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STATISTICAL ANALYSIS PLAN

An Open-Label Study to Evaluate the Efficacy and Safety of AG-348 in Regularly Transfused Adult Subjects with Pyruvate Kinase (PK) Deficiency

AG348-C-007

Version: 3.0

Date: 25-Sep-2020

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
AE	Adverse event
AESI	Adverse event of special interest
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ATC	Anatomical Therapeutic Chemical
BID	Twice daily
BMI	Body mass index
CI	Confidence interval
CRF	Case report form
CSR	Clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
DXA	Dual-energy x-ray absorptiometry
ECG	Electrocardiogram
eCRF	Electronic case report form
EOS	End of study
EOT	End of treatment
EPO	Erythropoietin
EQ-5D-5L	European quality of life five-dimensional descriptive system
FAS	Full Analysis Set
Hb	Hemoglobin
HLT	MedDRA High Level Term
HRQoL	Health-related quality of life
IC	Informed consent
LDH	Lactate dehydrogenase
LFT	Liver function test
LIC	Liver Iron Concentration
LLN	Lower limit of normal
MedDRA	Medical Dictionary for Regulatory Activities
MRI	Magnetic resonance imaging
MTF	Mean transfusion frequency

PD	Pharmacodynamic
PGIS	Patient Global Impression of Severity
PKD	Pyruvate kinase deficiency
PKDD	Pyruvate kinase deficiency diary
PKDIA	Pyruvate kinase deficiency impact assessment
PKR	Pyruvate kinase isoform R
PPS	Per-Protocol Set
PRO	Patient-reported outcome
PT	Preferred Term
QD	Once daily
QOD	Every Other Day
QTc	Heart rate-corrected QT interval
QTcB	Heart rate-corrected QT interval using the Bazett's formula
QTcF	Heart rate-corrected QT interval using the Fridericia's formula
RBC	Red blood cell
SAE	Serious adverse event
SAP	Statistical analysis plan
SD	Standard deviation
SOC	System Organ Class
TEAE	Treatment-emergent adverse event
TIBC	Total iron binding capacity
TRR	Transfusion reduction response
TT	Transfusion Trigger
ULN	Upper limit of normal
WBC	White blood cell
WHO	World Health Organization

1. VERSION HISTORY

This statistical analysis plan (SAP) describes the analysis associated with protocol AG348-C-007, Version 3.0 (dated 19-Mar-2019).

Table 1: Summary of Major Changes in Statistical Analysis Plan Amendments

Version	Version Date	Rationale and Summary of Changes
1.0	30-Apr-2018	Original version.
2.0	21-Aug-2020	Amendment rationale: to incorporate changes to the trial reflected in protocol amendments, to implement changes due to changes in data collection for the study. Major changes: 1. Changes due to protocol amendment 1, protocol v2.0 (15 October 2018) and protocol amendment 2, protocol v3.0 (19 March 2019) a. Updated sample size and power calculation. b. Added decision rule for hypothesis testing c. Updated the primary endpoint to be evaluated in the Fixed Dose Period only d. Updated the primary endpoint to consider subject as non-responder if discontinued before completing 12 weeks of treatment in Fixed Dose Period. e. IDMC was removed f. Updated exploratory objectives and endpoints. 2. Changes due to changes in data collection a. Analysis of study drug compliance based on days on treatment was removed as the diary data was incomplete b. Section 7.4.1, treatment compliance derivation was corrected to be based on tablets dispensed/returned to align with the data collected in the eCRF. Other important changes include: 1. Section 5, added safety analysis set and updated per-protocol set to include subjects who completing 12 weeks of treatment in Fixed Dose Period. 2. Section 6.2, updated power calculations and added hypothesis was added 3. Section 6.3.4, updated the baseline definition to align statistical methodology, as applicable, with that outlined in the SAP for AG348-C-006. 4. Section 6.3.5, added definition of dosing periods and optimized dose 5. Sections 6.4 and 6.5, updated general methodology and methods to handle missing data to align statistical methodology, as applicable, with that outlined in the SAP for AG348-C-006 6. Section 7.6.1, detailed derivations for primary endpoint were added 7. Section 7.6.2, Regression and GLMM analyses on RBC counts and transfusion episodes were removed 8. Section 7.6.3, subgroup analyses were added.

