Clinical Research Protocol

EQUIVALENCE OF TRIFERIC® (FERRIC PYROPHOSPHATE CITRATE) ADMINISTERED VIA HEMODIALYSATE AND INTRAVENOUSLY TO ADULT CKD-5HD PATIENTS

IND Number:	51,290
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Protocol Number:	RMFPC-20
Version:	1.0
Clinical Phase:	1/2
Investigational Drug:	Triferic® (Ferric Pyrophosphate Citrate)
Indication:	Maintenance of Iron and Hemoglobin in CKD-5HD Patients
Sponsor Signatory:	Raymond D. Pratt, MD FACP Chief Medical Officer Rockwell Medical, Inc.
Investigator:	
Original Protocol Date:	September 18, 2017

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LIST OF ABBREVIATIONS

AE	Adverse Event
ANCOVA	Analysis of Covariance
AUC	Area Under the Curve
CFR	Code of Federal Regulations
CIOMS	Council for International Organizations of Medical Science
CKD-5HD	Dialysis Dependent Chronic Kidney Disease
CLIA	Clinical Laboratory Improvement Amendments
Cmax	Maximum Serum Conentration
CMP	Clinical Monitoring Plan
CMS	Centers for Medicare and Medicaid Services
CRF	Case Report Form
CRO	Contract Research Organization
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DSMB	Data Safety Monitoring Board
eCRF	Electronic Case Report Forms
ESA	Erythropoiesis Stimulating Agent
FDA	Food and Drug Administration
FFR	Federal Financial Report
FPC	Ferric Pyrophosphate Citrate
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
HD	Hemodialysis
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
ICH E6	International Conference on Harmonisation Guidance for Industry, Good Clinical Practice: Consolidated Guidance
IDE	Investigational Device Exemption
IND	Investigational New Drug Application
IRB	Investigational Review Board
ISO	International Organization for Standardization
LSMEAN	Least-squares Means
MedDRA	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
MSDS	Material Safety Data Sheet
OHRP	Office for Human Research Protections
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SMC	Safety Monitoring Committee
SOC	System Organ Class
SOP	Standard Operating Procedure
UP	Unanticipated Problem
US	United States

PROTOCOL APPROVAL PAGE

Study Title:	Equivalence of Triferic® (Ferric Pyrophosphate Citrate) Administered via Hemodialysate and Intravenously to Adult CKD-5HD Patients
Protocol Number:	RMFPC-20
Version:	1.0
Date of Issue:	September 18, 2017
Sponsor Name and Address:	Rockwell Medical, Inc. 30142 S. Wixom Rd Wixom, MI 48393

I, the undersigned, have read and approve this protocol and agree on its content. It is confirmed that the information and guidance given in this protocol complies with scientific principles, the guidelines of Good Clinical Practice, the Declaration of Helsinki in the latest relevant version, and the applicable legal and regulatory requirements.

Sponsor Signatory:

Raymond D. Pratt, MD FACP
Chief Medical Officer

(Date)

INVESTIGATOR PROTOCOL AGREEMENT

Protocol Title: Equivalence of Triferic® (Ferric Pyrophosphate Citrate) Administered via Hemodialysate and Intravenously to Adult CKD-5HD Patients

Protocol Number: RMFPC-20 Version: 1.0

By my signature, I

- Confirm that my staff and I have carefully read and understand this protocol or protocol amendment and are thoroughly familiar with the appropriate use of the investigational drug described herein.
- Agree to comply with the conduct and terms of the study specified herein and with any other study conduct procedures provided by the Sponsor, Rockwell Medical.
- Agree to assume responsibility for the proper conduct of the study at this site, including complying with US Food and Drug Administration (FDA) regulations, the International Conference on Harmonisation (ICH) GCP guidelines, the Declaration of Helsinki, and all applicable rules, regulations, and federal, state, and local laws relating to the conduct of clinical studies and the protection of human subjects.
- Agree not to implement deviations from or changes to the protocol or protocol amendments without agreement from the Sponsor and prior submission to and written approval (where required) from the Institutional Review Board (IRB) or Ethics Committee (EC), except when necessary to eliminate an immediate hazard to the subjects, or for administrative aspects of the study (where permitted by all applicable regulatory requirements).
- Agree to onsite monitoring of the case report forms (CRFs) and source documents by Rockwell Medical or designee and to onsite inspection of CRFs and source documents by appropriate regulatory authorities, including but not limited to the FDA, local governing regulatory bodies, and IRB/EC inspectors.

Investigator's Signature Date

Print Name

PROTOCOL SUMMARY

Title: Equivalence of Triferic® (Ferric Pyrophosphate Citrate) Administered via Hemodialysate and Intravenously to Adult CKD-5HD Patients

Précis: An open-label four period, randomized, crossover study of Triferic iron administered via hemodialysis compared to Triferic administered intravenously pre- and post- hemodialyzer.

Objectives:

Primary Objective

1. To establish the equivalence of Triferic iron administered via dialysate (reference) to:
   - Triferic (6.5 mg) administered into the arterial blood line
   - Triferic (6.5 mg) administered into the venous blood line

Secondary Objectives

The secondary objective is:

- To explore the safety of IV Triferic administration in adult CKD-5HD patients

Number of Subjects 30

Endpoint Primary:

- Maximum observed plasma iron concentration (C_{max} Fe_{total})
- Area under plasma concentration-time curve ($AUC_{0\text{-}last}$ Fe_{total})
- Maximum observed transferrin bound plasma iron concentration (C_{max} $Fe_{TBI\ Calc}$)
- Area under plasma transferrin bound plasma iron concentration-time curve ($AUC_{0\text{-}last}$ $Fe_{TBI\ Calc}$)

Population: Stable, iron-replete adults with CKD-5HD

Phase: 1/2

Number of Sites enrolling participants: 1

Description of Study Agent : Triferic 4.5 mL ampules with 6.75 mg iron (1.5 mg Fe/mL) for IV use

Triferic 5 mL ampules with 27.2 mg Fe for addition to bicarbonate concentrate for hemodialysis

Study Duration: Approximately 3 months

Participant Duration: Up to 7 weeks including Screening, 4 hemodialysis sessions over 2 weeks (Treatment Period), and Follow-up

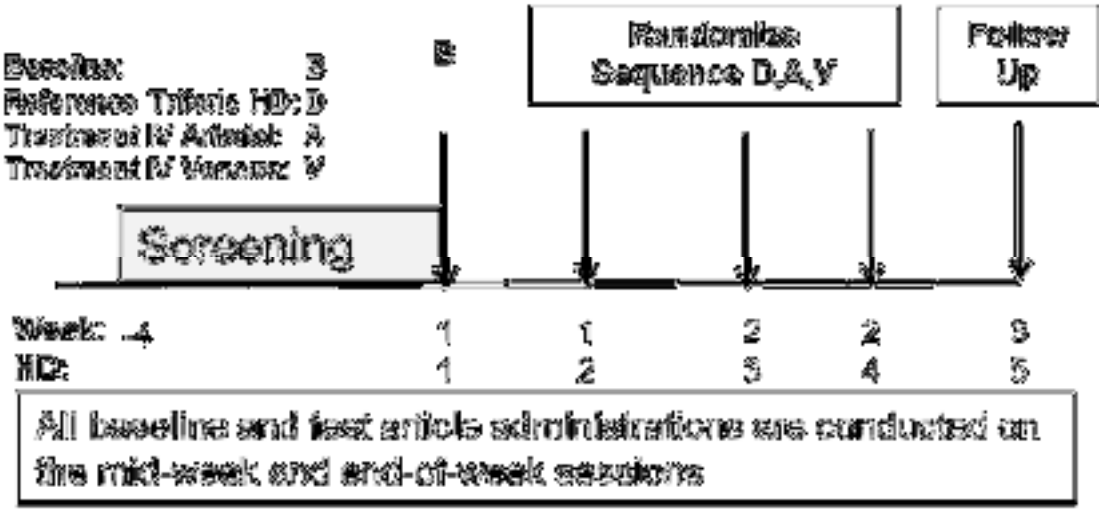
SCHEMATIC OF STUDY DESIGN

The study design is outlined in the figure below. A screening period of up to 4 weeks is included to allow for establishment of steady state plasma iron concentrations after withdrawing all oral and IV iron products. All baseline and test article administrations are conducted at the mid-week and end week HD session to avoid a preceding interdialytic interval of more than 2 days since this can result in greater fluid accumulation and hemodilution.

The study consists of 4 HD sessions . The study is an open-label, four period, three treatment, randomized crossover design study. Prior to the start the randomized treatment sequence, there is a baseline (no active treatment) session.

HD #1 will be standardized for all patients to collect basline serum iron concentrations (B). HD# 2, 3, and 4 will receive Triferic administered via dialysate (D), Triferic administered into the arterial (pre-dialyzer) blood line (A) or Triferic administered into the venous (post-dialyzer) blood line (V) in a randomized sequence.

Figure 1: Study Schematic



1 KEY ROLES

Sponsor Medical Monitor	Raymond D Pratt, MD FACP Chief Medical Officer Rockwell Medical Inc. 30142 S Wixom Rd Wixom MI 48393 USA Tel: 248 960 9009
Investigator(s)	TBD

2 INTRODUCTION: BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

2.1 BACKGROUND INFORMATION

The goals of iron therapy in adult patients with chronic kidney disease receiving chronic hemodialysis (CKD-5HD) are to avoid depletion of iron stores, prevent iron-restricted erythropoiesis and maintain hemoglobin levels while minimizing erythropoiesis-stimulating agent (ESA) therapy and avoiding blood transfusions that may sensitize patients and limit chances for a kidney transplant[1].

Iron supplementation is provided to patients receiving maintenance hemodialysis (HD) with intravenous (IV) iron. However, these IV iron products are ferric-hydroxide cores within a carbohydrate shell that do not donate iron directly to transferrin. Instead, the complexes must first be taken up by reticuloendothelial (RE) macrophages that free the iron from the carbohydrate shell for subsequent export via ferroportin. As a result, a considerable portion of the iron derived from these iron-carbohydrate complexes is sequestered within macrophages and is not readily available for transport to the erythroid marrow for use in hemoglobin synthesis. The use of IV iron, which is indicated for repletion of iron stores in iron deficiency anemia, is primarily used as a maintenance therapy in patients receiving HD. As a consequence, iron stores in adult patients, as measured by serum ferritin levels, have increased from 200 µg/L in the 1990s to almost 800 µg/L in 2013[5]. These changes in the management of anemia have led to concerns that increased use of IV iron may produce iron overload and contribute to inflammation, oxidative stress, endothelial dysfunction, cardiovascular disease, immune deficiency, and the risk of bacterial infections[2].

The guidelines for anemia management in CKD-5HD patients recommend iron repletion to maintain TSAT >20% and ferritin >100 µg/L. Ferritin levels should not be intentionally targeted to exceed 500 µg/L. It is expected that the majority of HD patients have a functional iron deficiency, which results from sequestration of iron in the reticuloendothelial system (RES) as a consequence of elevated hepcidin.

There are few studies that have examined chronic or maintenance IV iron therapy. In such studies, typically 1 to 2 mg/kg/week of elemental IV iron has been provided to keep the TSAT between 20 and 50% with serum ferritin levels between 100 and 800 µg/L. Because of the elevated hepcidin levels in CKD-5HD patients, the majority of administered IV iron ends up in RES storage sites and only a small proportion is used for erythropoiesis.

Triferic is approved in the US as a maintenance iron therapy. Triferic administered parenterally does not require processing by macrophages, and it donates iron directly to transferrin for optimal utilization in erythropoiesis, avoiding sequestration within RE macrophages. In adults, Triferic, administered at each HD session via dialysate, has been shown to maintain hemoglobin concentrations without increasing iron stores. Triferic does this by delivering 5-7 mg of elemental iron with each HD session, the amount typically lost as a consequence of retained blood in the dialyzer circuit plus other HD- and uremia-associated blood losses. The safety profile of Triferic in controlled clinical studies is similar to that of patients receiving placebo. There were few related adverse events (AEs) and no anaphylaxis in over 100,000 individual patient administrations. Safety data are included in the approved US package insert and in the Investigator's Brochure. Triferic added to the liquid bicarbonate concentrate for hemodialysis has been approved as the first maintenance iron therapy in adult CKD-5HD patients.

The goal of this study is to establish the equivalence of a new presentation of Triferic (6.75 mg iron in 4.5 mL WFI) administered into the pre-dialyzer and post dialyzer blood lines by use of the on machine infusion pump. This new presentation will allow patients who are dialyzed on machines that use solid bicarbonate bags or cartridges to benefit from Triferic iron replacement therapy.

