Statistical Analysis Plan

Ferric Citrate for the Prevention of Renal Failure in Adults with Advanced Chronic Kidney Disease (USRC 2021-002)

"FRONTIER"

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1. ABBREVIATIONS

Abbreviation or Term	Definition
AE	Adverse event
AESI	Adverse event of special interest
ASCVD	Atherosclerotic cardiovascular disease
CHF	Congestive heart failure
CKD	Chronic kidney disease
eGFR	Estimated Glomerular Filtration Rate
EOT	End of treatment
ESA	Erythropoiesis-stimulating agent
IV	Intravenous
KFRE	Kidney Failure Risk Equation
MedDRA	Medical Dictionary for Regulatory Activities
PP	Per protocol
PT	Preferred term
RBC	Red blood cell
SAE	Serious adverse event
SOC	Standard of care

Abbreviation or Term	Definition
TSAT	Transferrin saturation

2. INTRODUCTION

This statistical analysis plan (SAP) contains definitions of analysis sets and statistical methods for the planned analyses in protocol USRC-2021-002 (Version 5.0, 28JUL2022).

2.1. Overview of Trial Design

This is an investigator-initiated multicenter, randomized, double-blind, placebo-controlled, pragmatic clinical trial in patients with advanced chronic kidney disease (CKD) to determine the effect of ferric citrate versus placebo on the time to a composite endpoint of initiation of maintenance dialysis or all-cause mortality. Secondary objectives of this trial are to evaluate the impact of ferric citrate on time to all-cause hospitalization and individual components of the primary endpoint.

The study periods are:

- Screening Period: Up to 45 days prior to Day 1. Standard of Care (SOC) laboratory collection permitted up to 45 days (up to 90 days for estimated glomerular filtration rate [eGFR]) prior to Day 1.
- Treatment Period: Up to 9 months
- Safety follow-up Period: 30 days after end of treatment (EOT)

Subjects will be stratified by site and by eGFR (above or equal to/below 15 ml/min/1.73m^2) randomized in a 1:1 ratio to receive either ferric citrate or matching placebo.

All subjects will receive a fixed dose of two tablets orally taken with meals or snacks up to three times per day. The maximum daily dose is six tablets. Subjects will remain on study drug for up to 9 months followed by an additional 30-day safety follow-up period. Study drug will be stopped for subjects who initiate maintenance dialysis or receive a kidney transplant. Subjects who undergo maintenance dialysis or receive a kidney transplant will be removed from study with no follow-up visit, however, these subjects will have a vital status check (to ascertain mortality) performed 30-days following the relevant event.

Each randomized subject will complete five study visits (2 in-person and three phone/virtual or in-person). Study visits during the treatment period will be conducted as part of routine scheduled clinical encounters. Standard of care local laboratory results will be collected, including the monitoring of phosphorus and iron indices (TSAT and ferritin) at quarterly intervals.

Management of serum phosphorus and iron sufficiency will be directed at the discretion of the treating physician and/or Investigator independent of study drug with the exception of protocol required safety adjustments of study drug. There is no minimum allowable dose of study drug, and the prescribed dose of study drug will be adjusted only in the event of a safety or tolerability concern.

3. STATISTICAL CONSIDERATIONS

Data collected throughout the study will be summarized in tables with descriptive statistics and listed in by-subject listings. Missing information will be summarized by counts for each variable. Continuous variables will be summarized using number of subjects with data, mean, standard deviation, median, first and third quartile, minimum, and maximum. For categorical variables, the number and percentage of subjects in each category will be tabulated. Summaries will be provided by treatment group within appropriate analysis populations and by time point/time period, as appropriate.

3.1. Sample Size

The sample size for this study is driven by the primary endpoint, initiation of maintenance dialysis or all-cause mortality and is based on the observed data from the pilot study (Block, 2019) using nearly identical inclusion and exclusion criteria. The observed rate of the primary composite endpoint in the pilot study (all-cause mortality, maintenance dialysis or kidney transplant) for patients in the standard of care arm was 0.94 events per person-year of follow up. The observed hazard ratio was 0.42 (58% relative risk reduction, p=0.002) for the primary composite endpoint when comparing ferric citrate treated patients versus usual care.

Assuming 1 year recruitment and 0.75 years of follow up (9 months) for each individual and a hazard ratio of 0.60 (40% relative risk reduction), a sample size of 400 patients (200 per arm) provides 90% power with a 2-sided alpha p <0.05. Assuming a hazard ratio of 0.50 (50% relative risk reduction), a sample size of 225 patients provides 90% power with a 2-sided alpha p<0.05.

3.2. Populations for Analyses

The following analysis populations will be used in this study:

- Randomized population: defined as all randomized subjects.
- Full analysis population: defined as randomized subjects receiving one or more doses of study drug. This population will be analyzed based upon the randomized treatment.
- Safety population: defined as all subjects who received at least one dose of study drug. This population will be analyzed based upon the actual treatment received.
- Per protocol (PP) population: defined as full analysis subjects who received a non-zero dose of study medication at least 70% of their time in study and have no protocol deviations affecting the primary endpoint analyses. This population will be analyzed based upon the treatment received.