		9. Section 7.7, safety analyses were updated to align statistical methodology, as applicable, with that outlined in the SAP for AG348-C-006 and analyses for AE by dose at onset were added.
3.0	25-Sep-2020	Changes include: 1. Section 6.3.5, updated definitions of dosing periods to align with the definitions outlined in the SAP for AG348-C-006 2. Section 6.4.5 updated the visit window for DXA and LIC by MRI based on the data collection schedule in this study 3. Sections 7.3.2 and 7.5, added definition of mean transfusion frequency as per protocol 4. Sections 7.6.1, 7.6.1.1 and 7.6.2.1, updated the "change" and "Percentage change" to "reduction" and "Percentage reduction", respectively, for transfusion burden 5. Section 7.6.2.3, clarified the definition of transfusion-free in the Fixed Dose Period and added the analysis based on FAS 6. Section 7.8.5.1, corrected the analysis of PKDD to be by visit instead of weekly score and Section 6.3.4 corrected the definition of baseline to remove the exception for PKDD, based on the data collection schedule in this study. 7. Removed summary on PKR protein levels as it will be covered in a separate SAP.

2. INTRODUCTION

This SAP provides the detailed methodology for summary and statistical analyses of the data collected in Study AG348-C-007, except for pharmacokinetic and pharmacodynamic (PD) data, which will be described in a separate SAP. This document may modify the plans outlined in the protocol.

The clinical study report (CSR) will include all data up to the End of Study (EOS) for all subjects, which is defined as the time at which all subjects have completed the study or are lost to follow-up.

3. TRIAL OBJECTIVES AND ENDPOINTS

3.1. Objectives

3.1.1. Primary Objective

The primary objective of the study is to evaluate the efficacy of treatment with AG-348, as assessed by the reduction in transfusion burden.

3.1.2. Secondary Objectives

The secondary objective of this study is to evaluate the safety of treatment with AG-348.

3.1.3. Exploratory Objectives

The exploratory objectives of the study are the following:

- To determine the effect of AG-348 on markers of hemolysis, erythropoietic activity, and other indicators of clinical activity
- To determine the effect of AG-348 on markers of iron metabolism and indicators of iron overload
- To determine the effect of AG-348 on health-related quality of life (HRQoL), as determined using patient reported outcomes (PROs)
- To evaluate the pharmacokinetics of AG-348 after oral administration
- To evaluate the relationship of AG-348 pharmacokinetics to indicators of clinical activity
- To evaluate the change in the levels of PKR protein in whole blood

3.2. Endpoints

3.2.1. Primary Endpoint

The primary endpoint of this study is the proportion of subjects who achieve a reduction in transfusion burden, defined as a ≥33% reduction in the number of RBC units transfused during the 24 weeks of Part 2 compared with the historical transfusion burden standardized to 24 weeks (Standardized Control Period).

3.2.2. Secondary Endpoints

The secondary endpoints of the study are the following:

- Annualized total number of RBC units transfused during the study (both Part 1 and Part 2) compared with the historical transfusion burden
- Number of transfusion episodes during Part 2 compared with the Standardized Control Period
- Proportion of subjects who become transfusion-free, defined as 0 transfusions administered during Part 2
- Proportion of subjects who achieve Hb concentrations in the normal range at least once, 8 weeks or more after a transfusion in Part 2
- Safety endpoints, including the type, incidence, severity, and relationship to treatment of adverse events (AEs) and serious adverse events (SAEs), and AEs leading to treatment dose reduction, treatment interruption, and treatment discontinuation
- Laboratory tests over time (eg, serum chemistry, liver function tests [LFTs], hematology, coagulation, lipids, sex steroids, urinalysis), physical examination (PE) findings, dual-energy X-ray absorptiometry (DXA) scans (hip and lumbar spine), vital signs, and 12-lead electrocardiogram (ECG) data

3.2.3. Exploratory Endpoints

The exploratory endpoints of the study are the following:

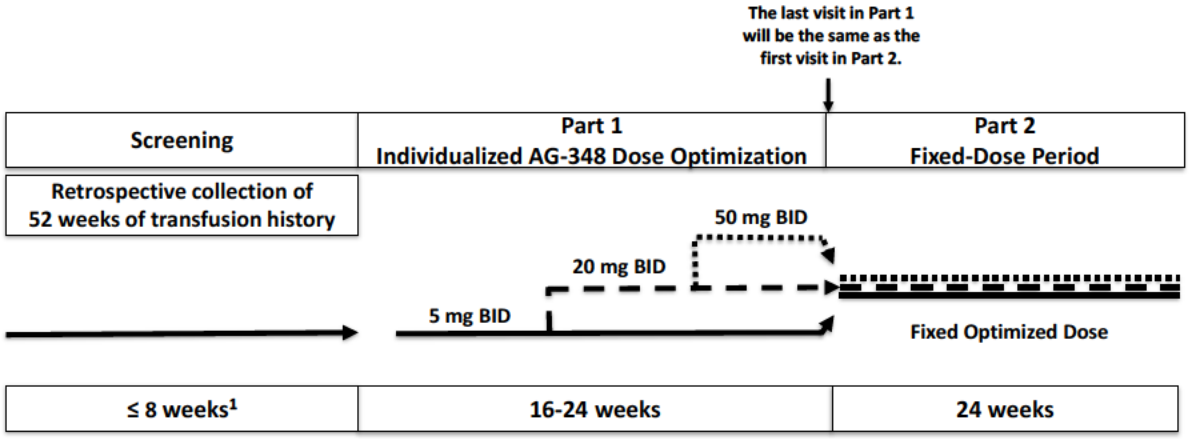
- Change from baseline in the following markers of hemolysis: bilirubin, lactate dehydrogenase (LDH), and haptoglobin levels
- Change from baseline in markers of erythropoietic activity
- Change from baseline in markers of iron metabolism and indicators of iron overload
- Change from baseline over time in HRQoL scores (ie, Pyruvate Kinase Deficiency Impact Assessment [PKDIA], Pyruvate Kinase Deficiency Diary [PKDD], EuroQol-5D-5L [EQ-5D-5L])
- Characterization of pharmacokinetic profile (drug concentrations over time) and determination of pharmacokinetic parameters of AG-348 (eg, AUC, C_{max}, and others as applicable) in Part 2
- Exposure-response (or pharmacokinetic-PD) relationship between relevant pharmacokinetic parameters and endpoints that are indicators of clinical activity
- Change from baseline in PKR protein level.