2.2 RATIONALE

Hemodialysis machines use a 3 stream process to combine acid concentrate containing Na, K, Cl, Ca, magnesium and buffer (acetic or citric acid) with bicarbonate and water to produce the final hemodialysate that is circulated through the hemodialyzer to remove uremic toxins and replace electrolytes in patients with chronic renal failure. In the USA, most dialysis centers mix the acid concentrate and bicarbonate concentrate in large quantities to be plumbed to individual machines where the concentrates are combined with reverse osmosis pure water to produce the final dialysate.

Triferic (ferric pyrophosphate citrate; FPC) is a water-soluble, mixed-ligand iron salt in which iron(III) is bound to pyrophosphate and citrate. It has a molecular formula of $Fe_4(C_6H_4O_7)_3(H_2P_2O_7)_2(P_2O_7)$ and a relative molecular weight of approximately 1313 Da. Triferic solution is a clear, slightly yellow-green sterile solution containing 27.2 mg iron(III) per 5 mL (5.44 mg iron(III) per mL) filled in a 5-mL low density polyethylene (LDPE) ampule. One ampule added to 2.5 gallons of bicarbonate concentrate provides a final hemodialysate concentration of 2 μM.

Dialysis units that do not mix acid and bicarbonate on site depend on individual acid concentrate containers and use a system of solid bicarbonate cartrdges (or bags) to produce the saturated bicarbonate concentrate on-line. Patients in these units (pediatrics, small hospital units and most centers outside the USA) cannot receive Triferic because it cannot be added to the solid bicarbonate.

To address the needs of this patient group, a new presentation of Triferic has been developed to allow slow administration of Triferic directly into the blood. A preliminary study (RMFPC-16) demonstrated the feasibility of this method and demonstrated that approximately 6.5 mg of Triferic iron is administered at each HD treatment. The new Triferic presentation contains 6.75 mg Triferic iron in 4.5 mL water for injection (1.5 mg Fe/mL), packaged in blowfill sealed LDPE leur lock ampules. Each ampule will provide a single treatment. The contents of the ampule will be drawn up into a syringe and connected to the arterial (pre-dialyzer) or venous (venous drip chamber post-dialyzer) lines via existing tubing. The pump on the dialysis machine is programmed to deliver the 4.5 mL contents of the vial over 3 hours to provide the equivalent amount of iron as transferred during a single HD treatment. Based on a residual volume of approximately 0.15 mL left in the delivery line upon completion of the IV infusion, each patient will receive 6.5 mg of Triferic.

The goal of this study is to establish equivalence between the hemodialysate administered Triferic iron and Triferic administered via direct IV pre-dialyzer or post-dialyzer.

2.3 Potential Risks and Benefits

2.3.1 KNOWN POTENTIAL RISKS

Triferic is generally well-tolerated. In controlled clinical studies, the safety profile is similar to patients receiving placebo treatment. The most common adverse reactions leading to treatment discontinuation included headache, asthenia, dizziness, constipation, nausea, hypersensitivity reactions, intradialytic hypotension, pruritus, and pyrexia. Adverse reactions reported in the treatment extension periods were similar to those observed in the randomized clinical studies.

Serious hypersensitivity reactions, including anaphylactic-type reactions, some of which have been life-threatening and fatal, have been reported in patients receiving parenteral iron products. Patients may present with shock, clinically significant hypotension, loss of consciousness, and/or collapse. Patients receiving iron should be monitored for signs and symptoms of hypersensitivity during and after hemodialysis until clinically stable.

Personnel and therapies should be immediately available for the treatment of serious hypersensitivity reactions.

Hypersensitivity reactions have been reported in 1 (0.3%) of 292 patients receiving Triferic in two randomized clinical trials.

2.3.2 KNOWN POTENTIAL BENEFITS

Triferic administered at each dialysis treatment can maintain hemoglobin concentrations without increasing body iron stores as measured by serum ferritin concentrations[3]. In one clinical study, patients receiving Triferic over 9 months required 35% less erythropoiesis stimulating agents (ESA) than patients receiving placebo[4].

3 OBJECTIVES AND PURPOSE

Primary Objective

2. To establish the equivalence of Triferic iron administered via dialysate (reference) to:
    - Triferic (6.5 mg) administered into the arterial blood line
    - Triferic (6.5 mg) administered into the venous blood line

Secondary Objectives

The secondary objective is:

- To explore the safety of IV Triferic administration in adult CKD-5HD patients

4 STUDY DESIGN AND ENDPOINTS

4.1 DESCRIPTION OF THE STUDY DESIGN

An open-label, four period, randomized, crossover study of Triferic iron administered via hemodialysis compared to Triferic administered intravenously pre- and post- hemodialyzer. (Figure 2).

Total duration of up to 7 weeks including Screening, Treatment Period, and Follow-up.

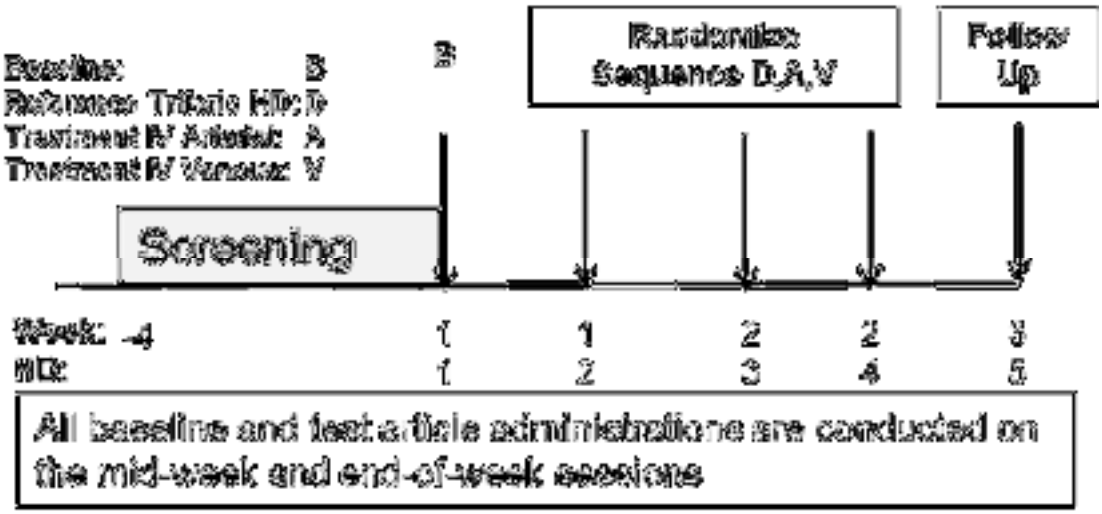
The study consists of 4 HD sessions . Prior to the start of the randomized treatments, there is a baseline (no active treatment) session.

HD #1 will be standardized for all patients to collect basline plasma iron concentrations (B). HD# 2, 3, and 4 will receive Triferic administered via dialysate (D), Triferic administered into the arterial (pre-dialyzer) blood line (A) or Triferic administered into the venous (post-dialyzer) blood line (V) in a randomized sequence. Treatments will be randomized according to a prepared schedule:

- Each patient will have their baseline diurnal variation of iron assessed during one HD treatments (HD #1).
- Each patient will receive Triferic administered via hemodialysate 2 µM (110 µg Fe/L) over 4 hrs at one hemodialysis session.
- Each patient will receive one 6.5-mg dose of Triferic administered IV over 3 hrs during hemodialysis via the unused heparin infusion line (pre-dialyzer).
- Each patient will receive one 6.5-mg dose of Triferic administered IV over 3 hrs during hemodialysis via infusion into the venous drip chamber (post-dialyzer).

Patients will undergo a follow-up visit within 7 days after their last dose of study drug.

Figure 2: Study Design



4.2.1 PRIMARY ENDPOINT

The primary endpoints for the study are:

- Maximum observed plasma iron concentration (C_{max} Fe_{total})
- Area under plasma concentration-time curve ($AUC_{0\text{-}last}$ Fe_{total})
- Maximum observed transferrin bound plasma iron concentration (C_{max} $Fe_{TBI\ Calc}$)
- Area under plasma transferrin bound plasma iron concentration-time curve ($AUC_{0\text{-}last}$ $Fe_{TBI\ Calc}$)

The primary equivalence analysis will be performed on the total plasma iron and transferrin bound iron (calculated), with the dialysate administered Triferic as the reference treatment and each of the IV administrations as test treatments. Non-compartmental PK parameters will be estimated for total plasma iron.

- Pharmacokinetic parameters will be determined using all appropriate available data for total plasma iron.

- The key PK parameters listed below will be calculated for plasma iron as data permit and as appropriate. Additional details of the PK analysis will be provided in the PK analysis plan. Exploratory modeling of the data may be utilized to refine parameter estimates.

C_{max}	The maximum drug concentration in plasma determined directly from individual concentration-time data
T_{max}	The observed time to reach maximum concentration
$AUC_{(0\text{-}t)}$	The area under the plasma concentration-time curve from time zero to the time of the last quantified concentration, calculated using the linear-up/log-down trapezoidal rule
$AUC_{(0\text{-}end)}$	The area under the plasma concentration-time curve from time zero to the end of each HD, calculated using the linear-up/log-down trapezoidal rule
λz	The terminal phase rate constant, estimated by linear regression through the terminal phase of the log concentration-time profile
$t_{½}$	The terminal phase half-life, calculated as: $t½ = \frac{\ln(2)}{\lambda z}$
CL	Total systemic clearance, calculated as: Total Dose/AUC_{inf}

4.2.2 SECONDARY ENDPOINTS

- Baseline corrected maximum observed plasma iron concentration (C_{max} $Fe_{total\ Corr}$)
- Area under the baseline corrected plasma concentration-time curve ($AUC_{0\text{-}last}$ $Fe_{total\ Corr}$)
- Baseline corrected maximum observed transferrin bound plasma iron concentration (C_{max} $Fe_{TBI\ Calc\ Corr}$)
- Area under the baseline corrected plasma transferrin bound plasma iron concentration-time curve ($AUC_{0\text{-}t}$ $Fe_{TBI\ Calc\ Corr}$)

4.2.3 EXPLORATORY ENDPOINTS

- Serum iron profile (total serum iron, TIBC, and TSAT) over time.

5 STUDY ENROLLMENT AND WITHDRAWAL

5.1 PARTICIPANT INCLUSION CRITERIA

Patients must meet all of the following criteria to be eligible for inclusion in the study:

1. The patient must be able to provide informed consent and have personally signed and dated the written informed consent document before completing any study-related procedures.
2. The patient must be 18-80 years of age inclusive at the time of consent.
3. The patient must have been undergoing chronic hemodialysis for chronic kidney disease for at least 3 months, and be expected to remain on hemodialysis and be able to complete the study.
4. The patient must have a Screening ferritin level of ≥100µg/L.

5. The patient must have a Screening transferrin saturation (TSAT) of 15-45%, inclusive.
6. The patient must have a Screening hemoglobin (Hgb) concentration ≥9.0 g/dL.
7. The patient must be undergoing hemodialysis at least 3x/week.
8. The patient must have at least a minimally adequate measured dialysis dose defined as single-pool Kt/V (dialyzer clearance of urea multiplied by dialysis time, divided by patient's total body water) ≥1.2, or $K_{ID}t/V$ (online dialyzer clearance measured using ionic dialysance multiplied by dialysis time, divided by patient's total body water) ≥1.2 measured within the 90 days prior to HD #1.
9. Patient is receiving, or can receive anticoagulation for dialysis by a single dose of unfractionated heparin or low molecular weight heparin pre-dialysis; or by intermittent IV heparin bolus.
10. The patient's vascular access for dialysis that will be used during the study must have stable function in the judgment of the Investigator.
11. The patient must agree to discontinue all iron preparations (oral and IV) for 14 days prior to the start of HD#1 and throughout the study.
12. Female patients must not be pregnant or breastfeeding. They must have been amenorrheic for the past year or be surgically sterile or agree to not become pregnant by continuous use of an effective birth control method acceptable to the Investigator for the duration of their participation in the study.

5.2 PARTICIPANT EXCLUSION CRITERIA

A patient will <u>not</u> be eligible for inclusion in the study if <u>any</u> of the following criteria apply:

1. The patient has had an RBC or whole blood transfusion within 4 weeks prior to Screening.
2. The patient requires a continuous infusion of heparin during standard hemodialysis.
3. The patient has had administration of IV or oral iron supplements (including multivitamins with iron or iron based phosphate binders) within 14 days prior to the start of HD #1. (The patient may subsequently become eligible if additional time elapses and all other eligibility criteria are met.).
4. The patient has known active bleeding from any site other than AV fistula or graft (e.g., gastrointestinal, hemorrhoidal, nasal, pulmonary, etc.).
5. The patient has a living kidney donor identified or living-donor kidney transplant scheduled to occur during study participation. (Note: Patients awaiting deceased-donor transplant need not be excluded.)
6. The patient is scheduled to have a surgical procedure during the study.
7. The patient has had a hospitalization within the 4 weeks prior to Screening (except for vascular access surgery) that, in the opinion of the Investigator, confers a significant risk of hospitalization during the course of the study.
8. The patient has a history of noncompliance with the dialysis regimen in the opinion of the Investigator.
9. The patient has a known ongoing active inflammatory disorder (other than CKD), such as systemic lupus erythematosus, rheumatoid arthritis, or other collagen-vascular disease, that currently requires systemic anti-inflammatory or immunomodulatory therapy.

