with removal of 900 mL blood. Plasma porphyrin levels decreased more slowly compared to ferritin, as is observed when patients with PCT and normal renal function undergo repeated phlebotomy. This reflects cessation of porphyrin accumulation followed by slow clearance from the liver of very large amounts of previously stored porphyrins. This patient achieved near normal plasma porphyrin levels within about 6 months, which approximates the 5-6 month time to remission observed with phlebotomy treatment of PCT patients with normal renal function.[30]

Case 2. In this patient, erythropoietin treatment combined with phlebotomies achieved a reduction in plasma porphyrins from 31-36 mcg/dL to 2.2 mcg/dL. Ferritin decreased from 449 to as low as 8.5. This patient was not initially anemic, but erythropoietin at 50 and later at 25 maintained adequate hematocrit and hemoglobin levels during most of the erythropoietin treatment period. Ferritin and plasma porphyrins decreased more slowly and with less separation in time than in Case 1, reflecting slower

response time in this case probably due to multiple concurrent conditions. Ferritin subsequently increased later to 109 (in the normal range) without a subsequent increase of PP.

Case 3. In this patient, PP level was initially as high as 124, however she did not require phlebotomy or epo during the study. She had CRT after 1 yr. and her PP dramatically decrease after this. Ferritin was slow to improve decreasing eventually to 590, there was a rebound increase on follows up however she did not have any symptoms and PP continues to be low.

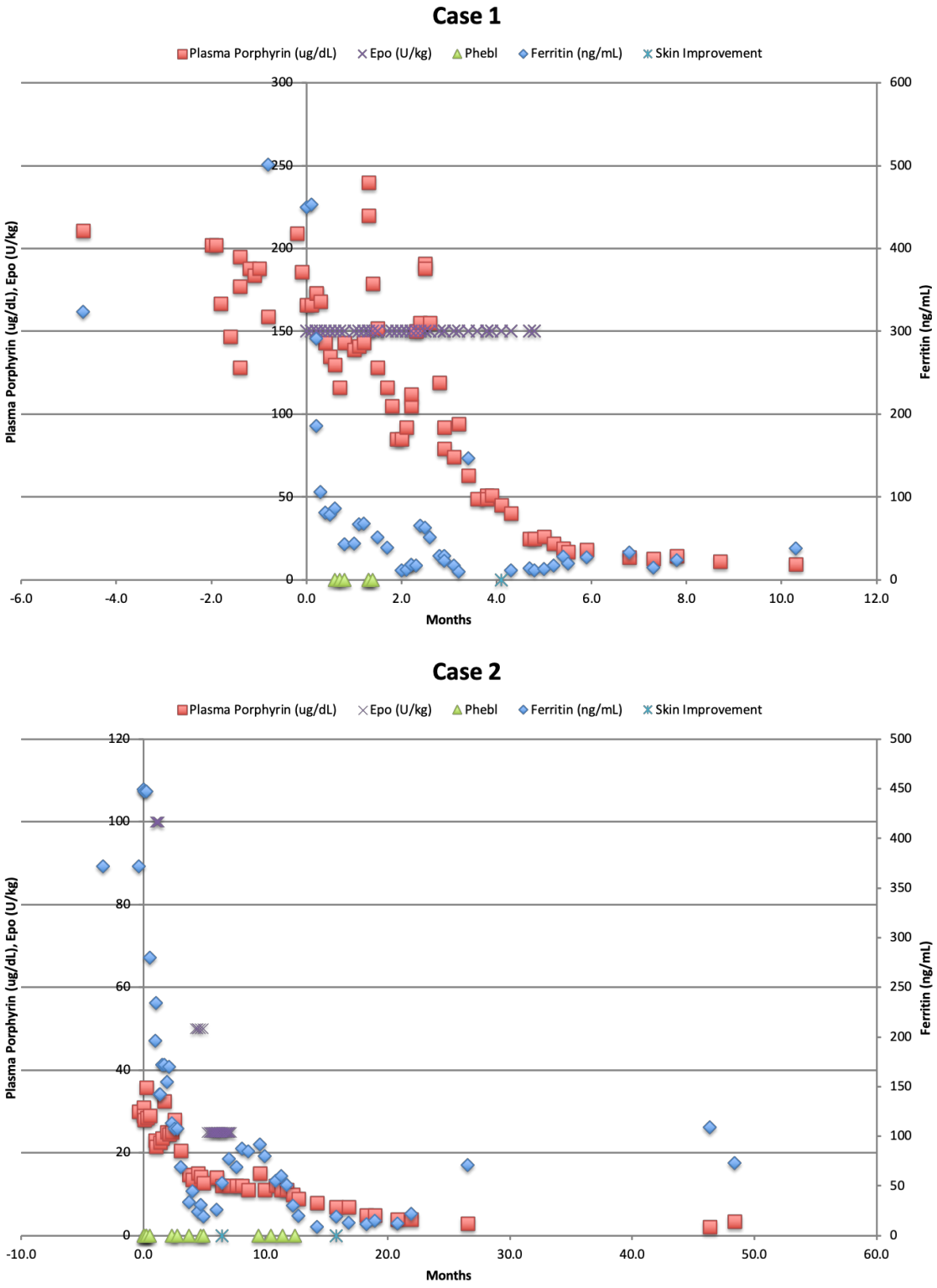
Case 4. This patient remained anemic despite treatment with erythropoietin, indicating that her anemia was due at least in part to factors other then erythropoietin deficiency. Because of her anemia she was not phlebotomized during the study to reduce iron, and she continued to have require transfusions. However, with erythropoietin treatment with erythropoietin of 100, increased to 150, her ferritin decreased considerably from 325 to as low as 70, and plasma porphyrins were gradually reduced from 122 to 42. These results indicate that erythropoietin stimulated erythropoiesis and mobilized excess storage iron sufficiently to produce improvement of PCT.

Case 5, This patient had an initial PP of 200 that increased to 875 after starting on epo at 50 and phlebotomies. Serum ferritin varied widely before and during treatment, ranging as high as 1240, probably reflecting acute phase responses as well as iron status. Plasma porphyrin levels were also erratic during treatment, which included 3 phlebotomies, but with continued treatment trended lower, eventually reaching levels as low as 18 after more than 2 years and later 4.4 mcg/dL with ferritin levels as low as 75. Results in this case indicate that improvement in PCT can result from phlebotomies but also from mobilization of excess iron stores by exogenous erythropoietin.