4. STUDY DESIGN

AG348-C-007 is a 2-part, multicenter, open-label, Phase 3 study consisting of a Dose Optimization Period (Part 1) followed by a Fixed-Dose Period (Part 2). The study will evaluate the efficacy and safety of treatment with AG-348 (hereinafter referred to as mitapivat) in a minimum of 20, with up to 40, adult subjects with PK deficiency who are regularly receiving blood transfusions.

An overview of the study design is provided in Figure 1.

Figure 1: Study Schema



BID = twice daily.

[1] Screening may be extended beyond 8 weeks if there is a delay in obtaining a subject's complete transfusion history or to ensure that the first dose of study drug can be administered 2-7 days after the most recent transfusion, upon approval by the Medical Monitor (or designee).

5. ANALYSIS DATA SETS

Only subjects who sign informed consent (IC) and are screened will be included in the analysis sets below.

- The Full Analysis Set (FAS) will include all subjects who receive at least 1 dose of study treatment.
- The Safety Analysis Set will include all subjects who receive at least 1 dose of study treatment. In this non-randomized study, the FAS and the safety analysis set are identical.
- The Per-Protocol Set (PPS) is a subset of the FAS and will include all subjects who complete 12 weeks of treatment in the Fixed Dose Period (ie, end date of the Fixed Dose Period – start date of the Fixed Dose Period +1 ≥84). See Section 6.3.5 for definition of start and end date of the Fixed Dose Period.

Table 2 summarizes the use of the analysis sets.

Table 2: Analysis Sets for Each Endpoint

Endpoints	Full Analysis Set (FAS)	Per-Protocol Set (PPS)	Safety Analysis Set
Demographic and other baseline characteristics	✓		✓
Disposition	✓		
Major protocol deviations	✓		
Exposure and concomitant therapies			✓
Efficacy	✓	✓ (primary and transfusion-free endpoints only)	
Safety			✓

6. GENERAL STATISTICAL CONSIDERATIONS

6.1. Randomization, Blinding, Unblinding, and Crossover

Not applicable. This is a non-randomized, open-label study.

6.2. Sample Size Determination and Decision Rules

6.2.1. Sample Size Determination

The following statistical hypothesis will be tested to address the primary objective:

$$H_1: \lambda_t \leq 0.1 \text{ vs } H_1: \lambda_t > 0.1$$

where λ_t is the transfusion reduction response (TRR) rate.

Due to the rarity of the disease and the small patient population, the sample size is largely driven by feasibility and the study will enroll a minimum of 20, with up to 40, subjects. The

power to reject H_0 at a 1-sided α=0.025 for different sample sizes and under different assumptions for the true TRR rate is shown in Table 3.

Table 3: Power Considerations

Sample Size	True TRR Rate		
	25%	**30%**	**35%**
20	0.38	0.58	0.75
30	0.49	0.72	0.88
40	0.70	0.89	0.97

6.2.2. Decision Rules

The study will have demonstrated the efficacy of mitapivat if the 1-sided p-value of the exact test for TRR rate is <0.025 and the TRR rate is >0.1.

6.3. Definitions

6.3.1. Study Drug and Study Treatment

Study drug and study treatment are both defined as mitapivat.

6.3.2. Start and End Dates of Study Drug and Study Treatment

The start of study treatment is the earliest date/time of administration of a non-zero dose of the study treatment.

The end of study treatment is the latest date/time of administration of a non-zero dose of the study treatment on or before the EOS date.