10. The patient has any febrile illness within one week prior to the start of HD #1 (e.g., oral temperature ≥100.4°F, 38.0°C). (The patient may subsequently become eligible at least 1 week after resolution of the illness.)
11. The patient has known bacterial, tuberculosis, fungal, viral, or parasitic infection requiring anti-microbial therapy or anticipated to require anti-microbial therapy during the patient's participation in this study.
12. The patient is known to be positive for HIV, hepatitis B, or hepatitis C (viral testing is not required as part of this protocol).
13. The patient has cirrhosis of the liver based on histological criteria or clinical criteria (e.g., presence of ascites, esophageal varices, multiple spider nevi, or history of hepatic encephalopathy).
14. The patient has ALT and/or AST levels consistently greater than twice the upper limit of normal at any time during the two months prior to the start of HD #1.
15. The patient currently has any malignancy other than basal or squamous cell skin cancer.
16. The patient has a history of drug or alcohol abuse within the 6 months prior to Screening.
17. The patient dosed in an investigational drug study within 30 days prior to the start of HD# 1.
18. The patient has any condition that, in the opinion of the Investigator, would make it unlikely for the patient to complete the study.

5.3 STRATEGIES FOR RECRUITMENT AND RETENTION

Patients will be recruited from local hemodialysis clinics. The study will be conducted on an outpatient basis. Patients will be compensated for participation according to local policies.

5.4 PARTICIPANT WITHDRAWAL OR TERMINATION

5.4.1 REASONS FOR WITHDRAWAL OR TERMINATION

A patient may be discontinued from the study for the following medical or administrative reasons:

- Occurrence of a treatment emergent adverse event (TEAE) that represents an unacceptable risk to the patient and when continued participation in the investigational study is not warranted, in the judgment of the Investigator. The Investigator must follow the patient until the TEAE resolves or satisfactorily stabilizes;
- Pregnancy;
- Initiation of a prohibited concomitant therapy without prior approval by the Investigator and or Sponsor; and/or
- Patient request.
- The Investigator may discontinue individual patients from the study at any time. Patients may voluntarily withdraw at any time. If possible, patients who are withdrawn should complete a Follow-up visit.

5.4.2 HANDLING OF PARTICIPANT WITHDRAWALS OR TERMINATION

Patients who withdraw or are withdrawn after enrollment may be replaced after consultation with the Sponsor. Patients who withdraw or are withdrawn prior to enrollment will be considered screen failures and will be replaced.

5.5 PREMATURE TERMINATION OR SUSPENSION OF STUDY

The study may be terminated by the Sponsor at any time. Patients will receive compensation for the number of sessions completed.

The study may be suspended for safety reviews at the discretion of the Sponsor or at the request of FDA. Once appropriate reviews are completed, the study may be resumed after appropriate protocol amendments or terminated.

6 STUDY AGENT

Triferic will be administered by qualified study personnel only in accordance with the procedures described in this protocol.

6.1 STUDY AGENT(S) AND CONTROL DESCRIPTION

6.1.1 ACQUISITION

Triferic 4.5 mL ampules with 6.75 mg iron (1.5 mg Fe/mL) for IV use will be provided by Rockwell Medical Inc.

Triferic 5 mL ampules with 27.2 mg Fe for addition to bicarbonate concentrate for hemodialysis will be provided by Rockwell Medical Inc.

All Triferic ampules are to be kept protected from light in the provided packages until use

6.1.2 FORMULATION, APPEARANCE, PACKAGING, AND LABELING

6.1.2.1 IDENTITY OF INVESTIGATIONAL PRODUCTS(S)

Triferic for addition to bicarbonate concentrate is supplied as sterile 5-mL ampules containing 5.44 mg/mL of iron in water for injection (WFI). Each 5 mL ampule contains 27.2 mg of Triferic iron.

Triferic for IV administration is supplied as sterile 4.5 mL ampules containing 1.5 mg Triferic iron/mL in WFI. Each ampule contains 6.75 mg Triferic iron in 4.5 mL.

6.1.2.2 LABELING

Study drug packaging will bear a label that meets applicable laws for an investigational drug, which includes, but is not limited to, the following information:

- Federal law statement
- Protocol number
- Lot number
- Storage information

6.1.3 PRODUCT STORAGE AND STABILITY

All study drug will be kept in a secured area with limited access.

Triferic ampules will be stored protected from light at controlled room temperature (20° to 25°C [68° to 77°F]; excursions will be permitted to 15° to 30°C [59° to 86°F] [See USP Controlled Room Temperature]).

6.1.4 PREPARATION

Triferic dosing solutions may be prepared up to 24 hrs prior to planned infusion start times.

6.1.4.1 TRIFERIC 2 µM VIA HD DOSING SOLUTION PREPARATION (TREATMENT D)

- For each patient, prepare the Triferic HD dosing solution by adding 5-mL of Triferic solution (5.44 mg iron/mL) to 2.5 gallons of bicarbonate concentrate or 2 mL/gallon of premixed 45X liquid bicarbonate concentrate.
- When delivered via a 45X hemodialysis machine, the concentration of Triferic in the final hemodialysate is 2 μM (110 μg Fe/L).
- Retain two (2) 5-mL aliquots of each patient's bicarbonate concentrate (after addition of Triferic) in polypropylene screw-cap tubes and store at approximately 4°C for possible dosing solution analysis.
- Administer the Triferic bicarbonate concentrate to each patient via hemodialysis for 4 hrs, with the Triferic bicarbonate concentrate replacing the patient's usual bicarbonate concentrate.
- Keep all Triferic solutions protected from light until use.

6.1.4.2 TRIFERIC 6.5 MG IV DOSING SOLUTION PREPARATION FOR ADMINISTRATION PRE-DIALYZER(TREATMENT A)

- The final volume of Triferic to be administered is 4.5 mL via the syringe pump on the dialysis machine. Based on a residual volume of approximately 0.15 mL left in the delivery line upon completion of the IV infusion, each patient will receive 6.5 mg of Triferic.
- The administration rate is 1.5 mL/hour for 3 hours.
- Prepare the solution by withdrawing 4.5 mL Triferic IV solution in a 10 mL syringe.
- Inject a small amount of the solution into the ***heparin*** line in order to displace the saline present in the line prior to initiating blood flow. (See Appendix 5)
- Mount the syringe on the heparin pump.
- Start infusion and administer over first 3 hours of the 4 hour HD session.
- Keep all Triferic solutions protected from light until use.

6.1.4.3 TRIFERIC 6.5 MG IV DOSING SOLUTION PREPARATION FOR ADMINISTRATION POST-DIALYZER (TREATMENT V)

- The final volume of Triferic to be administered is 4.5 mL via the syringe pump on the dialysis machine to the venous drip chamber. Based on a residual volume of approximately 0.15 mL left in the delivery line upon completion of the IV infusion, each patient will receive 6.5 mg of Triferic.
- The administration rate is 1.5 mL/hour for 3 hours.
- Prepare the solution by withdrawing 4.5 mL Triferic IV solution in a 10 mL syringe.
- Inject 0.5 mL of the solution into the connecting tubing in order to displace the air present in the line prior to initiating blood flow. (See Appendix 5)
- Mount the syringe on the heparin pump.
- Start infusion and administer over 3 hours of the 4 hour HD session.
- Keep all Triferic solutions protected from light until use.

6.1.5 DOSING AND ADMINISTRATION

This is a randomized open label treatment study. Patients and site personnel will be aware of each treatment administered.

The rationale for the doses chosen in this study has been presented in Section 1.5. Briefly, Triferic 2 μM (Treatment D) is the approved dose in adult CKD-5HD patients.

Triferic 6.5 mg over 3 hr (Treatment A and V doses) are the average amount of Triferic received from dialysate by adult CKD-5HD patients during a single 4-hour dialysis session.

6.1.6 ROUTE OF ADMINISTRATION

Triferic will be administered parenterally.

6.1.7 STARTING DOSE AND DOSE ESCALATION SCHEDULE

Each treatment consists of a single defined dose.

6.1.8 SELECTION AND TIMING OF DOSE FOR EACH PATIENT

Each treatment will be administered starting at approximately 8 AM on the applicable study day for each patient.

All start and stop times will be captured on the case report form (CRF).

6.1.9 DURATION OF THERAPY

Each treatment will be administered during a single 4 hour hemodialysis. Patients will remain in the clinic for an additional 8 hours to complete all required blood sampling.

6.1.10.4 TREATMENT COMPLIANCE

Because this is a parenteral administration study, the site is responsible for documenting compliance with study drug administration.

6.1.11 DEVICE SPECIFIC CONSIDERATIONS

See Appendix 2 for dialysis tubing connection diagrams.

6.2 STUDY AGENT ACCOUNTABILITY PROCEDURES

Study drug will be administered in accordance with the procedures of this protocol. Only authorized site personnel may supply or administer study drug and only patients enrolled in the study may receive study drug, in accordance with applicable regulatory requirements.

Drug accountability information collected may include but is not limited to:

- Receipt of study drug (date and quantity);
- Storage temperature log;
- Dispensation of study drug (date, time, quantity, and patient number);
- Return or destruction of study drug (date, quantity, and patient number);
- Initials of individual dispensing study drug; and
- Compliance assessment as outlined in Section 5.8.

At the conclusion of a site's participation in the study, all unused investigational drug shall be returned to the Sponsor or destroyed upon the Sponsor's request unless otherwise instructed by the Sponsor. A copy of the reconciled drug inventory record will be provided to the Sponsor or its designee, and the original will be retained at the site.

7 STUDY PROCEDURES AND SCHEDULE

7.1 STUDY PROCEDURES/EVALUATIONS

7.1.1 STUDY SPECIFIC PROCEDURES

Blood sampling for serum iron prarmeters will be obtained at specific times prior to, during and after hemodialysis according to the schedule in Table 1.

At each time point marked with an "X", collect 2 blood samples; one sample in a heparin coated PST (BD# 368056 or equivalent, Li Heparin 51 USP U/polymer plug) tube and one samplein a clot activator serum

separator tube (BD# 367981 3.5 mL tube with silica clot activator, polymer gel, silicone-coated interior or equivalent). The r samples will be processed according to the instructions in section 7.2.3 for the PK analysis of total iron and transferrin bound iron (TBI). One aliquot of serum will be sent to the clinical laboratory for determination of the serum iron profile(s).

The total anticipated blood volume for PK analyses is approximately 340 mL over 2 weeks.

Table 1: Blood sampling for PK analysis

	HD # 1	***HD #2***	***HD #3***	***HD # 4***
Week	***1***	***1***	***2***	***2***
Nominal Time[a]				
0 hr[b]	X	X	X	X
1 hr	X	X	X	X
2 hr	X	X	X	X
3 hr[c]	X	X	X	X
3.5 hr	X	X	X	X
4 hr[c,]	X	X	X	X
4.5 hr	X	X	X	X
5 hr	X	X	X	X
6 hr	X	X	X	X
8 hr	X	X	X	X
10 hr	X	X	X	X
12 hr	X	X	X	X

[a] PK sampling times on all treatments should occur within 5 min of the actual clock time of the first treatment HD in sequence. The exact time of each PK sample collection should be recorded on the CRF.

[b] The 0-hr sample for all visits should be obtained as close to 8 AM as practicable, just prior to the start of hemodialysis. The exact time of the start and stop of each Triferic administration should be recorded on the CRF.

[c] The 3 or 4-hr nominal time point is also the time of the end of Triferic infusion or dialysate administration, respectively. The PK sample for the 3 or 4-hr time point should be obtained within 5 min after the nominal time is reached..

7.1.2 STANDARD OF CARE STUDY PROCEDURES

Patients will receive their usual hemodialysis treatments as prescribed except for administration of Triferic on 3 of 4 HD treatments.

7.2 LABORATORY PROCEDURES/EVALUATIONS

7.2.1 CLINICAL LABORATORY EVALUATIONS

The primary analyte for PK analysis is total plasma iron (Fe) as measured by ICP-MS at QPS laboratories. Transferrin Bound Iron (TBI) will be will be determined using a LC-ICPMS assay at QPS laboratories.

Additional analytes at specified times include the serum iron profile (total serum iron, total iron binding capacity (TIBC), and transferrin saturation (TSAT)). These will be measured by the clinical laboratory.