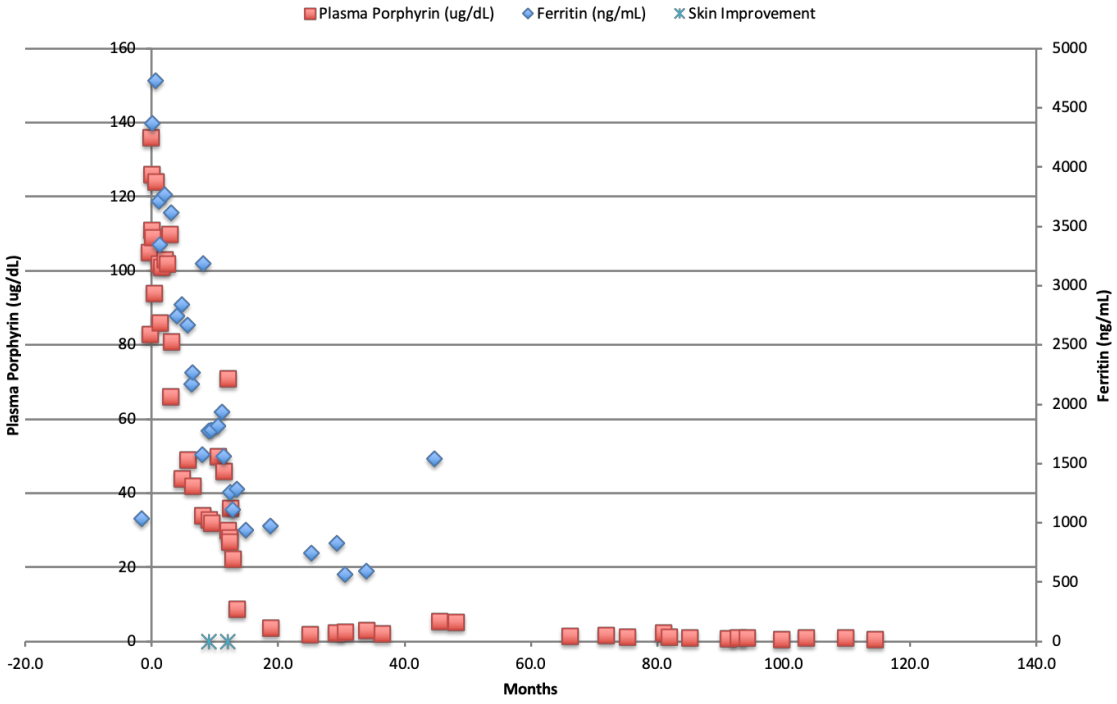
Case 6. This patient with minimal available data had PCT with markedly elevated plasma porphyrin levels. She was started on epo 100 IU/kg without phlebotomies, baseline porphyrin levels were not measured prior to enrollment into study (the only recorded reading was 269 ug/dl at 5.5 yrs. prior to the study). Her plasma porphyrins were undetectable at ~2 month into study (and remained undetectable), while ferritin remained elevated at 490.

In summary, in patients with clinical improvement, skin symptoms improved as early as 4 months (case 1) after initiation of Epo and therapeutic phlebotomy when the plasma porphyrin level falls to ~25% of initial level and continues to maintain remission of skin symptoms with porphyrin level falling to below 10% of initial level; this is also demonstrated in case 2. In both cases, plasma porphyrins falls as low as (2.2- 9.4) but doesn't reach normal level. Iron overload in these cases is corrected soon after initiation of phlebotomy (almost within a week of phlebotomy) as evident by the precipitous drop in ferritin, however plasma porphyrin have a more gradual decrease and clinical improvement of skin symptoms seemed to lag behind by at least several months (at least 4 months for case 1 and 6 months for case 2.

