	Pharmacokinetic Analysis Plan	**June 26, 2017**
	Study RMFPC-17	

Title:	Single Ascending Dose Study of Intraperitoneal Triferic (Ferric Pyrophosphate Citrate) in Patients on Chronic Peritoneal Dialysis
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Sponsor Approval:

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6/26/2017
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Author Approval:

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1. Introduction

This Pharmacokinetic Analysis Plan describes the analysis and reporting of the pharmacokinetic (PK) results for Study RMFPC-17. This is a Phase 1, open-label, single ascending dose study to assess the safety and pharmacokinetics of Triferic administered via peritoneal dialysate (PD) to iron-replete adult end-stage renal disease patients on maintenance peritoneal dialysis (CKD-5PD). Approximately 24 patients will be studied.

2. Objectives

The objectives of this study relevant to the pharmacokinetic analysis plan are as follows:

- **Primary**
    - To determine the pharmacokinetic profile, C_{max} and AUC_{0-t}, of Triferic iron administered intraperitoneally (IP).
- **Secondary**
    - To determine the dose proportionality of Triferic iron administered via PD.
    - Estimate absolute bioavailability of iron via PD
        - By proportionality of IP (C_{max} and AUC_{0-t}) to intravenous (IV) (C_{max} and AUC_{0-t}).
        - By quantitating iron remaining in the PD solution compared to that infused corrected.

3. Endpoints

- **Primary**
  - Assess the mean absolute C_{max}, $AUC_{0\text{-}t}$, $AUC_{0\text{-}end,}$ and AUC_{inf} of total serum iron, as data permits and as appropriate. Additional parameters to be determined may include T_{max}, CL, λz, and t½.
- **Secondary**
  - Dose proportionality of Triferic iron will be determined using the C_{max} and AUC parameters for serum iron.
  - The mass balance of iron absorbed from the PD fluid will be determined by two methods:
    - The absolute bioavailability of iron from Triferic administered via PD.
    - The difference in mass of Triferic iron in the PD fluid infused and removed at the end of the dwell will be measured to determine the mass balance.

4. Study Design

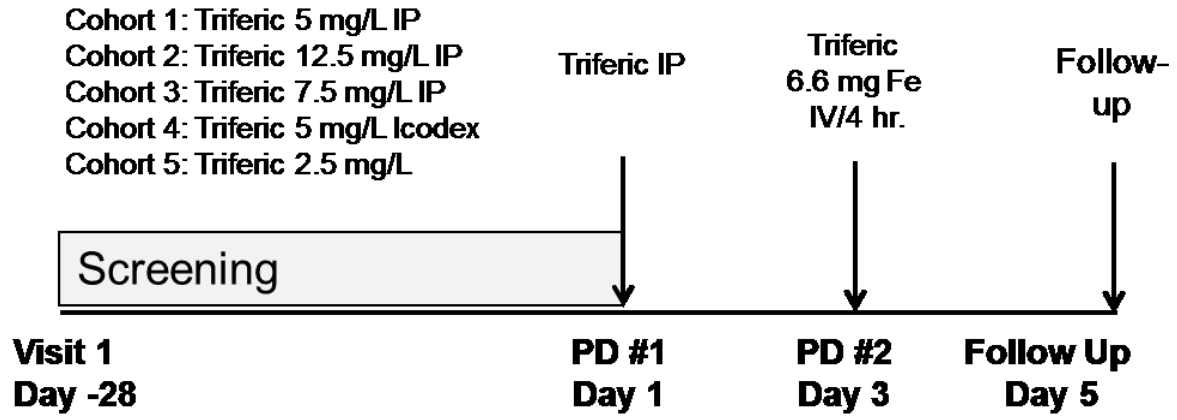
This is a Phase 1, open-label, single ascending dose study to assess the safety and pharmacokinetics of Triferic administered via PD to iron-replete adult end-stage renal disease patients on maintenance peritoneal dialysis (CKD-5PD). Approximately 24 patients will be studied.

Patients will be screened within 28 days of Baseline. All patients must provide informed consent to enter Screening. Patients will be advised to discontinue all iron supplements including multivitamins with iron. The study will be conducted as an outpatient study. Patients will attend the clinic for two (2) 12 hour treatment periods with a 48 hour interval between test article administrations. Patients will be randomly assigned to receive either a single dose of Triferic in the PD solution or a single IV dose of Triferic 6.6 mg over a 4 hour period.

Patients begin a long dwell time (12 hours) from approximately 08:00 to 20:00 hours on each day of dosing. After completion of PK measurements patient resume their usual dialysis prescription until the next scheduled administration of test article.

A follow-up assessment will be conducted within 1 week after the last administration of test article to assess safety parameters.