7.2.2 OTHER ASSAYS OR PROCEDURES

None

7.2.3 SPECIMEN PREPARATION, HANDLING, AND STORAGE

Supplies for the Study

3.5-mL Clot activator, serum separator vacutainer tubes (BD# 367981 or equivalent)

3.0 mL Plasma Separator tubes (BD# 368056 or equivalent)

3.5-mL screw top plastic sample transport tubes

5-mL screw top plastic transport tubes

PK Sample Collection and Processing Instructions

a) Collect 2 blood samples (one plasma and one serum) for each time point and invert serum tube 5-6 times and plasma tube 8-10 times.
b) After inversion, allow serum sample to clot for 1 hour at room temperature in upright position. After inversion, immediately centrifuge plasma sample as described in step C below.
c) Centrifuge both tubes for 15 min at manufacturer's recommended speed (usually 1000 to 1300 RCF). Do not use brake to stop centrifuge.
d) Carefully aspirate the supernatant (serum and plasma) at room temperature transfer each intotransfer tubes, taking care not to disturb the cell layer or transfer any cells. Use a clean pipette for each tube. The serum will be separated into two transfer tubes (Aliquot #1 and Aliquot #2), and the plasma will be separated into two transfer tubes (Aliquot #3 and Aliquot #4).
e) Inspect serum for turbidity. Turbid samples should be centrifuged and aspirated again to remove remaining insoluble matter.
f) Transfer at least 0.50 mL of serum and plasma into two or more labeled screw top plastic sample transport tubes. If only a small amount of serum or plasma has been obtained, ensure that the primary transport tube contains at least 500 µL. Store the tubes frozen at -70°C -80°C.
g) At all time points, there will be 4 tubes: Aliquot #1 (primary PK serum sample) and Aliquot #2 (serum iron profile sample), Aliquot #3 (primary PK plasma sample) and Aliquot #4 (back-up plasma PK sample). Any additional aliquots are to be stored frozen.
h) Once processed, send Aliquot #2 to the clinical laboratory for serum iron profile analysis. Store all remaining aliquots frozen until completion of the study or until directed by the Sponsor or designee to ship to the bioanalytical laboratory for analysis.

7.2.4 SPECIMEN SHIPMENT

Specimens are to be shipped to the bioanalytical laboratory frozen. Shipping instructions are provided in the study site manual.

7.3 STUDY SCHEDULE

Screening will occur on Study Day -28 through Study Day -1. Baseline assessments will occur on Study Day 1 (HD #1). Study Day 1 (HD #1) will take place at a mid-week HD session. All patients will undergo baseline evaluations at HD #1. Subsequent treatments will be dictated by the randomization schedule prepared for each patient. The randomized sequence of treatment administrations are listed in Table 2. Patients will be assigned randomly to the sequences. A total of 30 patients will ensure that each sequence is replicated at least 5 times.

Table 2: Study Design Sequence Allocation Table

Week	**1**	**1**	**2**	**2**
HD Session	**1**	**2**	**3**	**4**
Sequence	**Treatment**			
1	Baseline	D	A	V
2	Baseline	A	V	D
3	Baseline	V	D	A
4	Baseline	V	A	D
5	Baseline	D	V	A
6	Baseline	A	D	V

Baseline = Standard hemodialysis x 4 hours; D = 2 µM Triferic administered via hemodialysate over 4 hrs; V= 6.5 mg Triferic administered IV over 3 hrs during hemodialysis via an infusion port (post-dialyzer); A = 6.5 mg Triferic administered IV over 3 hrs during hemodialysis via the unused heparin infusion line (pre-dialyzer).

Please note that for any visit, if the visit is missed, if the patient arrives too late to begin hemodialysis at 8 AM, or hemodialysis and/or Triferic infusion are interrupted for more than 15 minutes, then the visit may be rescheduled to occur within the next 14 days and the remaining schedule adjusted accordingly. If Triferic was being administered, the administration should be discontinued and PK and serum iron profile samples should be drawn immediately, and no further PK or serum iron profile samples should be collected for that day.

7.3.1 SCREENING

The Screening Visit (Visit #1) should be conducted within 28 days prior to Randomization (Study Day 1). The date of Screening is considered to be the date that the first study-related screening assessment is performed. The following procedures will be performed at Screening:

- Obtain informed consent from the patient (must be done prior to any study procedures, including asking patients to discontinue any prohibited medications)
- Assign patient screening number
- Record patient demographics and medical history
- Record all current medications, and also record any other medications taken within 28 days prior to Study Day 1/HD #1
- Record the date of the patient's last RBC or whole blood transfusion
- Record height and pre- and post-dialysis weight
- Record pre- and post-dialysis vital signs (blood pressure, pulse, and temperature)
- Perform a brief physical examination
- Record ECG
- Collect laboratory samples if not performed during the previous month (including hematology, Chem-14, CRP, serum pregnancy test (if applicable), serum iron profile, transferrin, and ferritin)

- Confirm patient meets all assessable eligibility criteria
- Schedule the next study visit (Baseline/Study Day 1/Treatment HD #1) to occur within 28 days after Screening. Please remind the patients that they will need to come to each visit sufficiently early in order to complete pre-dialysis assessments and begin hemodialysis by 8 AM.
- Provide instruction to patients to discontinue any prohibited medications, such as oral iron supplements and multivitamins with iron.

7.3.2 TREATMENT: ENROLLMENT/BASELINE

On the day of the Baseline visit, a light breakfast may be served 30 minutes after the 0-hr PK sample collection, and a light lunch may be served any time after the 4-hr PK sample collection. Patients may eat a dinner and evening snack as determined by the study dietician.

The patient will be enrolled in the study prior to dialysis, once all inclusion/excusion criteria have been reviewed and eligibility is confirmed. A study-specific patient number will be assigned at that time.

PRE-DIALYSIS

- Assess and record medical conditions
- Record medications
- Perform a final review of inclusion/exclusion criteria and serum pregnancy test if required.
- Record vital signs (blood pressure, pulse, and temperature) and pre-dialysis weight within 30 minutes prior to the beginning of hemodialysis.
- Collect blood samples for PK, serum iron profile at approximately 8 AM (within 5 minutes prior to the start of hemodialysis) as indicated in Table 1. For the purpose of PK sampling times, the pre-dialysis sample will be designated to be the Hour 0 sample. All timing of all subsequent samples will be based on the actual start time of the dialysis. The exact time (clock time) of each sample collection should be recorded in the CRF.

DURING DIALYSIS

- Begin the patient's usual hemodialysis with standard bicarbonate concentrate at approximately 8 AM. Hemodialysis should last for 4 hours. Record the actual start time and stop time of hemodialysis.
- Collect PK, serum iron profile blood samples (the 1.0, 2.0, 3.0 and 3.5 hour samples) as indicated in Table 1. The exact time (clock time) of each sample collection should be recorded.
- Record vital signs (blood pressure, pulse, and temperature) as per site SOP during hemodialysis.
- Assess and record AEs.

POST-DIALYSIS

- Collect post-dialysis PK, serum iron profile blood samples at the time points indicated in Table 1 (4.0, 4.5 , 5.0, 6.0, 8.0, 10.0, and 12.0 hrs) to be sent to the bioanalytical laboratories. The 4.0-hr time point should occur within 5 minutes after the end of hemodialysis, and the subsequent time points should be timed from the end of hemodialysis (e.g., the 4.5-hr sample collection should occur 30 minutes after the end of hemodialysis). The exact time (clock time) of each sample collection should be recorded.
- Record vital signs (blood pressure, pulse, and temperature) and post-dialysis weight within 30 minutes after the end of hemodialysis.
- Assess and record AEs.
- Schedule the next treatment visits. Please remind the patients that they should eat the standardized meal discussed with the dietician on the evening before these visits; and that they will need to show up sufficiently early in order to complete pre-dialysis assessments and begin hemodialysis by 8 AM.

Remind patients to continue not to take any prohibited medications, such as oral iron supplements and multivitamins with iron.

Treatments in Sections 7.3.3, 7.3.4 and 7.3.5 will be administered in a randomized sequence.

7.3.3 TREATMENT HD: TRIFERIC VIA DIALYSATE

PK sampling times at Treatment 2-4 should occur within 5 min of the actual clock time of the corresponding PK sample at Treatment HD #1. The exact time of each PK sample collection must be recorded.

A light breakfast may be served any time after the 0-hr PK sample collection, and a light lunch may be served any time after the 4-hr PK sample collection. Patients may eat a dinner and evening snack. Patients may resume their usual diet after all sample collections are completed for each visit.

PRE-DIALYSIS

- Assess and record AEs.
- Record medications.
- Record vital signs (blood pressure, pulse, and temperature) and pre-dialysis weight within 30 minutes prior to the beginning of hemodialysis.
- Collect PK, serum iron profile blood samples at approximately 8 AM (within 5 minutes prior to the start of hemodialysis) as indicated in Table 1. The clock time of these samples should be approximately the same (within 15 minutes) as that of the Treatment 1, Hour 0 sample.

DURING DIALYSIS

- Begin the patient's usual hemodialysis, except with Triferic added to the bicarbonate concentrate, at approximately 8 AM. See Section 6.1.4.1 for details of the preparation of Triferic dosing solutions. The start time and stop time of hemodialysis should be approximately the same (within 15 minutes) as those from Treatment HD #1. Record the actual start time and stop time of hemodialysis.
- Collect PK, serum iron profile blood samples (the 1.0, 2.0, 3.0 and 3.5 hour samples) as indicated in Table 1. The exact time (clock time) of each sample collection should be recorded.
- Record vital signs (blood pressure, pulse, and temperature) according to the site SOP during hemodialysis.
- Assess and record AEs.

POST-DIALYSIS

- Collect post-dialysis PK, serum iron profile blood samples at the time points indicated in Table 1 (4.0, 4.5, 5.0, 6.0, 8.0, 10 and 12 hrs). The clock times of these samples should be approximately the same (within 15 minutes) as those of the corresponding HD #1 samples.
- Record vital signs (blood pressure, pulse, and temperature) and post-dialysis weight within 30 minutes after the end of hemodialysis
- Assess and record AEs.
- Confirm the scheduling of the next treatment visit. Please remind the patients that they will need to show up sufficiently early in order to complete pre-dialysis assessments and begin hemodialysis by 8 AM. Provide instruction to patients to continue not to take any prohibited medications, such as oral iron supplements and multivitamins with iron.

7.3.4 TREATMENT A: TRIFERIC IV VIA HEPARIN LINE PRE-DIALYZER

PK sampling times at Treatment 2-4 should occur within 15 min of the actual clock time of the corresponding PK sample at Treatment HD #1. The exact time of each PK sample collection must be recorded.

A light breakfast may be served any time after the 0-hr PK sample collection, and a light lunch may be served any time after the 4-hr PK sample collection. Patients may eat a dinner and evening snack. Patients may resume their usual diet after all sample collections are completed for each visit.

PRE-DIALYSIS

- Assess and record AEs.
- Record medications.
- Record vital signs (blood pressure, pulse, and temperature) and pre-dialysis weight within 30 minutes prior to the beginning of hemodialysis.
- Collect PK, serum iron profile blood sample at approximately 8 AM (within 5 minutes prior to the start of hemodialysis) as indicated in Appendix 2. The clock time of these samples should be approximately the same (within 15 minutes) as that of previous HD session, Hour 0 sample.

DURING DIALYSIS

- Begin the patient's usual hemodialysis with standard bicarbonate concentrate at approximately 8 AM. The start time and stop time of hemodialysis should be approximately the same (within 15 minutes) as that of each of the previous hemodialysis sessions. Record the actual start time and stop time of hemodialysis.
- At the same time that hemodialysis is started, begin administration of Triferic 6.5 mg intravenously over 3 hours (1.5 mL/hr.) into the heparin line (pre-dialyzer) via the machine heparin pump. See Section 6.1.4.2 for details of the preparation of Triferic dosing solutions. The rate of infusion should be adjusted to complete the infusion of 6.5 mg Triferic iron in 4.5 mL at 3 hours of the 4 hour HD period. Record the actual start time and stop time of the Triferic IV infusion.
- Collect PK, serum iron profile blood samples (the 1.0, 2.0, 3.0 and 3.5 hour samples) as indicated in Table 1. The exact time (clock time) of each sample collection should be recorded.
- Record vital signs (blood pressure, pulse, and temperature) according to the site SOP during hemodialysis.
- Assess and record AEs.

POST-DIALYSIS

- Collect post-dialysis PK, serum iron profile blood samples at the time points indicated in Appendix 2 (4.0, 4.5, 5.0, 6.0, 8.0,10.0 and 12.0 hrs). The clock times of these samples should be approximately the same (within 15 minutes) as those of the corresponding hemodialysis samples.
- Record vital signs (blood pressure, pulse, and temperature) and post-dialysis weight within 30 minutes after the end of hemodialysis.
- Assess and record AEs.
- Confirm the scheduling of the next treatment visit. Please remind the patients that they will need to show up sufficiently early in order to complete pre-dialysis assessments and begin hemodialysis by 8 AM. Provide instruction to patients to continue not to take any prohibited medications, such as oral iron supplements and multivitamins with iron.