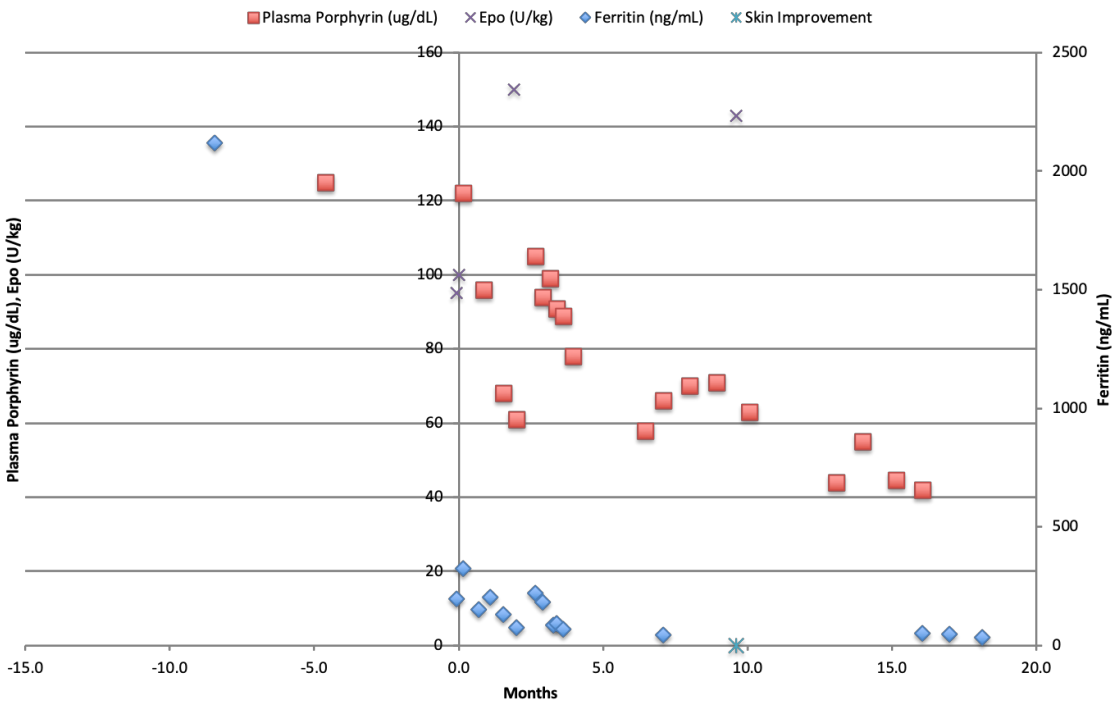
Figure 3: Porphyrin and Ferritin Levels



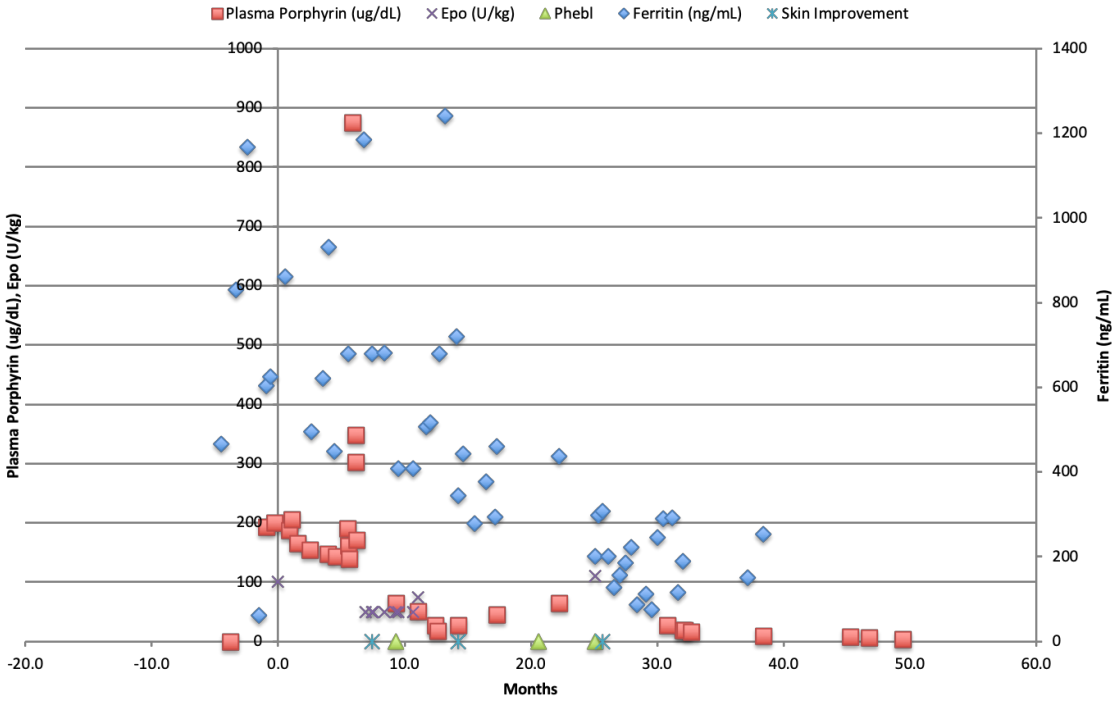
Case 3


Plasma Porphyrin (ug/dL)
Ferritin (ng/mL)
Skin Improvement
Plasma Porphyrin (ug/dL)
Ferritin (ng/mL)
Months
160
140
120
100
80
60
40
20
0
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
-20.0
0.0
20.0
40.0
60.0
80.0
100.0
120.0
140.0

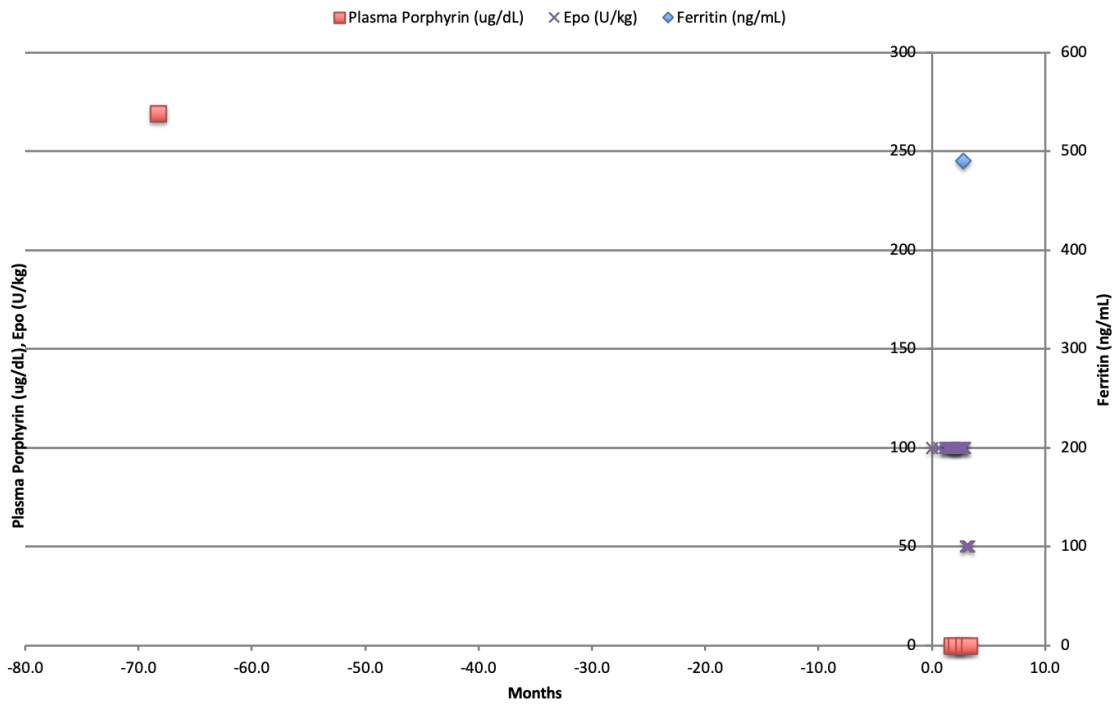
Case 4


Plasma Porphyrin (ug/dL)
Epo (U/kg)
Ferritin (ng/mL)
Skin Improvement
Plasma Porphyrin (ug/dL), Epo (U/kg)
Ferritin (ng/mL)
Months
160
140
120
100
80
60
40
20
0
2500
2000
1500
1000
500
0
-15.0
-10.0
-5.0
0.0
5.0
10.0
15.0
20.0

Case 5


Plasma Porphyrin (ug/dL)
Epo (U/kg)
Phebl
Ferritin (ng/mL)
Skin Improvement
Plasma Porphyrin (ug/dL), Epo (U/kg)
Ferritin (ng/mL)
Months
1000
900
800
700
600
500
400
300
200
100
0
1400
1200
1000
800
600
400
200
0
-20.0
-10.0
0.0
10.0
20.0
30.0
40.0
50.0
60.0