6.3.3. Study Day

The study day for assessments or events occurring on or after the start of study treatment (eg, AE onset, laboratory assessment) will be calculated as:

Study day=Date of the assessment or event−start of study treatment+1.

The study day for assessments or events occurring before the start of study treatment (eg, laboratory assessment during the Screening Period, medical history) will be negative and calculated as:

Study day=Date of the assessment or event−start of study treatment.

There is no study day 0. The study day will be displayed in data listings.

6.3.4. Baseline

Efficacy Evaluations

Baseline for transfusion burden will be determined based on the historical transfusion data during the 52 weeks before IC.

For HRQOL assessments, the last measurement before the start of study treatment will be used as the baseline.

For exploratory efficacy hematology laboratory parameters including Hb, markers of hemolysis, erythropoietic activity, iron metabolism and indicators of iron overload, the most recent non-missing measurements before Transfusion 0 (the most recent transfusion occurring in the Screening Period 2-7 days before the start of study treatment on Day 1) will be defined as the baseline to minimize the impact of transfusion on these hematology laboratory parameters.

Safety Evaluations

For alanine aminotransferase (ALT) and aspartate aminotransferase (AST), baseline is defined as the average of all assessments collected within 56 days before the start of study treatment. Baseline will be derived based on central laboratory data; if no central laboratory data are available before the start of study treatment, then local laboratory data will be used to derive the baseline.

For other laboratory assessments:

- Prior to deriving the baseline,
  - If there are multiple records with the same assessment day and time from the same laboratory, the average value will be used
  - If there are multiple records with the same assessment day and time from different laboratories, the value from the central laboratory will be used
- The baseline will then be the last value on or before the start of study treatment.

Triplicate ECGs are collected in this study; the baseline for each ECG measurement is the average of the last predose replicate measurements on or before the start of study treatment. Unscheduled assessments will not be included in the calculation of the average. The average of the replicate measurements will be determined after the derivation of the individual parameter at each time point.

For all other safety parameters, the last assessment on or before the start of study treatment will be used as the baseline.

If, per protocol, an assessment (efficacy or safety) is to be performed on study day 1, before the first dose of study treatment, and the assessment time, time of first dose of study treatment, or both, is missing (or not collected), it will be assumed that the assessment is performed before study treatment administration. Unscheduled assessments will be used in the determination of baseline; however, an unscheduled assessment on study day 1 will be considered to have been obtained after study treatment administration.

If no assessment meets the definition of baseline for an evaluation (efficacy or safety), the baseline will be set to missing.

6.3.5. On-Treatment Period and Optimized Dose

The on-treatment period starts on the date of the start of study treatment and ends 28 days after the end of study treatment.

Within the on-treatment period the following dosing periods are defined:

- **Dose Optimization Period (Part 1)** starts on the date of start of study treatment and ends
  - on the earliest date of EOS, the end of the on-treatment period, and the first day of the dose taper prescription if the subject discontinues study treatment before reaching the Part 2 Day 1 visit.
  - on the day of the Part 2 Day 1 visit, otherwise
- **Fixed Dose Period (Part 2)** starts 1 day after the end of the Dose Optimization Period and ends
  - on the first day of the dose taper prescription, if the subject enters the dose taper period, or
  - 4 days after the end of study treatment, otherwise.
- **Dose Taper Period** starts 1 day after the first day of the dose taper prescription and ends on the earlier date of EOS and the end of on-treatment period. This period is only applicable to subjects who go through the dose taper. The first day of the dose taper prescription is captured in the prescribed dose eCRF page as "planned treatment discontinuation".

Data listings will include all assessments and events, with those that occur outside of the on-treatment period flagged.

The optimized dose is defined as the dose prescribed at the Part 2 Day 1 visit (5 mg BID, 20 mg BID, or 50 mg BID).

6.4. General Methods

6.4.1. Data Handling After Cutoff Date

Not applicable.

6.4.2. Standard Derivations and Reporting Conventions

The following conversion factors will be used to convert days into weeks, months or years: 1 week=7 days, 1 month=30.4375 days, and 1 year=365.25 days.

The following derivations will be implemented.

- Age (years)=(year of given informed consent − year of birth), since only year of birth is collected in the eCRF.