7.3.5 TREATMENT V: TRIFERIC IV VIA POST-DIALYZER PORT

PK sampling times at Treatment 2-4 should occur within 15 min of the actual clock time of the corresponding PK sample at Treatment HD #1. The exact time of each PK sample collection must be recorded.

A light breakfast may be served any time after the 0-hr PK sample collection, and a light lunch may be served any time after the 4-hr PK sample collection. Patients may eat a dinner and evening snack. Patients may resume their usual diet after all sample collections are completed for each visit.

PRE-DIALYSIS

- Assess and record AEs.
- Record medications.
- Record vital signs (blood pressure, pulse, and temperature) and pre-dialysis weight within 30 minutes prior to the beginning of hemodialysis.
- Collect PK, serum iron profile blood samples at approximately 8 AM (within 5 minutes prior to the start of hemodialysis) as indicated in Appendix 2. The clock time of these samples should be approximately the same (within 15 minutes) as that of previous HD session, Hour 0 sample.

DURING DIALYSIS

- Begin the patient's usual hemodialysis with standard bicarbonate concentrate at approximately 8 AM. The start time and stop time of hemodialysis should be approximately the same (within 15 minutes) as that of each of the previous hemodialysis sessions. Record the actual start time and stop time of hemodialysis.
- At the same time that hemodialysis is started, begin administration of Triferic 6.5 mg intravenously in the post dialyzer venous drip chamber over 3 hours using the machine heparin pump. See Section 6.1.4.3 for details of the preparation of Triferic dosing solutions. The rate of infusion should be adjusted to complete the infusion of 6.5 mg Triferic iron at 3 hours of the 4 hour dialysis period. Record the actual start time and stop time of the Triferic IV infusion.
- Collect PK, serum iron profile blood samples (the 1.0, 2.0, 3.0 and 3.5 hour samples) as indicated in Appendix 2. The exact time (clock time) of each sample collection should be recorded.
- Record vital signs (blood pressure, pulse, and temperature) according to the site SOP during hemodialysis.
- Assess and record AEs

POST-DIALYSIS

- Collect post-dialysis PK, serum iron profile blood samples at the time points indicated in Appendix 2 (4.0, 4.5, 5.0, 6.0, 8.0,10.0 and 12.0 hrs). The clock times of these samples should be approximately the same (within 15 minutes) as those of the corresponding hemodialysis samples.
- Record vital signs (blood pressure, pulse, and temperature) and post-dialysis weight within 30 minutes after the end of hemodialysis.
- Assess and record AEs.
- Confirm the scheduling of the next visit.

7.3.6 FINAL STUDY VISIT

NOTE: Patients who are discontinued or withdraw from this study prior to Follow-up are to have Follow-up procedures and evaluations performed at the time of discontinuation or withdrawal.

The following assessments are to be completed during Follow-up:

- Assess and record AEs.
- Record concomitant medications.
- Record vital signs (blood pressure, pulse, and temperature).
- Collect safety laboratory samples (including hematology, and a pregnancy test (if applicable)).
- Discharge patient from the study.

7.3.7 EARLY TERMINATION VISIT

See Section 7.3.6

7.3.8 Schedule of Events Table

Assessments	Screening	HD #1Enrollment/ Baseline	HD # 2, #3, #4 Treatment D,A,V	Follow Up[f]
Visit #	**1**	**2**	**3,4,5,**	**6**
Target study day	*-28 to -1*	*1*	*3, 8,10,*	*13*
Informed consent	X			
Inclusion/exclusion criteria	X	X		
Demographics	X			
Medical history	X			
Height and pre- and post-HD weight[a]	X	X	X	
Vital signs	X	X	X	X
Physical examination	X			
ECG	X			
Serum pregnancy test (if applicable)	X			X
Hematology[b]	X			X
Chem-14, CRP[b]	X			
Serum iron profile[b,c]	X	X	X	
Ferritin, Transferrin	X			
Enrollment in study		X		
Triferic Administration[d]		Treatment B: Baseline Fe	Treatment D: Triferic 2 µM HD Treatment A: Triferic 6.5 mg Fe IV/3 hrs via pre dialyzer heparin line Treatment V: Triferic 6.5 mg Fe IV/3 hrs via post dialyzer port	
PK samples[e]		X	X	
Discharge from study[f]				X
Adverse events		X	X	X
Medications	X	X	X	X

[a] Height is measured at Visit 1 (Screening) only.
[b] Hematology, Chem-14, CRP (Visit 1/Screening only), the serum iron profile (total serum iron, TIBC, TSAT), and pregnancy test samples will be analyzed by the local clinical laboratory. Hematology and Chem-14 required only if not performed within 1 month prior to screening
[c] If an SAE occurs during or within 30 min after any Triferic infusion, a serum iron profile will be obtained as soon as the SAE is recognized.
[d] See Section 6.1.4 for instructions on the preparation and dosing of Triferic.
[e] See Section 7.1.1 for the schedule for obtaining PK samples. PK samples will be sent to and analyzed by the Central Laboratory. See Section 7.2.3 for instructions on the handling of the PK samples.
[f] Patients who are discontinued or withdraw from this study prior to Follow-up are to have Follow-up procedures and evaluations performed at the time of discontinuation or withdrawal.

7.4 JUSTIFICATION FOR SENSITIVE PROCEDURES

All procedures in this study are standard of care.

7.5 CONCOMITANT MEDICATIONS, TREATMENTS, AND PROCEDURES

7.5.1 PRIOR THERAPY

All prescription and non-prescription medications (including multivitamins and oral and IV iron products) taken within 28 days of Study Day 1/HD #1 will be documented in source documents and the CRF (based on patient report and/or medical records). The date of Screening is considered to be the date that the first study-related Screening assessment is performed.

7.5.2 CONCOMITANT THERAPY

All prescription and non-prescription medications taken from 28 days prior to Study Day 1/HD #1 to Follow-up must be documented in the source documents and CRF.

7.5.3 PROHIBITED MEDICATIONS

Oral and IV iron products are prohibited from 2 weeks prior to Baseline (Study Day 1/HD #1) until all blood samples have been collected for HD #4. This includes oral multivitamins containing iron.

Red blood cell and whole blood transfusions are prohibited from 4 weeks prior to Screening. Patients who receive a RBC transfusion after enrollment will be discontinued from the study.

7.5.4 PRECAUTIONARY MEDICATIONS, TREATMENTS, AND PROCEDURES

None

7.6 PROHIBITED TREATMENTS, AND PROCEDURES

Oral and IV iron are prohibited from screening through follow up, including oral multivitamin preparations containing iron and iron containing phosphate binders. Patients receiving prophylactic antibiotics are eligible for participation in the study. Patients who are receiving antibiotics for infectious processes are ineligible until the course of antibiotics have been completed.

7.7 PROPHYLACTIC MEDICATIONS, TREATMENTS, AND PROCEDURES

None

7.8 RESCUE MEDICATIONS, TREATMENTS, AND PROCEDURES

NA

7.9 PARTICIPANT ACCESS TO STUDY AGENT AT STUDY CLOSURE

Triferic is available to be administered via dialysate. Patients may continue to receive Triferic upon return to their dialysis unit if prescribed by their physician. The IV administration is not currently approved by the US FDA.

8 ASSESSMENT OF SAFETY

8.1 SPECIFICATION OF SAFETY PARAMETERS

Safety assessments will include the following:

- AEs and SAEs, both reported and observed
- Clinical laboratory tests
- Changes in physical examinations
- Vital sign measurements (blood pressure and pulse).

8.1.1 DEFINITION OF ADVERSE EVENTS (AE)

The Investigator is responsible for the detection and documentation of events meeting the definition of an AE or SAE as provided in this protocol.

At appropriate intervals, patients should be assessed for AEs and SAEs. After the patient has had an opportunity to spontaneously mention any problems, the Investigator should inquire about AEs by asking a non-leading question such as the following:

"How are you feeling?"

"Have you had any medical problems since your last assessment/visit?"

"Have you taken any new medicines since your last assessment/visit?"

An AE is any untoward medical occurrence in a clinical investigation patient administered a pharmaceutical product and which does not necessarily have a causal relationship with this product.

An AE can therefore be any unfavorable and unintended sign (including a clinically significant abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. Adverse events will be recorded on the AE CRF from the start of the HD #1 through the end of study participation or 7 days after the last dose of Triferic, whichever is later. Pre-treatment-emergent medical conditions will be captured on the medical history CRF, unless they meet seriousness criteria (Section 8.1.2).

An AE **does** include any:

- Exacerbation of a pre-existing illness;
- Increase in frequency or intensity of a pre-existing episodic event or condition;
- Condition detected or diagnosed after the start of study drug administration even though it may have been present prior to the start of the study; or
- Continuous persistent disease or symptoms present at baseline that worsen following the start of the study.
- Symptoms associated with a disease not previously reported by the patient will be recorded as an AE.

An AE **does not** include a/an:

- Medical or surgical procedure (e.g., surgery, endoscopy, tooth extraction, transfusion). Rather, the underlying condition that leads to the procedure is the AE that should be reported, unless the condition did not worsen during the study;
- Pre-existing diseases or conditions present or detected at the start of the study that do not worsen;
- Situations where an untoward medical occurrence has not occurred (e.g., hospitalization for cosmetic elective surgery, social and/or convenience admissions).

8.1.2 DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

Any SAE that occurs from the date of Screening to the date of the Follow-up visit or 7 days after the last dose of Triferic, whichever is later, will be reported on an SAE report form. For enrolled patients, SAEs are also recorded on the AE CRF page. An SAE is any AE occurring at any dose that results in any of the following outcomes:

1. Death;
2. A life-threatening AE;

NOTE: Life-threatening means that the patient was, in the view of the investigator, at immediate risk of death from the event as it occurred. This definition does not include an event that, had it occurred in a more severe form, might have caused death.

3. Inpatient hospitalization or prolongation of an existing hospitalization;

NOTE: Hospitalization for elective treatment of a pre-existing condition that did not worsen during the study is not considered an AE or SAE.

NOTE: Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization, or otherwise meets seriousness criteria, the event is an SAE.

NOTE: "Inpatient" hospitalization means the patient has been formally admitted to a hospital for medical reasons. This may or may not be overnight. It does not include presentation at a casualty or emergency room.

4. A disability/incapacity;

NOTE: The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, accidental trauma (e.g., sprained ankle) that may interfere or prevent everyday life functions but do not constitute a substantial disruption.

5. A congenital anomaly in the offspring of a patient who received drug; or
6. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the patient or may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Medical and scientific judgment should be used in deciding whether prompt reporting is appropriate in this situation.

8.1.3 DEFINITION OF PARAMETERS

Laboratory Assessments

All routine and safety blood samples will be analyzed by the site's local licensed clinical laboratory. The clinical laboratory tests that will be conducted at the local laboratory are as follows:

Hematology: complete blood count with platelet count, white blood cell (WBC) count and WBC differential.

Blood Chemistry: routine Chem-14 analysis (albumin, alkaline phosphatase, ALT/SGPT, AST/SGOT, bicarbonate/ carbon dioxide, bilirubin, BUN, calcium, chloride, creatinine, glucose, potassium, sodium, total protein), serum pregnancy test, and CRP.

Serum Iron Profile: total serum iron, TIBC, and TSAT. Ferritin and transferrin will be analyzed at Screening only.

The Investigator is responsible for determining whether out-of-range laboratory values are clinically significantly changed or not. If the Investigator determines that additional laboratory examinations are needed for a patient in Screening, then the patient is not considered eligible for the study until such values are considered clinically stable. All clinically significantly changed values of enrolled patients will be followed until resolution or stabilization.

Physical Examinations

Physical examinations will consist of assessments of the following: skin, ENT (ears, nose, and throat), head, eyes, lungs/chest, heart, abdomen, musculoskeletal, extremities, and neurologic.

Vital Signs and Weight

Vital signs will include sitting blood pressure (mm Hg), heart rate (beats per min), and temperature. Blood pressure and heart rate measurements will be obtained in the same position (supine or sitting) and in the same arm when possible, after being supine or sitting for at least 5 minutes.

8.2 CLASSIFICATION OF AN ADVERSE EVENT

8.2.1 SEVERITY OF EVENT

The Investigator must categorize the severity of each AE according to the following guidelines.

Mild:

The patient is aware of signs or symptoms but is able to perform activities of daily living.

Moderate:

The event is sufficiently discomforting to the patient that it interferes with the patient's performance of activities of daily living.

Severe:

Due to the event, the patient is unable to perform activities of daily living.