Any subject who is randomized to study intervention but does not receive study drug will be considered an early termination.

3.3. Missing or Incorrect Data

Discontinuation from study intervention is different from discontinuation from the study, and remaining study procedures will be completed as indicated by the study protocol unless the subject withdraws consent for participation in the study. The date of vital status for all patients who early term will be capped at 37 days post the early termination date. For all patients who complete the study through 9 months, the 10-month follow-up safety visit date will be capped at 307 days post study drug Day 1.

All data pertaining to the primary and secondary endpoints collected at any point during the study, both during study drug treatment and post-study drug treatment discontinuation, will be used for the primary analysis of the endpoints and its individual components.

3.4. Statistical Analyses

3.4.1. General Considerations

Data summaries for categorical variables such as serious adverse events (SAEs) and adverse events of special interest (AESI) will use descriptive statistics including incidence for subjects with, as well as counts for number of events. Data summaries for continuous variables such as laboratory parameters will use descriptive statistics for mean, standard deviation, median, first and third quartiles, minimum, and maximum. Change from baseline will be calculated, with the addition of a 95% confidence interval. Within-group and between group changes from baseline for continuous parameters will be assessed.

3.4.2. Baseline Characteristics

Descriptive statistics will be generated for demographic and pretreatment variables for the safety population defined in the protocol.

Medical history terms will be summarized by treatment group in the safety population. This will include:

- Congestive heart failure, defined as systolic or diastolic dysfunction, heart failure with reduced ejection fraction, or heart failure with preserved ejection fraction
- Diabetes, defined as type I or type II
- Atherosclerotic cardiovascular disease, defined as coronary artery disease, angioplasty, stent, myocardial infarction, cerebrovascular accident, or transient ischemic attack
- Hypertension
- Peripheral vascular disease defined as amputation or artery revascularization, stent, or angioplasty
- Dyslipidemia
- Anemia

- Secondary Hyperparathyroidism
- COVID-19 infection

3.4.3. Protocol Deviations

Important protocol deviations will be summarized for the full analysis population. For the purposes of this protocol, important deviations are defined as any subject who:

- Entered the study even though they did not satisfy entry criteria
- Received wrong treatment or incorrect dose (i.e., patient not stopping IP when protocol required, patient not decreasing IP when serum phosphorus and iron indices indicate)
- Received excluded concomitant treatment, including commercial Auryxia
- Does not discontinue prescribed phosphate-lowering therapy or oral/IV iron therapy in response to low phosphorus or elevated iron indices, per Section 7.1.2.1
- Does not receive quarterly monitoring of serum phosphorus and iron indices (with documented Investigator review).

A listing of subjects with important protocol deviations will be provided.

3.4.4. Analysis of Primary Endpoints

The primary endpoint is the time to composite endpoint of initiation of maintenance dialysis or all-cause mortality. A statistically significant hazard ratio less than 1 will be considered as confirmatory evidence of efficacy. The primary endpoint will be analyzed, based on the full analysis set, by Cox-proportional hazards regression model, with covariates included age, diabetes, atherosclerotic cardiovascular disease (ASCVD), congestive heart failure (CHF), and eGFR. The proportionality assumption of the model will be examined. HRs and corresponding 95% confidence intervals will be presented for the primary endpoint (initiation of maintenance dialysis or all-cause mortality). Analyses will be performed using the per protocol population as supportive.

3.4.5. Analysis of Secondary Endpoints

For the secondary endpoints listed below, Cox-proportional hazards model, using the same covariates as for the primary endpoint analysis, will be based on the full analysis set. Hazard ratios and corresponding 95% confidence intervals will be presented.

- Time to first event hospitalization reported as a SAE (excluding disease-related hospitalization [e.g., fistula replacement, dialysis initiation, kidney transplant] and elective procedures),
- Component of Primary – time to initiation of maintenance dialysis
- Component of Primary – time to all-cause mortality

Analyses will be performed using the per protocol population as supportive.

3.4.6. Tertiary Endpoint(s)

Tertiary endpoints include:

- Annualized rate of hospitalizations, incidence of hospitalizations, and proportion of days hospitalized prior to initiating maintenance dialysis
- Receipt of kidney transplant prior to experiencing a primary endpoint event
- Annualized incidence of receipt and cumulative dose of therapy for anemia including (individually, [ESA, intravenous iron, RBC transfusion]) prior to initiating maintenance dialysis
- Time to RBC transfusion

3.4.7. Subgroup analyses

Data will be analyzed within the following subgroups of interest, as appropriate:

- eGFR within 3 months of Screening:
    - ≥15 to ≤20 ml/min/1.73 m^2
    - <15 ml/min/1.73 m^2
- Diabetes versus Non-diabetic patients
- 3-variable Kidney Failure Risk Equation (KFRE) score [Tangri 2011] within 3 months of Screening:

- ≥20% over 2 years
- <20% over 2 years

- Per protocol subsets using thresholds of 50%, 60%, and 80% of time on study that a subject received a non-zero dose of study medication.