Case 6

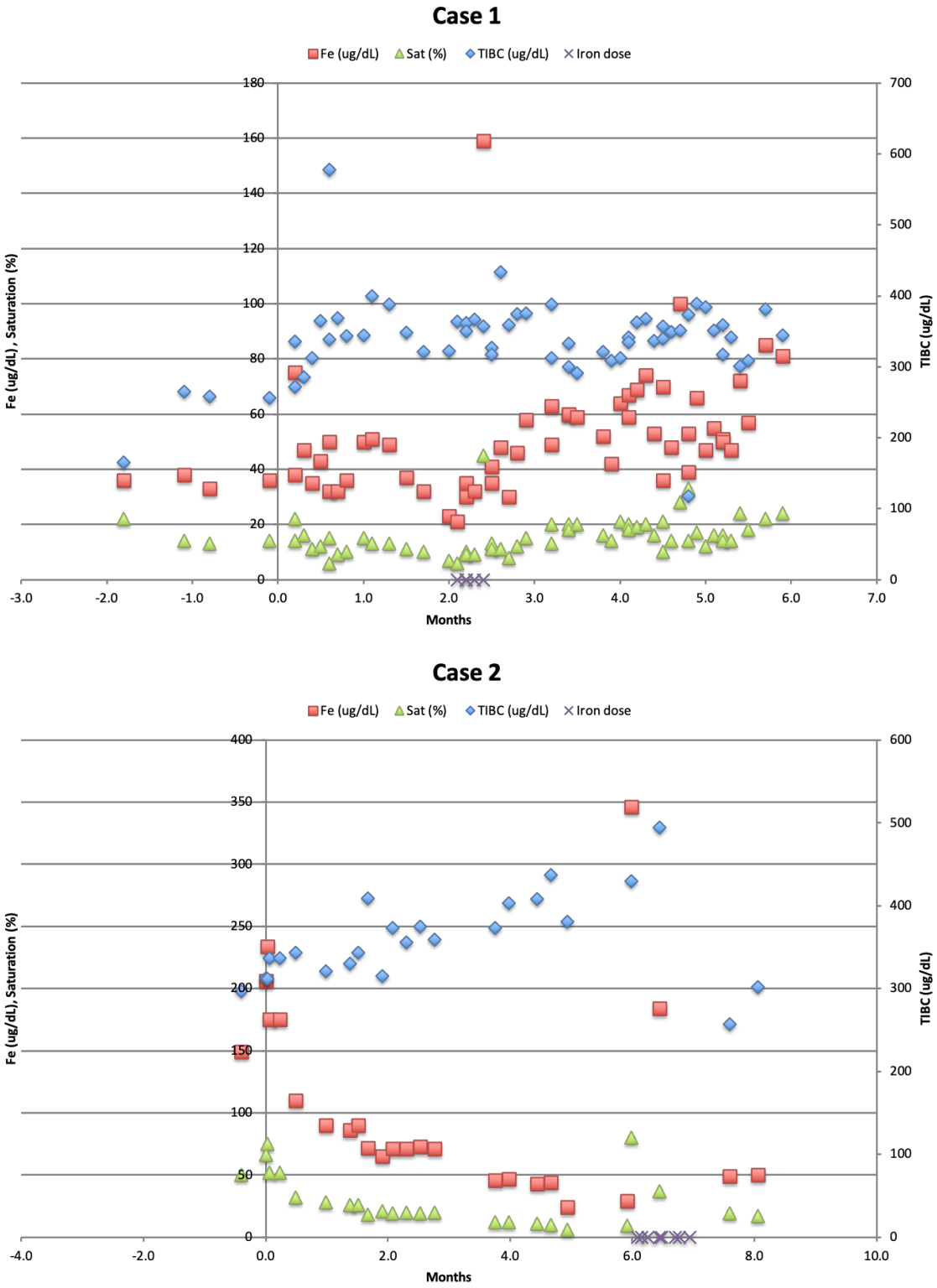

Plasma Porphyrin (ug/dL)
Epo (U/kg)
Ferritin (ng/mL)
Plasma Porphyrin (ug/dL), Epo (U/kg)
Ferritin (ng/mL)
Months
300
250
200
150
100
50
0
600
500
400
300
200
100
0
-80.0
-70.0
-60.0
-50.0
-40.0
-30.0
-20.0
-10.0
0.0
10.0

(d) Iron profile and supplementation

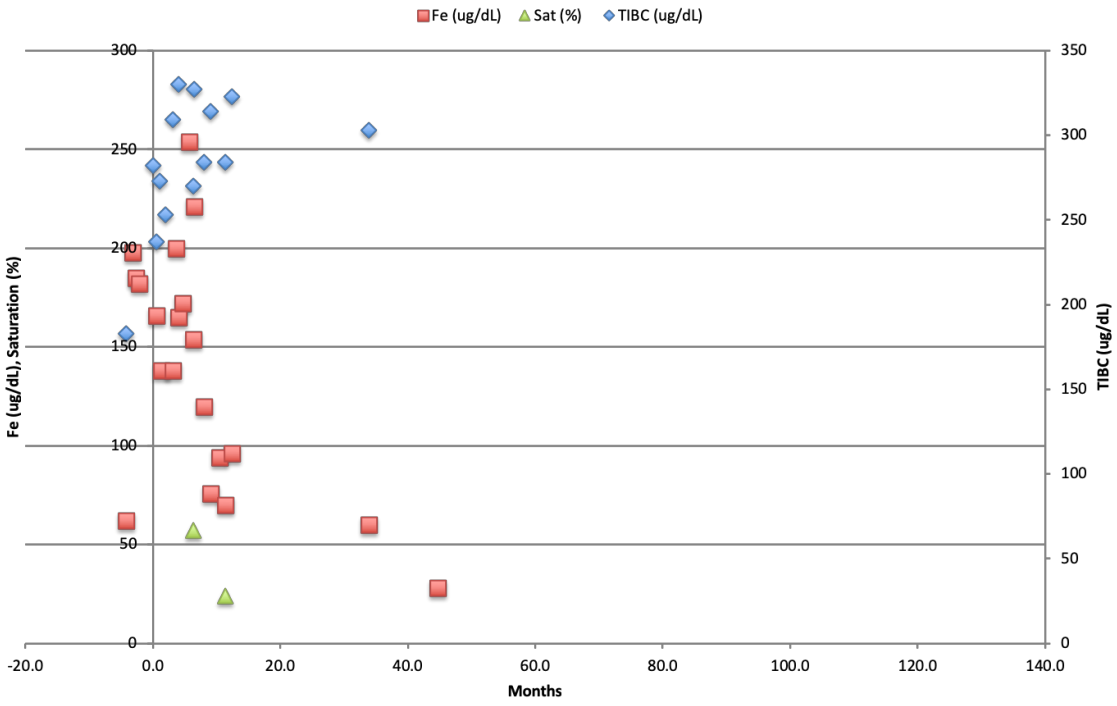
Figure 4 showed the effects of iron dose on iron profile. For case 1, after completion of phlebotomy within the first 2 months, patient was given iron supplementation for low iron (serum iron dropped to lowest value of 21). Iron supplementation lasted for approximately 1 week, iron level initially increased to 159 (high normal) after last iron dose, however this subsequently normalized to between 30-100. Case 2 had iron supplementation after 6 months of study for low iron of 29 (ferritin levels around 50s during this time, NL), patient had numerous phlebotomies prior to this with most recent phlebotomy at 3.8 month. Iron levels increased to 346, then steadily decrease to 50s at 7.6 months (last dose of iron supplementation at 6.9 months). Case 3 did not require iron supplementation, after patient's CRT at 12 months, her iron profile normalized with iron level at 96 (NL). She was followed up to 45 months (2.75 yrs. after her transplant) at which time she was found to have low serum iron level at 28, there were not reports of iron supplementation at this time. Case 4 had iron supplementation at 6.9 month of study for low iron that was undetectable, ferritin level at that time was at 45 with plasma porphyrin at 66, dose of epo at 150. Patient's iron level normalize to 40-50s at the following iron level recheck at 13 months, unfortunately we do not have data soon after the iron supplementation was started at 6.9 months to determine how soon after supplementation was given for iron level to normalize. Case 5 had numerous iron supplementations prior to study and from 3.6-4.6 months. His iron level went from 29 to 78-134 after receiving iron supplementation during the study. His clinical course was complicated due to medical non-adherence, his iron levels fluctuates between 30s-220s after iron supplementation. Case 6 did not receive iron supplementation during the study.