8.2.2 RELATIONSHIP TO STUDY AGENT

The relationship of each AE to the study drug administration will be assessed by the Investigator after careful consideration, and according to the following guidelines.

No, Not Related:

This category is applicable to those AEs that are clearly due to extraneous causes (concurrent drugs, environment, etc.) and do not meet the criteria for drug relationship listed under UNLIKELY, POSSIBLY, PROBABLY, AND DEFINITELY RELATED.

Unlikely Related:

This category applies to those AEs that are judged to be unlikely to be related to the study drug administration. An AE may be considered to be UNLIKELY RELATED when it meets at least two (2) of the following criteria:

a) It does not follow a reasonable temporal sequence from administration of the study drug.

b) It could readily have been produced by the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient.

c) It does not follow a known or expected response pattern to the study drug.

d) It does not reappear or worsen when the study drug is re-administered.

Possibly Related:

This category applies to those AEs that are judged to be perhaps related to the study drug administration. An AE may be considered POSSIBLY RELATED when it meets at least one (1) of the following criteria:

a) It follows a reasonable temporal sequence from administration of the study drug.

b) It could not readily have been produced by the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient.

c) It follows a known or expected response pattern to the study drug.

Probably Related:

This category applies to those AEs that are felt with a high degree of certainty to be related to the study drug administration. An AE may be considered PROBABLY RELATED if it meets at least two (2) of the following criteria:

a) It follows a reasonable temporal sequence from administration of the study drug.

b) It could not be reasonably explained by the known characteristics of the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient.

c) It disappears or decreases on cessation or reduction in study drug dose. There are exceptions when an AE does not disappear upon discontinuation of the drug, yet drug relatedness clearly exists (e.g., bone marrow depression, fixed drug eruptions, tardive dyskinesia, etc.).

d) It follows a known or expected response pattern to the study drug.

Definitely Related:

This category applies to those AEs that are incontrovertibly related to study drug administration. An AE may be assigned to this category if it meets at least the first three (3) of the following criteria:

a) It follows a reasonable temporal sequence from administration of the study drug.

b) It could not be reasonably explained by the known characteristics of the patient's clinical state, environmental or toxic factors, or other modes of therapy administered to the patient.

c) It disappears or decreases on cessation or reduction in study drug dose. There are exceptions when an AE does not disappear upon discontinuation of the drug, yet drug relatedness clearly exists (e.g., bone marrow depression, fixed drug eruptions, tardive dyskinesia, etc.).

d) It follows a known or expected response pattern to the study drug.

e) It reappears or worsens when the study drug is re-administered.

8.2.3 EXPECTEDNESS

The Medical Monitor will be responsible for determining whether an SAE is expected or unexpected. An SAE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study agent in the package insert or the research investigatorsbrochure (RIB).

8.3 TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

Prompt notification to the Sponsor regarding SAEs is essential so that ethical and regulatory responsibilities and legal obligations can be satisfied. The Investigator must report SAEs according to the following time frames:

Death or Life-Threatening Event:

Initial notification must be sent to the Sponsor within 24 hrs of the investigational site learning of the death or life-threatening event (regardless of causality).

Complete SAE information (i.e., all SAE pages) must be sent to the Sponsor within 48 hrs.

Follow-up information must be sent to the Sponsor within 48 hrs of receipt of the information by the investigational site.

All Other SAEs:

Complete SAE information (i.e., all SAE pages) must be sent to the Sponsor within 48 hrs of site study personnel learning of the event.

Follow-up information must be sent to the Sponsor within 48 hrs of receipt of the information by the investigational site.

Serious Adverse Event Information to Report:

All information available regarding an SAE must be submitted in the timeframes indicated in Section 8.3. At a minimum, SAE reports must contain the patient's study identifier, the SAE term, and the name of the person reporting the event to the Sponsor. Optimally, the initial report will also include the onset date, relationship to study drug, and a brief narrative of the event.

The Investigator must record all relevant information regarding an AE/SAE in the applicable sections of the CRF. It is not acceptable for the Investigator to send photocopies of the patient's medical records in lieu of completion of the appropriate AE/SAE pages. However, there may be instances when copies of medical records for certain cases are requested by the Sponsor. If medical records are submitted to the Sponsor then all patient personal identifiers must be completely and thoroughly redacted prior to submission. Each page of medical records should be labeled with the patient's study identifier.

8.4 REPORTING PROCEDURES

8.4.1 ADVERSE EVENT REPORTING

Adverse events, including SAEs, will be recorded on the AE CRF from the start of the first dose of Triferic through the end of study participation or 7 days after the last dose of Triferic, whichever is later. Pre-treatment-emergent medical conditions will be captured on the medical history CRF, unless they meet seriousness criteria.

The Investigator should attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis should be documented as the AE and/or SAE.

8.4.2 SERIOUS ADVERSE EVENT REPORTING

Any SAE that occurs from the date of Screening to the Follow-up visit or 7 days after the last dose of Triferic, whichever is later, will be reported on an SAE report form. For enrolled patients, SAEs are also recorded on the AE CRF page.

CONTACT THE MEDICAL MONITOR BY PHONE, EMAIL, OR FAX (1 866 250 5488) WITHIN THE TIMEFRAME SPECIFIED IN SECTION 8.3 TO NOTIFY THE SPONSOR OF ANY SAEs.

All SAEs (related and unrelated) will be recorded from the time of the Screening visit until the date of the Follow-up visit or 7 days following the last dose of Triferic, whichever is later. Any SAEs considered possibly, probably, or definitely related to the investigational product and discovered by the Investigator or site personnel at any interval after completion of the study should also be reported. All SAEs must be reported to the Sponsor within 48 hrs (24 hrs for deaths and life-threatening events) of the site's first awareness of the event. The Investigator must complete, sign and date the SAE pages, verify the accuracy of the information recorded on the SAE pages with the corresponding source documents, and send a copy by fax (1 866 250 5488) or email to the Sponsor.

Additional follow-up information, if required or available, should be sent to the Sponsor within 48 hrs of receipt. Follow-up information should be provided using a follow up SAE report, and the follow-up SAE report should be placed with the original report in the appropriate section of the CRF/study file.

The Investigator is encouraged to discuss with the Sponsor any AEs for which the issue of seriousness is unclear or questioned.

Yhe Sponsor is responsible for notifying the relevant regulatory authorities of certain events. Multiple inquiries between the Sponsor and the study site may be necessary for report preparation.

It is the Principal Investigator's responsibility to notify the Institutional Review Board (IRB), Independent Ethics Committee (IEC) or the relevant local regulatory authority of all SAEs that occur at his or her site. Investigators will also be notified of all unexpected, serious, drug-related events (7- and 15-Day Safety Reports) that occur during any clinical trials. Each site is responsible for notifying their IRB, IEC or the relevant local regulatory authority of these additional SAEs.

Clinical Laboratory Abnormalities and Other Abnormal Assessments as Adverse Events

Clinically significant abnormal laboratory findings or other abnormal assessments that are associated with a diagnosis, unless judged by the Investigator as more severe than expected for the patient's condition, or that are present or detected before study drug administration and do not worsen after study drug administration, should not be reported as AEs. Instead, the diagnosis with which they are associated should be assessed for whether it constitutes an AE, and reported accordingly. For example, if a patient experiences leukocytosis or hypoxia associated with a diagnosis of pneumonia, it is not necessary to report these in addition to reporting the pneumonia unless they are more severe than expected.

If not known to be associated with a diagnosis, abnormal laboratory findings (e.g., clinical chemistry or hematology) or other abnormal assessments (e.g., ECGs or vital signs) that are judged by the Investigator as clinically significant must be recorded as AEs or SAEs if they meet the definition of an AE and also reported as SAEs if they meet the criteria for seriousness.

The Investigator should exercise his or her medical and scientific judgment in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

Follow-up of Adverse Events

After the initial AE report, the Investigator is required to proactively follow each patient and provide further information to the Sponsor on the patient's condition. All AEs documented at a previous visit/contact that are designated as ongoing will be reviewed at subsequent visits/contacts.

All AEs that are ongoing at the conclusion of the patient's participation will be followed up until resolution, until the condition stabilizes, or until the patient is lost to follow-up. The appropriate AE/SAE source document and CRF page(s) will be updated. If a patient dies during participation in the study or during the 7 days following the patient's last dose of Triferic, a copy of any post-mortem findings, including histopathology, should be obtained, if available, and forwarded to Rockwell Medical.

8.4.3 POST-STUDY ADVERSE EVENTS

Investigators are not obligated to actively seek new AEs or SAEs that begin more than 7 days after the last dose of Triferic. The Investigator should notify the Sponsor of any SAEs that begin following study completion only if the event is considered related to study drug.

8.4.4 EVENTS OF SPECIAL INTEREST

No events of special interest are anticipated in this study.

8.4.5 REPORTING OF PREGNANCY

A negative pregnancy test during screening for all women under age 60 years is required for study eligibility. Any verified pregnancy after initial exposure to study drug in a patient on study must be immediately reported to the Investigator and in turn to the Sponsor or its designee per study reporting procedures, and the patient must discontinue study drug administration. Pregnancy during the study period will be reported and followed until final resolution (i.e., delivery or early termination). Any treatment-emergent birth defect or congenital anomaly will be reported to the Sponsor or its designee per study reporting procedures immediately as an SAE.

8.5 STUDY HALTING RULES

There are no pre-specified study halting rules.

The study can be stopped at any time if any of the following circumstances occurs:

- An SAE occurring during Triferic administration and considered related to study drug;
- Signs of possible hypersensitivity during infusion of study drug as evidenced by any of the following, that is not consistent with the patient's ongoing medical history:
    - Hypotension, respiratory difficulty, angioedema, generalized pruritis and flushing;
    - GI: Nausea, vomiting, diarrhea and abdominal pain;
    - CV: Decreased cardiac output leading to hypoperfusion and shock;
    - Metabolic: Acute metabolic acidosis; or
    - CNS: Depressed sensorium;
    - Serum iron levels ≥500 µg/mL regardless of symptomology; or
    - Hepatic injury as evidenced by any acute combination of elevated bilirubin, AST or ALT levels.

If any of these events occurs, a safety review committee will review the entire study's safety data before a decision is made to terminate the study.

8.6 SAFETY OVERSIGHT

There is no DSMB for this study. The Medical Monitor will be responsible for the review of safety information. The study may be temporarily halted if an SAE determined to be probably or definitely related to study drug is reported.

9 CLINICAL MONITORING

Study Initiation, Monitoring and Closeout Visits

Representatives of the Sponsor will perform a number of on-site visits to the study center, from prior to initiation of the study at the site until after the study has been completed. These visits will include but not be limited to review of the site for adequacy to conduct the trial, review of study data, CRFs, and supportive source documents, and drug accountability.

Throughout the course of the study, Sponsor representatives will also make frequent contacts with the Investigator and designated site personnel. As part of the data review it is expected that source documents (e.g., hospital records, office records) will be made available for review by the Sponsor. The study documents may also be similarly evaluated by auditors representing the Sponsor. For these purposes, the Investigator will make CRFs, source documents and study files available when requested.

The study will be terminated and the study center will be closed when all completed original CRFs have been collected, all data discrepancies resolved, and drug accountability has been reconciled. It will be the responsibility of the Investigator to notify the IRB/EC that the study has been completed.

The Sponsor has the right to terminate the study for non-adherence to protocol, unavailability of the Investigator or his or her study staff for the Sponsor or its representatives, or for administrative reasons, at any time. In that event, the Sponsor will notify the Investigator in writing that the study is to be discontinued. The Investigator will comply with the Sponsor's written instructions for study discontinuation, which will include the following:

- Date discontinuation will occur,
- Rationale for discontinuation,
- Instructions on how discontinuation is to be performed,
- Instructions for patients participating in the study, and
- Instructions for retention of study documents.

In addition to monitoring by the Sponsor or its designees, the study may be audited by representatives of the U.S. Food and Drug Administration (FDA) or other applicable regulatory agencies, who will also be allowed access to study documents. The Investigator should immediately notify the Sponsor of any proposed or scheduled audits with any regulatory authorities.

10 STATISTICAL CONSIDERATIONS

10.1 STATISTICAL AND ANALYTICAL PLANS

A formal statistical analysis plan for the determination of equivalence of dialysate administered Triferic iron (reference) compared to IV administered Triferic iron pre-dialyzer (test 1) and Post-dialyzer (test 2) will be prepared and completed prior to database lock.

10.2 STATISTICAL HYPOTHESES

The statistical test to be used to determine equivalence of test to reference will be a two one-sided t-test (TOST) that requires a 90% confidence interval of the ratio of the geometric means obtained from a mixed effects model with sequence, period and treatment as fixed effects and subject (sequence) as a random effect, to be within the boundaries of 0.8 to 1.25.