  The integer part of the calculated age will be used for reporting purposes.
- Body mass index (BMI; kg/m^2)=weight (kg)/height $(m)^2$
- Duration (in days) from a reference date (eg, start date of study treatment) =
  - date of event − reference date + 1, if the date of the event is on or after the reference date

- date of event − reference date, if the date of the event is before the reference date

Reporting conventions will be as follows:

- Mean and median will be displayed to one more decimal place than the raw data.
- SD will be displayed to two more decimal places than the raw data.
- Percentages will be displayed to 1 decimal place (however, percentages corresponding to 0 counts will be reported as 0 rather than 0.0 and 100 percent will be reported as 100 rather than 100.0).
- p-values will be reported with 4 decimal places; all p-values should be specified to be 1-sided or 2-sided.
- Unless otherwise specified, rounding will be performed to the closest integer/first decimal using the common mid-point between the two consecutive values, eg, 5.11 to 5.14 will be rounded to 5.1, and 5.15 to 5.19 will be rounded to 5.2.
  - Non-zero percentages that are <0.1 before rounding will be displayed as "<0.1", eg, 0.09 will be reported as <0.1 rather than as 0.1.
  - p-values<0.0001 before rounding will be displayed as "<0.0001", eg, a p-value of 0.00009 will be displayed as <0.0001 rather than as 0.0001.

6.4.3. Pooling of Data Across Sites

In order to provide overall estimates of treatment effects, data will be pooled across sites. The "site" factor will not be considered in statistical models or subgroup analyses given the high number of participating sites in contrast to the anticipated small number of subjects treated at each site.

6.4.4. Continuous and Categorical Variables

Continuous variables will be summarized using descriptive statistics, ie, number of non-missing values, mean, SD, median, quartiles, minimum, and maximum.

Categorical variables will be summarized by frequency distributions (number and percentage of subjects within a given category in the analysis data set). Unless otherwise specified, the calculation of percentages will include the "missing" category. Therefore, counts of missing observations will be included in the denominator and presented as a separate category. For summaries by visit, percentages will be based on the number of subjects with data available for that visit, unless otherwise specified.

6.4.5. Unscheduled Visits

Generally, data collected at unscheduled visits will be included and summarized for both safety and efficacy analyses in the same manner as the data collected at scheduled visits. Data collected at unscheduled visits will be included in by-subject listings together with the data collected at scheduled visits.

Summaries of outliers [eg, worst value, worst change from baseline, worst Common Terminology Criteria for Adverse Events (CTCAE) grade] during the on-treatment period for safety endpoints such as AEs, laboratory measurements and ECG parameters will include data from both scheduled and unscheduled visits.

Individual longitudinal plots for laboratory measurements during the on-treatment period will include data from both scheduled and unscheduled visits.

Descriptive statistics (mean, SD, median, quartiles, minimum, maximum) by visit will be provided for DXA and MRI results with windows derived based on the rules described below.

Analysis Visit Windows

For the evaluation of HRQOL endpoints, the analysis visit windows will be derived based on the target study day for the scheduled visits as follows. Note that based on the scheduled of assessments, a Week 4 Visit, for example, will have a target study day of 1+(4×7)=29.

- Visit windows will be implemented for scheduled visits after Day 1.
- For analysis visit(n):
  - Start day of visit window =1+end day of window for visit(n−1). If n=1, start day of the visit window is study day 2
  - End day of visit window = [(target day for analysis visit(n) + target day for analysis visit(n+1))/2]–1 for visits before the Fixed Dose Period Week 24 visit. The end day of the visit window for the Fixed Dose Period Week 24 visit is the end of the Fixed Dose Period.

For DXA scan results and LIC by MRI, the analysis visit window for

- the Part 2 Day 1 Visit will start 85 days before the start of the Fixed Dose Period and end on day 85 of the Fixed Dose Period or EOS, whichever is earlier
- the Fixed Dose Period Week 24 Visit will start on day 86 ofthe Fixed Dose Period and end on the EOS.
- Results outside visit windows will not be summarized.

Derivation of Values at Scheduled Postbaseline Visits Based on Analysis Visit Windows

For HRQOL data, assessments (scheduled or unscheduled) within the visit windows and on or before the end of the Fixed Dose Period will be used.

If multiple assessments are identified within a visit window for a parameter, the following rules will be applied:

- The assessment measured closest to the target study day of the scheduled visit will be used
- If there are multiple assessments equidistant to the target study day
  - the average value will be used for efficacy laboratory parameters

- the later assessment will be used for HRQOL endpoints

6.5. Methods for Handling Missing Data

6.5.1. Adverse Event and Concomitant Medication Start Dates

If the end date is non-missing and the imputed start date is after the end date, the end date will be used as the start date.

(1) Missing day only

- If the month and year are the same as the month and year of the date of the start of study treatment, the date of the start of study treatment will be used.
- If the month and year are before the month and year of the date of the start of study treatment, the last day of the month will be used.
- If the month and year are after the month and year of the date of the start of study treatment, the first day of the month will be used.

(2) Missing day and month

- If the year is the same as the year of the date of the start of study treatment, the date of the start of study treatment will be used.
- If the year is before the year of the date of the start of study treatment, 31 December will be used.
- If the year is after the year of the date of the start of study treatment, 01 January will be used.

(3) Missing day, month, and year

- The date of the start of study treatment will be used.