In summary, to maintain response to Epo, relative iron deficiency is corrected with supplementation, the amount of which varies on a case by case basis. For patients with known iron supplementation (case 1, 2, 5), iron profile and ferritin levels were monitored to prevent iron overload. Iron supplementation was successful to normalize iron and ferritin levels, and at least for cases 1 and 2, supplementation did not seem to worsening skin symptoms or increase plasma porphyrin levels (case 5 had tremendous fluctuations in plasma porphyrin levels and multiple recurrences of skin lesions throughout clinical course, complicated by medical non adherence as discussed previously, making it difficult to correlate these effects to iron supplementation).

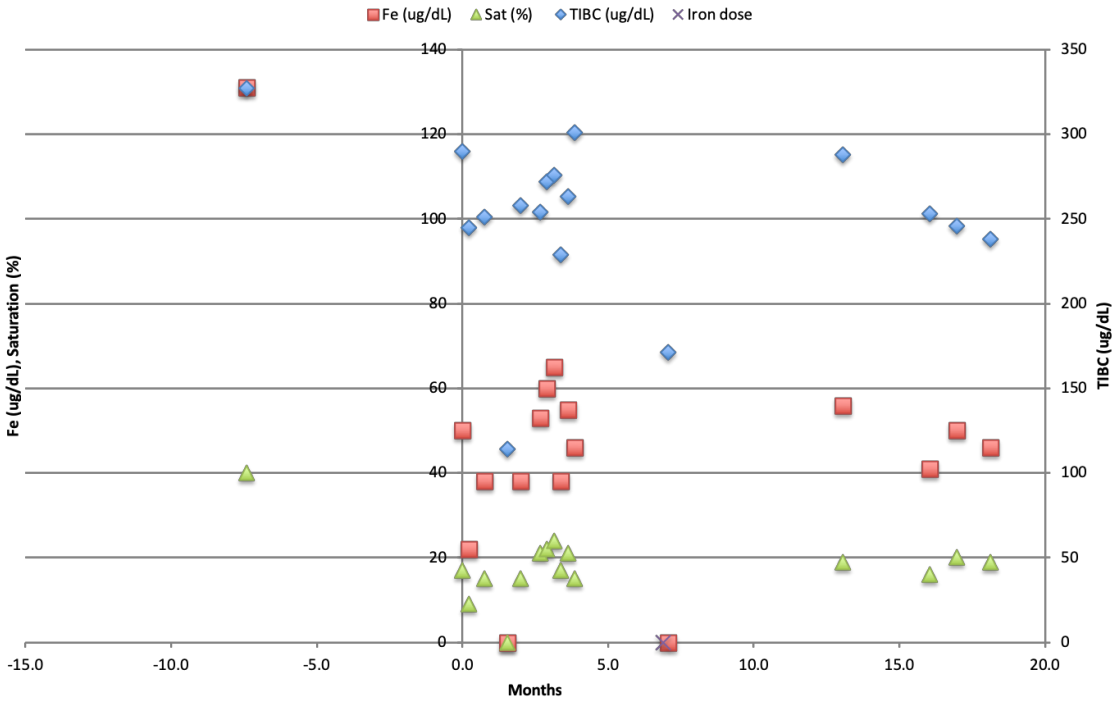
Figure 4: Effects of Iron Supplementation on Iron Profile