Test of significance will be based on 5%, one-sided (α=0.05)

10.3 ANALYSIS DATASETS

The primary analysis dataset for this study will be will include all randomized patients who provide data for the baseline iron profile and receive at least 1 dose of study drug and have sufficient PK samples to include in the PK assessments.

The safety dataset will include all randomized patients who received at least one dose of Triferic by HD or IV.

10.4 DESCRIPTION OF STATISTICAL METHODS

10.4.1 GENERAL APPROACH

Equivalence of Triferic administered via dialysate compared to each of the two intravenous administrations will be demonstrated using a 3 period crossover design. The first HD will be used to collect time matched baseline samples. HD #2, 3 and 4 will administer Triferic via HD, pre-dialyzer and post-dialyzer in one of six predefined random sequences. Equivalence will be assessed according to the principals outlined in the FDA Guidance document Statistical Approaches to Establishing Bioequivalence (2001). Because iron is a endogenous metal, baseline time corrected changes in serum total iron from T=0 to T=last observation will be used for a secondary endpoint. A complete PK stastical analysis plan will be completed prior to database lock.

Exploratory compartmental modeling may be used to improve the parameter estimates.

10.4.1 SAFETY ANALYSES

Demographics and safety analyses will be presented using descriptive statistics.

10.4.2 PLANNED INTERIM ANALYSES

There is no interim analysis.

10.4.7.1 SAFETY REVIEW

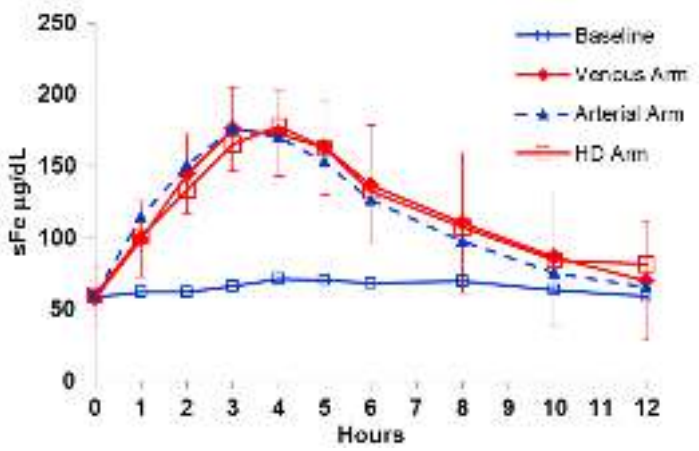
NO DSMB is required for this study. Safety will be assessed by the site investigators and the Sponsor's Medical Monitor.

10.5 SAMPLE SIZE

The sample size for this study was determined from the analysis of the PK data from Study RMFPC-16 (A Preliminary Equivalence Study of Triferic Administered via Dialysate and by 2 IV Methods).

The study was a 4 HD, 3 treatment randomized design with the the first period being the time matched basal serum iron profile (Baseline) and the reference and two test treatments randomized. The serum iron concentrations versus time data are presented in Figure 3.

Figure 3: RMFPC-16 Serum Iron Concentrations over Time



The statistical analysis summary of C_{max}, $AUC_{0-\infty}$ and AUC_{0-last}, form this study is presented in Table 3. The intra-subject variability for AUC_{0-last} on the natural log scale is estimated to be 0.34, for AUC_{inf} it is 0.28 and for C_{max} it is 0.15. This estimate is derived from the following table that supports the analysis from study RMFPC-16.

Table 3 Summary of Statistical Analysis, Study RMFPC-16 using baseline corrected PK Parameters.

Treatment	Parameter	Units	Geometric LSM (Test)	Geometric LSM (Reference)	Test/ Reference (%)	90% Confidence Interval
Pre-dialyzer	Cmax	µg/dL	229	217	105	(96.66, 114.77)
	AUClast	h*µg/dL	1710	1670	102	(87.82, 119.34)
	AUCinf	h*µg/dL	2390	2060	116	(78.99, 170.42)
Post-dialyzer	Cmax	µg/dL	215	217	98.7	(90.59, 107.56)
	AUClast	h*µg/dL	1660	1670	99.4	(85.24, 115.85)
	AUCinf	h*µg/dL	2040	2060	99.3	(67.51, 146.2)

Based on the results of RMFPC-16 the following estimates of sample size have been calculated:

$\underline{AUC_{last}}$

In a two one-sided test(TOST) analysis for additive equivalence in the natural log scale of two-sample normal means with bounds -0.233 and 0.233 for the mean difference and a significance level of 0.05, assuming a mean difference of 0.05 and a common standard deviation of 0.151, a sample size of 14 per group is required to obtain a power of at least 0.9.

Cmax

In a two one-sided test(TOST) analysis for additive equivalence in the natural log scale of two-sample normal means with bounds -0.233 and 0.233 for the mean difference and a significance level of 0.05, assuming a mean difference of 0.05 and a common standard deviation of 0.083, a sample size of 14 per group will give an approximate power of is required to obtain a power of at least 0.99).

10.5.1 FINAL SAMPLE SIZE JUSTIFICATION

Because of the variability in AUC, and the difficulty of recruiting sufficient numbers of HD patients for this study. Hence, per simulation of a replicate study design of the TOST, assuming an intra-subject variabililty of 0.34, a total of 24 subjects completing should provide approximately 85% power to establish equivalence in both AUC_{0-t} and Cmax.

Up to 36 patients will be recruited to accommodate for up to a possible 20% drop out rate in this 7 week study.

The sample size estimation report is provided as Appendix 1.

10.6 MEASURES TO MINIMIZE BIAS

10.6.1 ENROLLMENT/ RANDOMIZATION/ MASKING PROCEDURES

Because the two formulations are different it is not feasible to conduct a double-blind study. The safety profile of Triferic by HD is similar to IV administration, therefore patients will not experience any differential effects from either treatment.

11 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

All records of this clinical study must be retained by the Investigator, including, but not limited to, the following:

- Protocol and all protocol amendments,
- All signed versions of the Statement of Investigator, Form FDA 1572,
- All drug accountability records,
- All IRB/EC approvals, correspondence and reports,
- Signed and dated informed consent forms for each patient,
- Completed CRFs for each patient,
- Copies of any other material distributed to patients,
- Any advertisements for this study,
- The Investigator's final report to the IRB/EC, and
- Source documents pertaining to the study, including, but not limited to, any operative reports, laboratory results, radiographic films, tracings, and computer discs, files or tapes.

12 QUALITY ASSURANCE AND QUALITY CONTROL

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

Following written SOPs, the monitors will verify that the clinical trial is conducted and data are generated, documented (recorded), and reported in compliance with the protocoland GCP,(Good Clinical Practices).

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

13 ETHICS/PROTECTION OF HUMAN SUBJECTS

13.1 ETHICAL STANDARD

This study will be conducted under a US Investigational New Drug (IND) Application. All applicable US regulations governing human subject protection must be followed. All ethical and regulatory requirements necessary to comply with the principles of GCP for the conduct and monitoring of clinical investigations must be followed.

13.2 INSTITUTIONAL REVIEW BOARD

A copy of the proposed ICF should be submitted to the Sponsor for review and comment prior to submission to the reviewing IRB. The ICF must be approved by the IRB and contain all elements required by all applicable federal, state, local, and institutional regulations or requirements prior to consenting a patient. Authorization to use or disclose Personal Health Information (PHI) in accordance with requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA) should be covered in the ICF or in a separate document to be signed by the patient.

The Investigator will be responsible for obtaining written informed consent from the potential patients prior to any study-specific screening and entry into the study. The research study will be completely explained to each prospective study patient. The Investigator or designee must explain that the patient is free to refuse to enter the study, and free to withdraw from it at any time for any reason.

Institutional Review Board or Independent Ethics Committee Approval

In accordance with 21 CFR Parts 50 and 56, the Investigator agrees to provide the appropriate IRB with all appropriate material, including a copy of the protocol, ICF, and any proposed advertisement for the study prior to the start of the study.

The proposed ICF and any proposed advertisement must also be agreed to by Rockwell Medical. The site may not begin consenting, screening or enrolling patients until the Investigator has obtained IRB approval of the protocol and ICF and the Sponsorhas received documentation of each.

The Investigator will supply to the Sponsor a list of the names, professions, and affiliations of IRB members to demonstrate compliance with membership requirements. If the Investigator or a sub-investigator is a routine voting member of the IRB, the Sponsor will be provided with a statement from the IRB that the Investigator/
sub-investigator did not and will not vote on any IRB decisions pertaining to this clinical investigation.

During the course of the study, the Investigator shall make timely and accurate reports to the IRB on the progress of the trial, at intervals not exceeding one year, as well as satisfying any other local IRB regulations regarding reporting. Furthermore, at the completion or early termination of the study, a final report should be made to the IRB by the Investigator within the applicable IRB time frames.

Any significant changes or revisions in the study protocol or any changes that may alter patient risk must be approved by the Sponsor (and may require FDA/other regulatory agency review and/or approval) and must be approved in writing by the IRB prior to implementation. The Investigator must also receive a written notice of approval from the Sponsor prior to initiating the revised changes to the study protocol. A protocol change intended to eliminate an apparent immediate hazard may be implemented immediately, provided that

the Sponsor is immediately notified and an amendment is subsequently provided by the Sponsor and approved by the IRB.

It is the Investigator's obligation to maintain an IRB correspondence file, to provide copies of all documents to the Sponsor, and to make this available for review by the Sponsor or its designated representatives as part of the study monitoring process.

13.3 INFORMED CONSENT PROCESS

13.3.1 CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

See Section 13.2 Institutional Review Board or Independent Ethics Committee Approval.

13.3.2 CONSENT PROCEDURES AND DOCUMENTATION

Informed consent is a process that is initiated prior to the individual's agreeing to participate in the study and continues throughout the individual's study participation. Discussion of risks and possible benefits of participation will be provided to the participants and their families. Consent forms will be IRB-approved and the participant will be asked to read and review the document. The investigator will explain the research study to the participant and answer any questions that may arise. All participants will receive a verbal explanation in terms suited to their comprehension of the purposes, procedures, and potential risks of the study and of their rights as research participants. Participants will have the opportunity to carefully review the written consent form and ask questions prior to signing. The participants should have the opportunity to discuss the study with their surrogates or think about it prior to agreeing to participate. The participant will sign the informed consent document prior to any procedures being done specifically for the study. The participants may withdraw consent at any time throughout the course of the trial. A copy of the informed consent document will be given to the participants for their records. The rights and welfare of the participants will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

13.4 PARTICIPANT AND DATA CONFIDENTIALITY

All US-based investigational sites and laboratories or entities providing support for this study, must, where applicable, comply with HIPAA. An investigational site that is not a Covered Entity, as defined by HIPAA, must provide documentation of this fact to the Sponsor.

13.4.1 RESEARCH USE OF STORED HUMAN SAMPLES,SPECIMENS OR DATA

Data collected for this study will be analyzed and stored by the Sponsor. Serum samples will be stored at the clinical sites until a decision by the sponsor to destroy the samples.

All data collected is anonomized and may be provided to investigators for additional analysis upon acceptance of a protocol for data analysis.

13.5 FUTURE USE OF STORED SPECIMENS

When the study is completed, all retained samples will be destroyed upon expiration of the required retention time.

14 DATA HANDLING AND RECORD KEEPING

14.1 DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Case report forms (CRFs) will be supplied by the Sponsor or its designee and should be handled in accordance with instructions from the Sponsor.

In accordance with the US 21 CFR 312.62, a CRF, whether paper or electronic, must be completed for each patient enrolled in the study. All data collected for each study patient will be recorded on CRFs provided or approved by the Sponsor.

CRFs need not be completed by the Investigator, but all entries in CRFs are the responsibility of the Investigator and entry of CRF data must be made under the supervision of the Investigator. The Investigator is responsible for ensuring the accuracy, completeness, legibility (if paper), and timeliness of all data reported in the CRFs and all required reports for each study patient. The Investigator is also responsible for maintaining any source documentation related to the study, including, but not limited to, any operative reports, laboratory results, radiographic films, ECG tracings, and computer discs, files or tapes. The Investigator must retain a copy of all CRFs.

14.2 STUDY RECORDS RETENTION

All records of this clinical study must be retained by the Investigator, including, but not limited to, the following:

- Protocol and all protocol amendments,
- All signed versions of the Statement of Investigator, Form FDA 1572,
- All drug accountability records,
- All IRB/EC approvals, correspondence and reports,
- Signed and dated informed consent forms for each patient,
- Completed CRFs for each patient,
- Copies of any other material distributed to patients,
- Any advertisements for this study,
- The Investigator's final report to the IRB/EC, and

Source documents pertaining to the study, including, but not limited to, any operative reports, laboratory results, radiographic films, tracings, and computer discs, files or tapes.