Figure 1 Study Design Schematic



5. Pharmacokinetic Sample Collection

Blood samples will be collected at 0, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, and 24 hr post dose following drug administration on Day 1 and Day 3 (Figure 1).

6. Bioanalytical Methods

6.1. Clinical Laboratory

Total serum iron, TSAT, total iron binding capacity (TIBC), and ferritin values will be determined by the clinical laboratory using established methods on the Roche COBAS platform using a Gen2 Fe assay. Under acidic conditions, iron is liberated from transferrin. Lipemic samples are clarified by the detergent. Ascorbate reduces the released Fe3+ ions to Fe2+ ions which then react with FerroZine to form a colored complex. The color intensity is directly proportional to the iron concentration and can be measured photometrically. The quantification range for the assay is 5 – 1000 µg/dL.

6.2. Bioanalytical Laboratory

6.2.1. Total Serum Iron

Total serum iron will be measured using ICP-MS at QPS laboratories (Gronigan, The Netherlands) using a validated serum assay.

6.2.2. Serum Hepcidin

Hepcidin samples will be analyzed by Mass Spectroscopy at the laboratory of Dorine Swinkels (www.hepcidinanalysis.com).

7. Pharmacokinetic Population

The PK Population will include all enrolled subjects who receive at least 1 dose of study drug and have sufficient PK samples (a sample at the end of administration and at least 3 samples during the elimination phase) to include in the PK assessments.

8. Pharmacokinetic Analyses

8.1. Noncompartmental Pharmacokinetic Analysis

All pharmacokinetic analysis and reporting will be performed according to applicable Nuventra SOPs and protocol specifications. Programming of tables, figures, and listings will be performed by Nuventra using R version 3.3.1 or later (R Foundation for Statistical Computing, Vienna, Austria). Pharmacokinetic parameters will be calculated by Nuventra using Phoenix® WinNonlin® 6.3 or later (Certara USA, Inc. (Princeton, NJ)) using actual sampling times.

The measured serum iron profile parameters and associated endpoints for PK analyses ("serum iron endpoints") comprise the following:

- Total serum iron (sFe)

Additional serum iron endpoints will be summarized by treatment:

- Transferrin saturation (TSAT)
- Total iron-binding capacity (TIBC)
- Serum ferritin
- Serum hepcidin

Listings of serum iron endpoints by subject and treatment with nominal blood sample collection times, as well as derived actual sampling times will be provided. Serum iron endpoint data will be tabulated and summarized using descriptive statistics (including N, mean, SD, CV%, median, minimum, maximum, and geometric mean) for each treatment.

Figures of the time course of individual serum iron endpoints will be presented on linear and semi-logarithmic scales, as appropriate. Figures illustrating the time course of mean/median serum iron endpoints for each treatment will be overlaid and presented for relevant comparisons on linear and semi-logarithmic scales, as appropriate. In addition, overlay plots of individual serum iron endpoint profiles will be generated for all subjects by treatment.

All serum iron endpoints and PK parameters for total serum iron will be summarized, as described above, using actual concentrations.

The following pharmacokinetic parameters will be estimated for total serum iron, as appropriate and as data permit:

C_{max}	The maximum drug concentration in serum determined directly from individual concentration-time data
T_{max}	The observed time to reach maximum concentration
T_{last}	The observed time of the last quantified concentration
AUC_{last}	The area under the serum 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 serum concentration time curve from time zero to the end of the study drug infusion, calculated using the linear-up/log-down trapezoidal rule
AUC_{inf}	Area under the concentration-time curve from time-zero extrapolated to infinity, calculated as: $AUC\,\text{inf} = AUClast + \frac{Clast}{\lambda z}$ where Clast is the last quantified concentration in the terminal elimination phase.
λz	The terminal phase rate constant; estimated by linear regression through the terminal phase of the log concentration-time profile
t½	The terminal phase half-life calculated as: $t½ = \frac{\ln(2)}{\lambda z}$
CL	Clearance after intravenous administration, calculated as: $CL = \frac{Dose}{AUC_{inf}}$
CL/F	Clearance after extravascular administration, calculated as: $CL = \frac{Dose}{AUC_{inf}}$
F	Bioavailability, calculated as: $F = \frac{AUC_{inf}\,IP}{AUC_{inf}\,IV} * \frac{DoseIV}{DoseIP}$

Note that the abbreviations and definitions listed above may differ slightly from the protocol in the phrasing; however, the equations and methods used to generate these parameters do not differ from the protocol.

No value for λz, AUC_{inf}, t½, or CL will be reported for cases that do not exhibit a terminal log linear phase in the concentration versus time profile. PK parameters will be calculated for subjects with detectable concentrations for at least 4 time points. Additional PK parameters may be calculated, as necessary, to fully characterize the PK profiles of total iron.