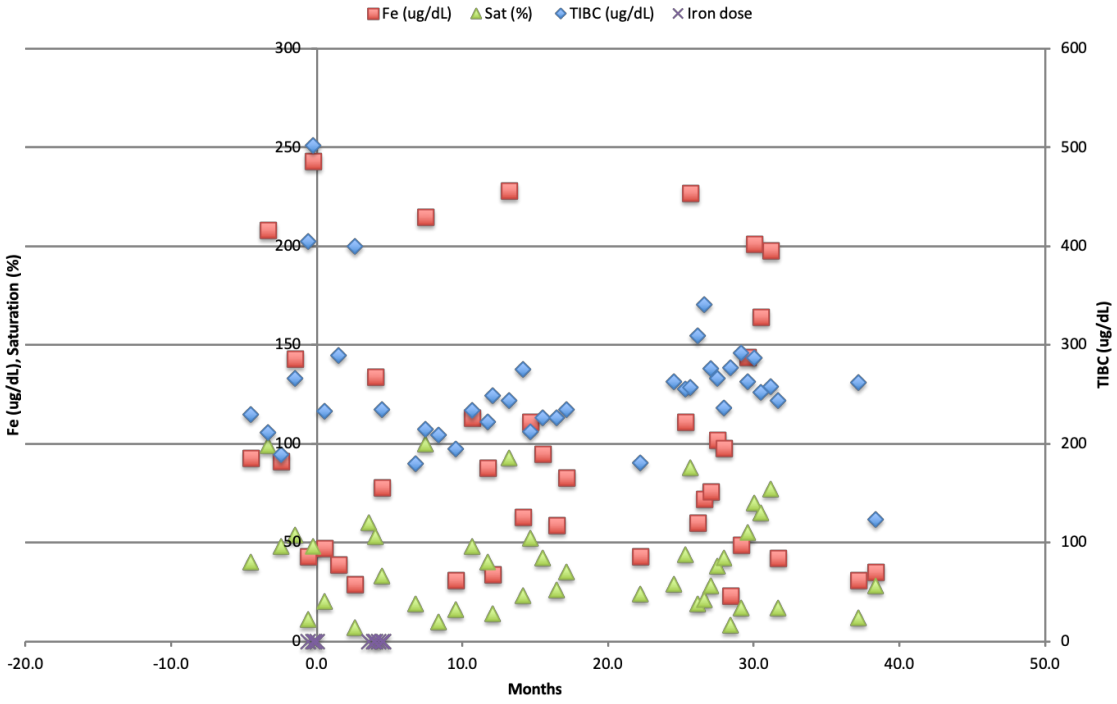
Case 3


Fe (ug/dL)
Sat (%)
TIBC (ug/dL)
Fe (ug/dL), Saturation (%)
TIBC (ug/dL)
Months
300
250
200
150
100
50
0
350
300
250
200
150
100
50
0
-20.0
0.0
20.0
40.0
60.0
80.0
100.0
120.0
140.0

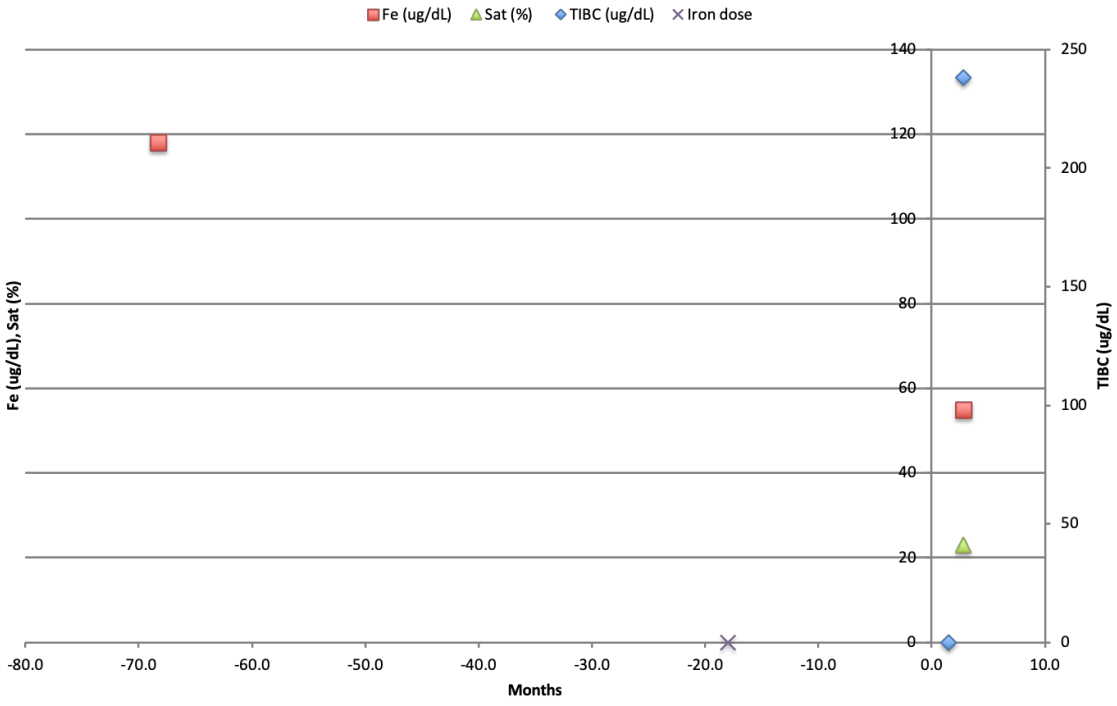
Case 4


Fe (ug/dL)
Sat (%)
TIBC (ug/dL)
Iron dose
Fe (ug/dL), Saturation (%)
TIBC (ug/dL)
Months
140
120
100
80
60
40
20
0
350
300
250
200
150
100
50
0
-15.0
-10.0
-5.0
0.0
5.0
10.0
15.0
20.0

Case 5


Fe (ug/dL)
Sat (%)
TIBC (ug/dL)
Iron dose
Fe (ug/dL), Saturation (%)
TIBC (ug/dL)
Months
300
250
200
150
100
50
0
600
500
400
300
200
100
0
-20.0
-10.0
0.0
10.0
20.0
30.0
40.0
50.0

Case 6


Fe (ug/dL)
Sat (%)
TIBC (ug/dL)
Iron dose
Fe (ug/dL), Sat (%)
TIBC (ug/dL)
Months
140
120
100
80
60
40
20
0
250
200
150
100
50
0
-80.0
-70.0
-60.0
-50.0
-40.0
-30.0
-20.0
-10.0
0.0
10.0

(e) Adverse events summary

Case 1 did not have any adverse events reported. Case 2 was reported to have angina pain relieved with nitroglycerin, this occurred 2 months after he had been off erythropoietin since his hgb had stabilized to around 12. High levels of hemoglobin induced by erythropoietin can have adverse effects in patients with CAD and other vascular disease, but such high levels were not achieved in this patient. An episode of pseudomonas peritonitis was also thought to be unrelated to treatment. Case 3 also developed peritonitis, a known complication of peritoneal dialysis. Anemia and PCT resolved after CRT. Case 4 was reported to have slow GI bleed, however it was unclear if this was related to erythropoietin therapy. We do not have any information regarding workup for her GI bleed. Case 5 had multiple skipped dialysis sessions and complications from medical non-adherence including alcohol related pancreatitis. Case 6 had CABG done within a month of starting on erythropoietin therapy and received 3 units transfusion while hospitalized for the CABG. It was not clear if she had prior comorbid cardiovascular conditions and had already been planning for the CABG at the time she was started on erythropoietin therapy. There were no reports of adverse events related to the initiation of erythropoietin on this patient.