The period of time these documents must be maintained is governed by US law and, when applicable, non-US regulations. All records are to be retained by the investigator for a minimum of two (2) years after the FDA has approved the new drug application, or after the Sponsor has notified the Investigator in writing that all investigations of the drug have been discontinued. However, because of international regulatory requirements, the Sponsor may request retention for a longer period of time. Therefore, the Sponsor or its designee will inform the Investigator when these documents may be destroyed. The investigator must obtain written approval from the Sponsor prior to destruction of any records.

The Investigator must advise the Sponsor in writing if the records are to be moved to a location other than the Investigator's archives. If the Investigator leaves the institution or study center, the records shall be transferred to an appropriate designee, at the study center, who assumes the responsibility for record retention. Notice of such transfer shall be documented in writing and provided to the Sponsor.

In the event of accidental loss or destruction of any study records, the Investigator will immediately notify the Sponsor in writing. The Sponsor or its designee must be notified in writing at least 30 days prior to the intended date of disposal of any study records related to this protocol.

Investigator's Final Report

Shortly after completion of the Investigator's participation in the study, the Investigator will submit a written report to Rockwell Medical. This report may be a copy of the Investigator's end-of-study report to their IRB, which will include, but not be limited to, notification that the study has concluded, the number of

patients enrolled/ treated, and the number of AEs and SAEs that occurred during the study. The report to the IRB will be consistent with the applicable IRB regulations and time frames.

14.3 PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, GCP, or MOP requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH E6:

4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3

5.1 Quality Assurance and Quality Control, section 5.1.1

5.20 Noncompliance, sections 5.20.1, and 5.20.2.

Except for a change that is intended to eliminate an apparent immediate hazard to a study patient, the protocol shall be conducted as specified. Any such change must be reported immediately to the Sponsor and to the IRB according to the applicable IRB/EC policy.

The investigator must notify the IRB of any and all protocol deviations according to the applicable IRB/EC policy. Protocol 'waivers' will not be granted.

Written documentation of all protocol deviations must be kept in the study center file and provided to the Sponsor. Examples of possible protocol deviations include, but are not limited to:

- Failure to obtain required informed consent,
- Failure to collect, report or file AE reports,
- Performance of an unapproved study procedure,
- Performance of research at an unapproved location,
- Failure to file protocol modifications, and
- Failure to adhere to an approved protocol.

14.4 PUBLICATION AND DATA SHARING POLICY

All information obtained as a result of this study or during the conduct of this study will be regarded as confidential. All unpublished information relating to this drug or to the operations of the Sponsor, including clinical indications, formula, methods of manufacture, and any other related scientific data provided to or developed by the Investigator, is confidential and shall remain the sole property of the Sponsor. The Investigator agrees to use the information for the purpose of carrying out this study and for no other purpose, unless prior written permission from the Sponsor is obtained. The Sponsor has full ownership of the CRFs and database resulting from this study.

The Investigator agrees that results from this study may be used by the Sponsor for purposes of domestic and international new drug registration, for publication, and to inform medical and pharmaceutical professionals. Regulatory authorities will be notified of the Investigator's name, address, qualifications, and extent of involvement.

17 LITERATURE REFERENCES

Reference List

1. III. Clinical practice recommendations for anemia in chronic kidney disease in children. *Am J Kidney Dis* 47:S86-108, 2006

2. Fishbane S, Mathew A, Vaziri ND: Iron toxicity: relevance for dialysis patients. *Nephrol Dial Transplant* 29:255-259, 2014

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4. Gupta A, Lin V, Guss C *et al.*: Ferric pyrophosphate citrate administered via dialysate reduces erythropoiesis-stimulating agent use and maintains hemoglobin in hemodialysis patients. *Kidney Int* 88:1187-1194, 2015

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APPENDIX 1. VERSION CONTROL

Version	Date	Significant Revisions

APPENDIX 2: SAMPLE SIZE ESTIMATION

Sample Size Estimation

NUVENTRA

December 20, 2016 Authored by: sue walker

Triferic Iron BE Sample Size Calculation: Absolute Total Iron

Sample Size Estimation

Background

This document is to provide sample size estimation for Triferic 2 µM using results from study RMFPC16.

Primary Objective

To evaluate the bioequivalence of Triferic 2 µM via hemodialysate. The primary objective will be evaluated using the following endpoints:

Absolute Total Iron maximum observed plasma drug concentration (Cmax),

Absolute Total Iron area under the plasma concentration-time curve (AUC),

For the purpose of sample size generation, analyses will be performed on natural log-transformed data resulting in the two one-sided hypothesis (alpha=0.05) tests. Upon back transformation we get the following hypothesis tests below:

H_0: $g\mu_{(test)}/g\mu_{(ref)} < 0.8$ vs. H_a: $g\mu_{(test)}/g\mu_{(ref)} \geq 0.8$ And

H_0: $g\mu_{(test)}/g\mu_{(ref)} > 1.25$ vs. H_a: $g\mu_{(test)}/_g\mu_{(ref)} \leq 1.25$

where $g\mu_{(test)}/g\mu_{(ref)}$ is the geometric mean ratio of the natural log-transformed data. Note that this is analogous to the following in the natural log scale:

H_0: $\mu_{(test)} - \mu_{(ref)} < -0.223$ vs. H_a: $\mu_{(test)} - \mu_{(ref)} \geq -0.223$ And

H_0: $\mu_{(test)} + \mu_{(ref)} > 0.223$ vs. H_a: $\mu_{(test)} + \mu_{(ref)} \leq 0.223$

Assumptions

The true effect is measured by the ratio of the geometric means of each PK parameter and the corresponding 90% confidence intervals.

The intent is to analyze the baseline corrected.

The assumption is that the natural log of each PK parameter is normally distributed. Note that this document will focus on inferences and estimates in the natural log state.

The statistical test to be used to determine bioequivalence of test to reference will be a two one-sided t test(TOST) that requires a 90% confidence interval of the ratio of the geometric means obtained from a mixed effects model with sequence, period and treatment as fixed effects and subject(sequence) as a random effect, to be within the boundaries of 0.8 to 1.25.

Test of significance will be based on 5%, one-sided (α=0.05)

The sample size considerations do not account for possible drop-out rates. Sample size assumes all completers.

The true ratio of the means for each PK parameter is expected to be 1.00, to account for small deviations, a change of 3% or 5% is incorporated into the sample size calculation.

The variability for AUC last, and AUC inf is estimated to be 0.151 and 0.272 respectively, And for Cmax it is 0.0825. This estimate is derived from the following table that supports the analysis from Study .

Summary of Stat Analysis of Study RMFPC-16 using Absolute Total Iron PK Parameters

Pharmacokinetic Parameter	Comparison	Ratio	Lower 90% Confidence Limit	Upper 90% Confidence Limit
AUCINF_obs	A.Triferic 6.6 mg IV(Pre dialyzer) vs Triferic 2 uM HD	0.960	0.781	1.181
	B.Triferic 6.6 mg IV(Post dialyzer) vs Triferic 2 uM HD	1.039	0.827	1.305
AUClast	A.Triferic 6.6 mg IV(Pre dialyzer) vs Triferic 2 uM HD	0.984	0.889	1.090
	B.Triferic 6.6 mg IV(Post dialyzer) vs Triferic 2 uM HD	1.010	0.912	1.118
Cmax	A.Triferic 6.6 mg IV(Pre dialyzer) vs Triferic 2 uM HD	1.016	0.961	1.074
	B.Triferic 6.6 mg IV(Post dialyzer) vs Triferic 2 uM HD	1.023	0.967	1.081 1

Intra-subject Variability Estimates

Param	Intra subject Variability Estimates (Sqrt of residual variance from model)
AUCINF_obs	0.272
AUClast	0.151
Cmax	0.0825

Standard Study Design

Standard crossover with each treatment represented once.

AUClast

In a two one-sided test(TOST) analysis for additive equivalence in the natural log scale of two-sample normal means with bounds -0.233 and 0.233 for the mean difference and a significance level of 0.05, assuming a mean difference of 0.05 and a common standard deviation of 0.151, a sample size of 14 per group is required to obtain a power of at least 0.9.

AUCinf

In a two one-sided test(TOST) analysis for additive equivalence in the natural log scale of two-sample normal means with bounds -0.233 and 0.233 for the mean difference and a significance level of 0.05, assuming a mean difference of

0.05 and a common standard deviation of 0.28, a sample size of 32(43) per group is required to obtain a power of at least 0.8(0.9).

Cmax

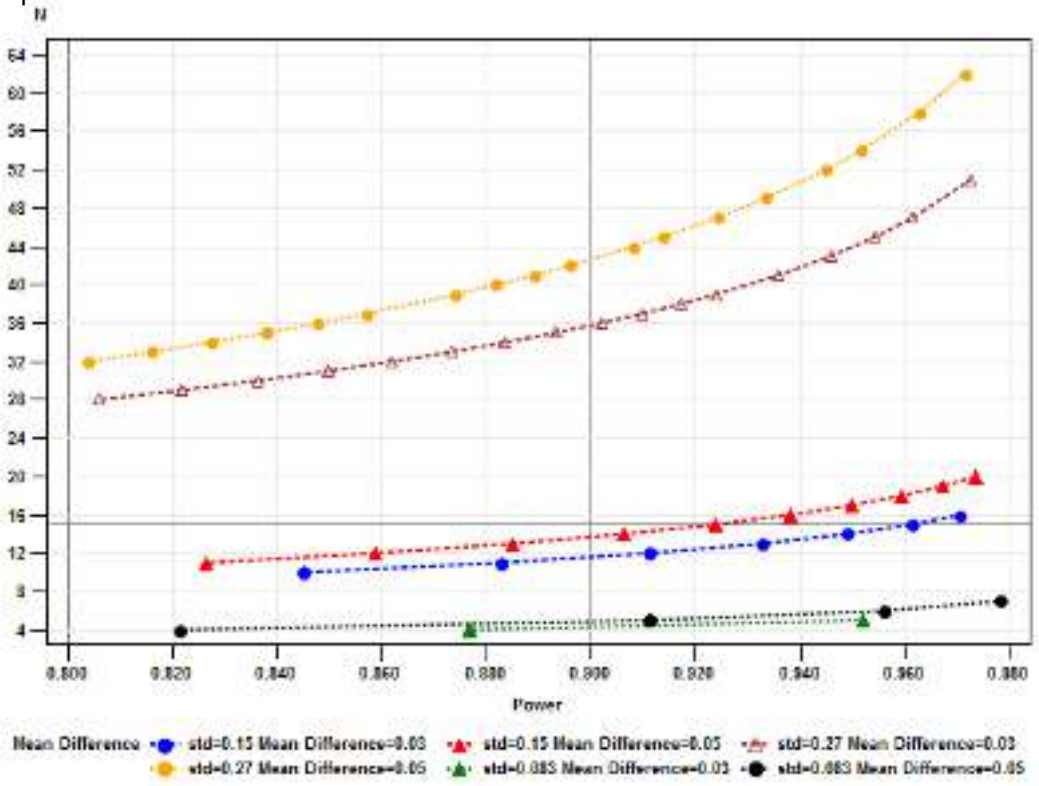
In a two one-sided test(TOST) analysis for additive equivalence in the natural log scale of two-sample normal means with bounds -0.233 and 0.233 for the mean difference and a significance level of 0.05, assuming a mean difference of

0.05 and a common standard deviation of 0.083, a sample size of 14 per group will give an approximate power of is required to obtain a power of at least 0.99).

Computed N Per Group					
Index	Mean Diff	Std Dev	Nominal Power	Actual Power	N Per Group
1	0.03	0.150	0.98	0.983	18
2	0.03	0.150	0.90	0.911	12
3	0.03	0.150	0.80	0.845	10
4	0.03	0.270	0.98	0.981	55
5	0.03	0.270	0.90	0.902	36
6	0.03	0.270	0.80	0.806	28
7	0.03	0.083	0.98	0.981	6
8	0.03	0.083	0.90	0.952	5
9	0.03	0.083	0.80	0.877	4
10	0.05	0.150	0.98	0.983	22
11	0.05	0.150	0.90	0.906	14
12	0.05	0.150	0.80	0.826	11

13	0.05	0.270	0.98	0.981	68
14	0.05	0.270	0.90	0.902	43
15	0.05	0.270	0.80	0.804	32
16	0.05	0.083	0.98	0.990	8
17	0.05	0.083	0.90	0.911	5
18	0.05	0.083	0.80	0.821	4

Sample Size versus Power



Conclusions

AUCinf demonstrated significantly higher variability than either AUClast or Cmax. If we focus on AUC last and Cmax then it would be easier to power for a formal BE using (maybe around 16 subjects). If we use AUCinf, then approaches described in the other sample size document where the std=.28 may make more sense. Since Cmax has such a smaller std as compared to either AUC, powering on AUC will result in an overall power consistent with the AUC power (for instance 0.90*0.99=0.89).

APPENDIX 3: DIALYSIS TUBING CONNECTIONS