Individual PK parameters will be listed by subject and treatment. Summary statistics of PK parameters will be tabulated by treatment. The following summary statistics will be presented for PK parameters: arithmetic mean, CV%, SD of the arithmetic mean, median, minimum,

maximum, N, geometric mean, and CV% of the geometric mean. T_{max} and T_{last} will be presented as median, minimum, and maximum.

8.2. Quantitation of Iron Administered

An estimate of the amount of iron transferred from the PD solution to the circulation will be obtained in two different analyses:

- The C_{max} and AUC_{0-t} for sFe at each dose level compared to the C_{max} and AUC_{0-t} for the Triferic IV administration will be used to calculate the relative bioavailability of Triferic iron during the PD period.
- A sample of the initial dosing solution and the final PD drainage will be analyzed for iron content using ICP-MS (conducted by the bioanalytical lab). The difference between the dosing solution and the final drainage (corrected for volume effects) is a direct measure of Triferic iron absorbed during the PD dwell.

9. Imputation of BLQ values

For calculation of mean concentrations and generation of mean concentration time profiles, all below the limit of quantification (BLQ) values will be set to zero except when an individual BLQ falls between two quantifiable values, in which case it will be treated as missing data.

For the pharmacokinetic analysis and individual concentration vs time plots, a concentration that is BLQ is assigned a value of zero if it occurs in a profile before the first measurable concentration. If a BLQ value occurs after a measurable concentration in a profile and is followed by a value above the lower limit of quantification, then the BLQ is treated as missing data. If a BLQ value occurs at the end of the collection interval (after the last quantifiable concentration) it is treated as missing data. If two BLQ values occur in succession after C_{max}, the profile is deemed to have terminated at the first BLQ value and any subsequent concentrations are omitted from pharmacokinetic calculations.

In circumstances where alternative approaches to handling BLQ data are necessary, the relevant modifications will be appropriately documented in the final PK Report.

10.Significant Figures

Significant figures for concentrations and PK parameters, with the exception of T_{max}, will be generally reported as 3 significant figures. All associated summary statistics for these parameters will also be reported to 3 significant figures with the exception of N which will be reported as an integer. T_{max} will be reported to 2 decimal places.

11. Tables, Listings, and Figures

Lists of Tables, Figures, Listings, and Appendices to be generated with the analyses are provided below. Additional Listings, Tables, and Figures may be generated, as necessary, to fully characterize and explore the available data.

Note: Text in parenthesis is for clarification purposes, and may not appear in the TLF caption

11.1. List of Tables

- Subject Demographics and Baseline Characteristics
- Subject Accountability Table
- Absolute Total Iron Data and Summary Statistics by Sample Collection Time, Cohort, and Route of Administration
- Absolute Total Iron Data and Summary Statistics by Sample Collection Time, Cohort, and Route of Administration (Clinical Laboratory Data)
- Transferrin Saturation Data and Summary Statistics by Sample Collection Time, Cohort, and Route of Administration
- Total Iron Binding Capacity Data and Summary Statistics by Sample Collection Time, Cohort, and Route of Administration
- Serum Ferritin Data and Summary Statistics by Sample Collection Time, Cohort, and Route of Administration
- Serum Hepcidin Data and Summary Statistics by Visit and Cohort
- Noncompartmental Pharmacokinetic Parameters for Total Iron by Cohort and Route of Administration
- Noncompartmental Pharmacokinetic Parameters for Total Iron by Cohort and Route of Administration (Clinical Laboratory Data)

11.2. List of Figures

- Mean Total Iron vs Time by Cohort with Route of Administration Overlaid
- Mean Total Iron vs Time by Route of Administration with Cohorts Overlaid
- Overlay Plot of Individual Subject Total Iron vs Time with Data Separated by Cohort and Route of Administration
- Mean Total Iron vs Time by Cohort with Route of Administration Overlaid (Clinical Laboratory Data)
- Mean Total Iron vs Time by Route of Administration with Cohorts Overlaid (Clinical Laboratory Data)
- Overlay Plot of Individual Subject Total Iron vs Time with Data Separated by Cohort and Route of Administration (Clinical Laboratory Data)

11.3. Listings and Appendices

- Listing of Total Iron Data for Noncompartmental Analysis
- Listing of Total Iron Data for Noncompartmental Analysis (Clinical Laboratory Data)
- Listing of Transferrin Saturation Data
- Listing of Total Iron-binding Capacity Data
- Listing of Ferritin Data
- Listing of Hepcidin Data
- Individual Subject Total Serum Iron vs Time Plots with Route of Administration Overlaid
- Individual Subject Total Serum Iron vs Time Plots with Route of Administration Overlaid (Clinical Laboratory Data)
- Individual λz Plots for Total Iron Noncompartmental Analysis