Overall, erythropoietin therapy appears to be safe in all cases discussed. Two cases had reports of cardiovascular events (case 2 with angina and case 6 had CABG done), however these events were not directly linked to the use of erythropoietin.

DISCUSSION OF SPECIFIC AIM 2

Specific Aim 2 for this study was to develop at least provisional clinical guidelines to the use of erythropoietin for treatment of PCT associated with hemodialysis. Aspects of treatment that were intended to be included in such guidelines were (a) frequency of monitoring of plasma porphyrin and ferritin levels during treatment, (b) indications for erythropoietin dose adjustments, (c) indications for therapeutic phlebotomy and (d) indications for iron supplementation to prevent or treat an absolute or relative iron deficiency that may develop with erythropoietin treatment.

(a) Frequency of monitoring of plasma porphyrin and ferritin levels during treatment-Our study showed that ferritin drops precipitously after initiation of phlebotomy and epogen while plasma porphyrin drops more gradually and clinical symptoms does not seem to improve until at least 4 months after initiation of therapy, when plasma porphyrin is at 25% of pretreatment level. Therefore, ferritin would need to be monitor more frequently and at least earlier during treatment. Our protocol had patients ferritin monitored as frequent as every 3 days. This may be indicated initially due to the precipitous drop in ferritin level, however, in practice it is probably more feasible to monitor at least once weekly after initiation of therapy. Plasma porphyrin can be monitored at less regular interval, as the clinical response of skin improvement doesn't usually occur until at least 4 months of therapy.

(b) Indications for erythropoietin dose adjustments- Erythropoietin in our study is used to maintain hemoglobin/hematocrit levels sufficient to support phlebotomy. This is ideally

to maintain hemoglobin/hematocrit above 9.5/28.5%. Doses were adjusted on a case by case basis, usually by increments of 25U/kg of epogen, with maximal dose of up to 150 U/kg.

(c) Indications for therapeutic phlebotomy- Therapeutic phlebotomy in our study is used in combination with epogen to treat moderate or marked iron overload as indicated by (a) an initial ferritin in excess of 1000 ng/ml in a patient with anemia (that is due to erythropoietin deficiency) not previously treated with erythropoietin, (b) normal or increased ferritin during treatment with erythropoietin in a patient in whom anemia has responded to erythropoietin but in whom plasma porphyrins have not decreased or have leveled off after an initial decrease.

(d) indications for iron supplementation to prevent or treat an absolute or relative iron deficiency that may develop with erythropoietin treatment-
An iron supplement was permitted if iron saturation and ferritin were below normal or there is evidence of a poor response to erythropoietin and if there is evidence of a clear decrease in plasma porphyrins.

Chapter 10 Limitations

This study is limited by retrospective review of the data. As such there were missing information especially pertaining to pre and post treatment lab measures, and incomplete data recording of some cases including total phlebotomy quantities, transfusion amounts, and iron supplementation dosages. Patients were loss to follow up which limits our interpretation of clinical data such as PCT symptoms correlating with plasma porphyrin and ferritin levels. Additionally, patient non adherent limits our study, as in Case 5, for which multiple missed dialysis treatment sessions and hospitalizations for comorbid medical conditions limits our interpretation of treatment effectiveness. This study is also limited by a lack of randomization of treatment.

Chapter 11 Future Directions

Erythropoietin is demonstrated to decrease transfusion requirements in most but not all patients with PCT associated with ESRD. Future studies should include more rigorous collection of transfusion data during both control and treatment periods. Failure of erythropoietin treatment response (as noted with Case 4) should be further investigate. Hepcidin is a major regulator of iron absorption and/or mobilization. Future studies may investigate the role of hepcidin on anemia of ESRD and how this result in PCT refractory to erythropoietin and phlebotomy treatment.

Chapter 12 Conclusion

In this pilot crossover study of 6 patients with PCT associated with ESRD, we demonstrated that erythropoietin treatment helps with mobilizing iron overload in these

patients, improved anemia to allow for therapeutic phlebotomy, with subsequent improvement of PCT symptoms.

Clinical improvement may appear as early as 4 months after initiation of erythropoietin and therapeutic phlebotomy, when plasma porphyrin level falls to ~25% of initial level and continues to maintain remission of skin symptoms with porphyrin level falling to below 10% of initial level.

Erythropoietin therapy appears to be safe in all cases discussed. Two cases had reports of cardiovascular events, however these events were not directly linked to the use of erythropoietin.

In cases with treatment refractoriness, relative iron deficiency is a confounder, that can be treated with judicious iron supplementation, however despite this, one case was still refractory to treatment, which suggested additional factors contributing to treatment refractoriness.

Chapter 13 Supervision and Facilities

Dr. Karl Anderson was the primary supervising investigator. This study was conducted at the University of Texas Medical Branch with support from a grant from the FDA Office of Orphan Product Development and utilized resources provided by the UTMB General Clinical Research Center. Several patients were studied at other sites (University Kidney Center, Houston, TX; Clear Lake Kidney Center, Friendswood, TX; The Kidney Center, Boston, MA). UTMB Institutional Review Board approved the study, and written informed consent was obtained from all subjects.

Chapter 14 Human Subjects

Patients were potentially eligible for this study if they were 18 years of age or older, were receiving chronic dialysis therapy at a center that agreed to assist in sample and data collection, had well documented PCT (as defined earlier), and in the absence of treatment with erythropoietin, had either anemia or a transfusion requirement sufficient to contraindicate treatment by repeated phlebotomy. Anemia sufficient to contraindicate phlebotomies was defined either as hemoglobin below 9.5 mg/dl and not due to an identifiable or treatable cause other than end stage renal disease, or a higher hemoglobin level maintained by blood transfusions.

Exclusion criteria included known hypersensitivity to erythropoietin, human albumin or mammalian cell-derived products, uncontrolled hypertension, uncontrolled seizure disorder, significant hematologic disease other than anemia due to end stage renal disease, or any other condition that might increase the risk to the patients or decrease the chances of obtaining satisfactory data to achieve the objectives of the study.