3.5. Study attrition and disposition

We will summarize study attrition by treatment group, including patients randomized, received study drug, and completed. We will identify those who ended the study due to study endpoint (death, initiation of maintenance dialysis, transplant), adverse event, withdrawal of consent, lost to follow up, investigator decision, and all other reasons.

3.6. Safety Analyses

Hypotheses are not provided for safety outcomes. Observed measures of the following biochemical results will be reported at each visit and change from baseline at each visit: serum phosphorus, ferritin, TSAT, hemoglobin, and eGFR. Additionally, for hemoglobin and serum phosphorus, the proportion of patients within clinical standard of care range, using last on treatment value will be reported.

3.6.1. Analysis of Adverse Events

Analyses of safety data will use the safety population and will only be conducted for SAEs, related SAEs, AESI, and laboratory values.

Adverse Events of Special Interest:

- Iron overload is defined as any liver biopsy findings or MRI imaging consistent with this diagnosis in the setting of persistent TSAT >45%.
- Clinically significant hypophosphatemia, defined as serum phosphorus <2.5 mg/dL.

No statistical inference testing will be done for adverse events.

AE analyses, by subject incidence and event counts, will be summarized by preferred terms, within system organ class, by number and percentage for all subjects in the safety population.

All SAEs will be coded using Medical Dictionary for Regulatory Activities (MedDRA). All verbatim terms will be reconciled to preferred terms by the Medical Monitor. Treatment-emergent SAEs will be summarized by system organ class and preferred term (PT) for each treatment group. SAEs will also be summarized by their maximum severity.

A summary and listing of all deaths and cause of death will be provided overall and by treatment group.

3.6.2. Safety Sub-Group Analyses

There are no safety subgroup analyses planned. If appropriate, selected safety analyses may be conducted using the subgroups from the primary analyses defined above.

3.7. Planned Interim Analyses

There are no planned interim analyses for this study.

3.8. Sensitivity / Exploratory Analyses

Primary and secondary endpoint analysis using the mITT population may be conducted using the percentage of time on study a subject received a non-zero dose of study drug as a covariate.

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4. REFERENCES

1. Block G.A., Block M.S., Smits G, Mehta R, Isakova T, Wolf M, Chertow G.M. A Pilot Randomized Trial of Ferric Citrate Coordination Complex for the Treatment of Advanced CKD. *JASN* 2019;30:1495-1504.
2. Tangri N, Stevens LA, Griffith J, et al. A predictive model for progression of chronic kidney disease to kidney failure. JAMA. (2011) (15): 1553-9.

5. SELECT TABLE SHELLS

Table X: Selected Demographic, Clinical, and Laboratory Characteristics at baseline

Variable		Ferric Citrate	Placebo	Significance
eGFR<15 (%)				
Age (mean year)				
Male (%)				
Race (%)	Black			
	White			
	Other			
Ethnicity (n, %)	Hispanic			
	Non-Hispanic			
CKD Cause (%)	Diabetes			
	Hypertension			
	Glomerular disease			
	Polycystic disease			
	Other			
Comorbidity, %				
ASCVD				
Congestive Heart Failure				
Diabetes				
Hypertension				
Peripheral vascular disease				
Cerebrovascular disease				
Secondary hyperparathyroidism				
Dyslipidemia				
Anemia				
Transplant status, %				
Listed or Referred				
Kidney Failure Risk Equation				
Laboratory (mean±SD)				
Creatinine				
eGFR, ml/min per 1.73m^2				

Ferritin, ng/ml				
TSAT				
Phosphorus				

Notes: Therapies and laboratory values estimated during the screening period, prior to Day 1 of the study. Values to be provided as mean (standard deviation) or proportion (count) as appropriate.

Abbreviations: TSAT, transferrin saturation

Table X: Primary Outcome Measures

Table X: Secondary Outcome Measures

Table X: All Adverse Events

Type of AE	Ferric Citrate (n, % with event)	Placebo (n, % with event)
Serious AEs		
Related Serious AEs		
Adverse Events of Special Interest (AESI)		

Abbreviations: AE, adverse event.

Table X: Specific Adverse Events

System Organ Class	Serious AE		Related Serious AE	
	Ferric Citrate (n, % with event)	Placebo (n, % with event)	Ferric Citrate (n, % with event)	Placebo (n, % with event)
Blood and lymphatic system				
Cardiac disorders				
Endocrine disorders				
Eye Disorders				
GI disorders				
General				
Hepatobiliary				
Immune System				
Infections and infestations				
Injury (poisoning)				
Metabolism				
Musculoskeletal				
Neoplasms				

Nervous system				
Psychiatric				
Renal and urinary				
Reproductive				
Respiratory				
Skin				
Vascular				
All Others				

Abbreviations: AE, adverse event.

Additional tables and figures without specific table or figure shells:

Study Disposition Table

Consented

Screened

    Screen Failures

Early Terminations

    Reasons for ET (including endpoints)

Completed 9 months

    Endpoints

Safety Follow-Up (10 months)