6.5.2. Adverse Event and Concomitant Medication End Dates

If the start date is non-missing and the imputed end date is before the start date, the start date will be used as the end date. If an imputation for an AE end date results in an AE end date that is after the EOS date, the AE will be considered as ongoing at the EOS date.

(1) Missing day only

- The last day of the month will be used.

(2) Missing day and month

- 31 December will be used.

(3) Missing day, month, and year

- The event will be regarded as ongoing.

6.5.3. Exposure

No imputation will be done for the date of the first dose of study drug.

If the date of the last dose of study drug is missing or partially missing, it will be imputed as follows (separately for each study drug):

- If the last date of study drug is completely missing and there is no End of Treatment Disposition eCRF page for the study drug AND there is no death date, the subject should be considered to be ongoing and the EOS date for the analysis will be used as the last dosing date.
- If the last date of study drug is completely or partially missing and there is EITHER an End of Treatment Disposition eCRF page for the study drug OR a death date (on or before the EOS date), then the imputed last dose date is:

  =Last day of the year, if only the year is available and Year <Year of min (EOT date, death date)

  =Last day of the month, if both the year and month are available and Year=Year of min (EOT date, death date) and Month <Month of min (EOT date, death date)

  =min (EOT date, death date), for all other cases

7. STATISTICAL ANALYSES

7.1. Subject Disposition

For all subjects screened in the study, the following will be summarized:

- Number of subjects screened in the study
- Frequency (number and percentage) of subjects who discontinued the study before start of study treatment, overall and by reason for discontinuation. Percentages will be calculated based on the number of subjects screened in the study.

In addition, the frequency of subjects in each of the analysis sets described in Section 5 will be summarized. Percentages will be calculated only for analysis sets that are a subset of the FAS or a subset of the safety analysis set.

The following summaries will be presented based on the FAS:

- Frequency of subjects treated in each geographic region, country, and site
- Frequency of subjects with study drug ongoing
- Frequency of subjects who discontinued study drug, overall and by the reason for discontinuation of study drug
- Frequency of subjects who completed the study
- Frequency of subjects ongoing in the study

- Frequency of subjects who discontinued the study, overall and by the reason for study discontinuation

The frequency of subjects with disposition reason, in each epoch, due to reasons associated with COVID-19 will further be summarized under the main reason for discontinuation.

Disposition for all screened subjects will be provided in by-subject listings.

7.2. Protocol Deviations

All major protocol deviations that impact the safety of the subjects, the conduct of the study, or the evaluation of the study results will be reported based on the FAS. These will include:

- Subjects treated despite not satisfying the eligibility criteria
- Subjects who develop withdrawal criteria while on the study but are not withdrawn
- Subjects who receive an excluded concomitant medication

In addition, for each category of major protocol deviations, those related to COVID-19 will be summarized.

Major protocol deviations will be provided in a by-subject listing.

7.3. Demographic and Other Baseline Characteristics

The following summaries will be presented based on the FAS, unless otherwise specified.

7.3.1. Demographics and Physical Measurements

Demographic characteristics and physical measurements at baseline will be summarized as follows:

- Demographic characteristics
  - Sex: male, female (child-bearing potential status will be summarized for female subjects)
  - Race: American Indian or Alaska Native, Asian, Black or African American, Native Hawaiian or other Pacific Islander, White, other, unknown
  - Ethnic origin: Hispanic or Latino, not Hispanic or Latino, not reported
  - Age (years): summary statistics
- Age categories:
  - <65, ≥65 years
  - <35, ≥35 years
- Physical measurements
  - Height (cm)
  - Weight (kg)

- BMI (kg/m^2)

Demographic data for all screened subjects will be provided in a by-subject listing.

7.3.2. Disease Characteristics

The following baseline characteristics of the underlying disease will be summarized based on the data entered in the eCRF:

- Baseline Hb concentration
- Baseline ferritin level
- DXA scan results by location (femoral total and adjusted spine): bone mineral density (BMD) and their corresponding T-scores and Z-scores. Frequency of subjects with T-scores in 3 categories (≤-2.5, >-2.5-<-1, ≥-1.0)
- UGT1A1 genotype
- Prior splenectomy status (Yes, No; if Yes, age of splenectomy)
- Prior cholecystectomy status (Yes, No; if Yes, age of cholecystectomy)
- Prior chelation status (Yes, No); the status is "Yes" if a subject has received chelation therapy within 52 weeks before the first dose of study treatment.
- PKR genotype (missense/missense, missense/non-missense)
- Transfusion History during the 52 weeks before IC:
  - Number of transfusion episodes
  - Number of transfusion episodes standardized to 24 weeks and categories (≤6, >6)
  - Number of RBC units transfused
  - Number of RBC units transfused standardized to 24 weeks and categories (≤6, >6)