References

1. Bunn HF. Erythropoietin. *Cold Spring Harbor perspectives in medicine.* 2013;3(3):a011619.
2. Kalantar-Zadeh K. History of Erythropoiesis-Stimulating Agents, the Development of Biosimilars, and the Future of Anemia Treatment in Nephrology. *American journal of nephrology.* 2017;45(3):235-247.
3. Phillips JD, Bergonia HA, Reilly CA, Franklin MR, Kushner JP. A porphomethene inhibitor of uroporphyrinogen decarboxylase causes porphyria cutanea tarda. *Proceedings of the National Academy of Sciences of the United States of America.* 2007;104(12):5079-5084.
4. Besur S, Hou W, Schmeltzer P, Bonkovsky HL. Clinically important features of porphyrin and heme metabolism and the porphyrias. *Metabolites.* 2014;4(4):977-1006.
5. Day RS, Eales L, Disler PB. Porphyrias and the kidney. *Nephron.* 1981;28(6):261-267.
6. Vyoral D, Petrak J. Hepcidin: a direct link between iron metabolism and immunity. *The international journal of biochemistry & cell biology.* 2005;37(9):1768-1773.
7. Barany P, Divino Filho JC, Bergstrom J. High C-reactive protein is a strong predictor of resistance to erythropoietin in hemodialysis patients. *American journal of kidney diseases : the official journal of the National Kidney Foundation.* 1997;29(4):565-568.
8. Shieh S, Cohen JL, Lim HW. Management of porphyria cutanea tarda in the setting of chronic renal failure: a case report and review. *Journal of the American Academy of Dermatology.* 2000;42(4):645-652.
9. Day RS, Eales L. Porphyrins in chronic renal failure. *Nephron.* 1980;26(2):90-95.
10. Gibson GE, McGinnity E, McGrath P, et al. Cutaneous abnormalities and metabolic disturbance of porphyrins in patients on maintenance haemodialysis. *Clinical and experimental dermatology.* 1997;22(3):124-127.
11. Adamson JW, Eschbach JW. Treatment of the anemia of chronic renal failure with recombinant human erythropoietin. *Annual review of medicine.* 1990;41:349-360.
12. Bonomini M, Del Vecchio L, Sirolli V, Locatelli F. New Treatment Approaches for the Anemia of CKD. *American journal of kidney diseases : the official journal of the National Kidney Foundation.* 2016;67(1):133-142.
13. Scholnick PL, Epstein J, Marver HS. The molecular basis of the action of chloroquine in porphyria cutanea tarda. *The Journal of investigative dermatology.* 1973;61(4):226-232.
14. Kostler E, Wollina U. Therapy of porphyria cutanea tarda. *Expert opinion on pharmacotherapy.* 2005;6(3):377-383.
15. Ryali ME, Whittier WL. Bullous skin lesions in a patient undergoing chronic hemodialysis. *Seminars in Dialysis.* 2010;23(1):83-87.
16. Albalate M, Farinas MC, Octavio JG, Perez JH, Goicoechea M, Caramelo C. Development of porphyria cutanea tarda in a hemodialysis patient after reactivation of hepatitis C virus infection. *Nephron.* 2001;88(2):170-173.

17. Kelly MA, O'Rourke KD. Treatment of porphyria cutanea tarda with phlebotomy in a patient on peritoneal dialysis. *Journal of the American Academy of Dermatology.* 2001;44(2 Suppl):336-338.
18. Lee PK, Abrahams I, Bickers DR. Porphyria cutanea tarda occurring in a patient with renal failure, systemic lupus erythematosus and chronic hepatitis C infection treated with hemodialysis. *Cutis.* 1999;64(4):237-239.
19. Poux JM, Demontis R, Cadranel JF, Ghazali A, Fievet P, Nordmann Y. Porphyria cutanea tarda in a dialyzed patient with hepatitis C virus infection: dramatic efficacy of small repeated phlebotomies. *The American journal of medicine.* 1997;103(2):163-164.
20. Peces R, Enriquez de Salamanca R, Fontanellas A, et al. Successful treatment of haemodialysis-related porphyria cutanea tarda with erythropoietin. *Nephrology Dialysis Transplantation.* 1994;9(4):433-435.
21. Sarkell B, Patterson JW. Treatment of porphyria cutanea tarda of end-stage renal disease with erythropoietin. *Journal of the American Academy of Dermatology.* 1993;29(3):499-500.
22. Stevens BR, Fleischer AB, Jr, Piering FF, Crosby DL. Porphyria cutanea tarda in the setting of renal failure: Response to renal transplantation. *Archives of Dermatology.* 1993;129(3):337-339.
23. Piazza V, Villa G, Galli F, et al. Erythropoietin as treatment of haemodialysis-related porphyria cutanea tarda. *Nephrology Dialysis Transplantation.* 1992;7(5):438-442.
24. Yaqoob M, Smyth J, Ahmad R, et al. Haemodialysis-related porphyria cutanea tarda and treatment by recombinant human erythropoietin. *Nephron.* 1992;60(4):428-431.
25. Anderson KE, Goeger DE, Carson RW, Lee SM, Stead RB. Erythropoietin for the treatment of porphyria cutanea tarda in a patient on long-term hemodialysis.[Erratum appears in N Engl J Med 1990 May 31;322(22):1616]. *New England Journal of Medicine.* 1990;322(5):315-317.
26. Toriyama T, Kawahara H. [Small volume plasma exchange therapy in patients with porphyria cutanea tarda on maintenance hemodialysis]. *Nihon rinsho Japanese journal of clinical medicine.* 1992;50 Suppl:423-428.
27. Ruggian JC, Fishbane S, Demento FJ, Maesaka JK, Frei GL. Porphyria cutanea tarda in a patient on chronic ambulatory peritoneal dialysis. *Journal of the American Society of Nephrology : JASN.* 1996;7(3):397-402.
28. Riccioni N, Donati G, Soldani S, Scatena P, Arcabasso GD. Treatment of hemodialysis-related porphyria cutanea tarda with small repeated phlebotomies. *Nephron.* 1987;46(2):125-127.
29. Poh-Fitzpatrick MB, Lamola AA. Direct spectrofluorometry of diluted erythrocytes and plasma: a rapid diagnostic method in primary and secondary porphyrinemias. *The Journal of laboratory and clinical medicine.* 1976;87(2):362-370.
30. Singal AK et al. Low-dose hydroxychloroquine is as effective as phlebotomy in treatment of patients with porphyria cutanea tarda. Clin Gastroenterol Hepatol. 2012 Dec;10(12):1402-9.

Vita (Heading 2, style: TOC 2)

<The vita is a brief biographical sketch of the writer. It should include the writer's date and place of birth; name of parents; schools and colleges attended; degrees awarded; publications and experience in teaching, business, and industry; military service, if any; and a permanent address.>

Permanent address: <nn Street Name, City, State Zip>

This dissertation was typed by <the author, or typist's name>.