  Value standardized to 24 weeks = value for the 52-week period×24/52
  - Mean Transfusion Frequency (MTF) = 52 weeks / number of transfusion episodes
  - Average duration between transfusions = (last transfusion date – first transfusion date +1 – number of transfusions) /(number of transfusions –1)
  - Average RBC units transfused (unit/transfusion)
  - Individual Transfusion Trigger (TT) as reported by the investigator
- Transfusions on or after IC and before start of study treatment
  - Number of transfusion episodes
  - Time from last transfusion to start of study treatment = start of study treatment – date of last transfusion

- Number of RBC units transfused
- Average of RBC units transfused (unit/transfusion)

In the calculations of number of transfusion episodes, transfusions received over up to 3 consecutive days will be counted as 1 episode.

Data on disease characteristics will be provided in by-subject listings.

7.3.3. Medical History

Medical history will be summarized in frequency tabulations according to the latest version of the Medical Dictionary for Regulatory Activities (MedDRA) by System Organ Class (SOC) and Preferred Term (PT).

Medical history will be provided in by-subject listings.

7.3.4. Prior Therapies

The following summaries will be presented based on the safety analysis set.

Prior medications are defined as medications (from the Prior and Concomitant Medications eCRF) that are started before the start of study treatment.

All non-study medications will be coded according to the Anatomical Therapeutic Chemical (ATC) code and PT using the latest version of the World Health Organization (WHO) Drug Dictionary. All prior medications will be summarized in frequency tabulations according to the WHO ATC third level and PT.

Prior medications will be provided in a by-subject listing.

Prior procedures are defined as procedures (from the Surgical History and Concomitant Procedures eCRF) that are started before the start of study treatment.

The prior procedures will be coded according to the latest version of the MedDRA by SOC and PT and will be summarized in frequency tabulations by SOC and PT.

Prior procedures and surgical history of splenectomy will be provided in by-subject listings.

Prior transfusions are collected in the Transfusion History eCRF and will be summarized as described in Section 7.3.2.

7.4. Exposure to Study Drug and Compliance

The following summaries will be presented based on the safety analysis set.

7.4.1. Treatment Duration and Exposure

The frequency of subjects with an optimized dose of 5 mg BID, 20 mg BID or 50 mg BID will be summarized by treatment arm.

Duration of exposure will be summarized as a continuous variable as well as in categories (>0-8, >8-16, >16-24, >24-32, >32-40, and >40 weeks), where

Duration of exposure = end date of study drug – start date of study drug +1.

Study drug compliance will be summarized based on percentage of tablets taken, where

- Percentage of tablets taken = 100×(total number of tablets administered)/(total number of tablets intended)
- Total number of tablets administered = total number of tablets dispensed – tablets returned
- Duration of prescription = end date of prescription – start date of prescription +1
- Number of tablets intended during each prescription: for each new prescription, prescribed dosing frequency × duration of the prescription. Prescribed dosing frequency takes value of 0.5, 1, and 2 for every other day (QOD), once daily (QD) and twice daily (BID), respectively.
- Total number of tablets intended=sum of number of tablets intended over all prescriptions

Percentage of tablets taken will be summarized. The frequency of subjects whose compliance is <80% , 80-100%, >100-120%, and >120% will be summarized.

7.4.2. Dose Modifications

The summary of dose modifications will include:

- The frequency of subjects with at least 1 dose reduction
- Summary of reasons for dose reduction

Dose reduction is defined as the prescribed dose being decreased from the previous dose collected in the Prescribed Dose eCRF. Dose prescriptions and modifications will be provided in a by-subject listing.

7.5. Concomitant Therapies

The following summaries will be presented based on the safety analysis set.

Concomitant medications are defined as non-study medications (from the Prior and Concomitant Medications eCRF) that are started during the on-treatment period or are started before the start of the study treatment and end or remain ongoing during the on-treatment period.

All non-study medications will be coded according to ATC code and PT using the latest version of the WHO Drug Dictionary. All concomitant medications will be summarized in frequency tabulations according to WHO ATC third level and PT.

Concomitant procedures are defined as procedures (from the Prior and Concomitant Procedures eCRF) that are started during the on-treatment period or are started before the start of the study treatment and end or remain ongoing during the on-treatment period.

The concomitant procedures will be coded by the latest version of MedDRA by SOC and PT and will be summarized in frequency tabulations by SOC and PT.

Concomitant transfusions are collected in the “On Study Transfusions” eCRF page.

On-study transfusions in each dosing period (Dose Optimization Period, Fixed Dose Period, Dose Taper Period) will be summarized using descriptive statistics:

- Number of transfusion episodes
- Number of RBC units transfused
- MTF(weeks) = (end of dosing period – the start of dosing period +1)/[7×(number of transfusions in the dosing period)]
- Average duration between transfusions = [last transfusion date in the dosing period– first transfusion date in the dosing period +1 – number of transfusions in the dosing period) /(number of transfusions in the dosing period –1)
- Average RBC units transfused unit/transfusion)

In the calculations of number of transfusion episodes, transfusions received over up to 3 consecutive days will be counted as 1 episode.

7.6. Efficacy Analyses

The following analyses will be based on the FAS, unless otherwise specified.

7.6.1. Primary Endpoint

The primary endpoint is the reduction in transfusion burden (transfusion reduction response, TRR), defined as a ≥33% reduction in total number of RBC units transfused during the Fixed Dose Period (on-study transfusion burden) standardized to 24 weeks compared with the historical transfusion burden standardized to 24 weeks.

- Historical transfusion burden standardized to 24 weeks (units/24-weeks) = total number of transfused RBC units during the 52 weeks before IC ×24/52.
- On-study (Fixed Dose Period) transfusion burden standardized to 24 weeks = 24×total number of transfused RBC units in the Fixed Dose Period / (total number of days in the Fixed Dose Period/7).

Transfusion reduction response will be evaluated based on percentage reduction in transfusion burden standardized to 24 weeks:

- Percentage reduction in transfusion burden standardized to 24 weeks = [Historical transfusion burden standardized to 24 weeks – On-study (Fixed Dose Period) transfusion burden standardized to 24 weeks] / historical transfusion burden standardized to 24 weeks.

Subjects who complete at least 12 weeks of treatment in the Fixed Dose Period (ie, end date of the Fixed Dose Period - start date of the Fixed Dose Period +1 ≥84) will be consider responders if the percentage reduction in transfusion burden is ≥33%. Subjects who discontinue the study before completing 12 weeks of treatment in the Fixed Dose Period will be considered non-responders.

7.6.1.1. Primary Analyses

The frequency of subjects with TRR will be summarized based on the FAS along with the 1-sided p-value of binomial exact test and the 2-sided 95% exact CI using the Clopper-

Pearson method (exact CI for a binomial proportion as computed by default by the FREQ procedure using the EXACT option).

The frequency of subjects with TRR and the 2-sided 95% exact CI will also be summarized by optimized dose. If the number of subjects with an optimized dose is small (<10% of subjects), the results by optimized dose may not be interpretable.

Historical transfusion burden standardized to 24 weeks, on-study (Fixed Dose Period) transfusion burden standardized to 24 weeks and its reduction and percentage reduction from historical transfusion burden standardized to 24 weeks will be summarized.

The frequency of subjects with different categories of percentage reduction will be provided (<0, 0 to <20, 20 to <33, 33 to <50, ≥50%).

7.6.1.2. Sensitivity Analyses

To evaluate the impact of early discontinuation in the primary analysis a sensitivity analysis will be conducted based on the PPS. The frequency of subjects with TRR will be summarized along with the 2-sided 95% exact CI using the Clopper-Pearson method.

7.6.2. Secondary Endpoints

7.6.2.1. Annualized total number of RBC units transfused during the study compared with the historical transfusion burden

Annualized historical transfusion burden = total number of RBC units transfused during the 52 weeks before IC.

Annualized total number of RBC units transfused (units/52-weeks) during the study will be evaluated based on two calculations:

- Including data up to EOS:

  =52× total number of RBC units transfused during the entire study/ [(date of EOS – date of start of study treatment + 1)/7].

- Including data up to the end of Fixed Dose Period:

  =52× total number of RBC units transfused during the Dose Optimization and Fixed Dose Periods combined/ [(end date of the Fixed Dose Period – date of start of study treatment + 1)/7].

The annualized historical transfusion burden, the annualized total number of RBC units transfused and its reduction and percentage reduction from the annualized historical transfusion burden will be summarized.

The frequency of subjects with different categories in percentage reduction will be provided (<0, 0 to <20, 20 to <33, 33 to <50, ≥50%).

7.6.2.2. Number of transfusion episodes

Number of historical transfusion episodes standardized to 24 weeks = total number of transfusion episodes in 52 weeks before IC ×24/52.

Number of transfusion episodes during the Fixed Dose Period standardized to 24 weeks = 24×total number of transfusion episodes in the Fixed Dose Period / (total number of days in the Fixed Dose Period/7).

In these calculations, transfusions received over up to 3 consecutive days will be counted as 1 episode.

The number of historical transfusion episodes standardized to 24 weeks, the number of transfusion episodes during the Fixed Dose Period standardized to 24 weeks and its change and percent change from the number of historical transfusion episodes standardized to 24 weeks will be summarized.

7.6.2.3. Transfusion-free

A subject is considered to become transfusion-free in a dosing period if he/she does not receive transfusions during that period.

The frequency of subjects who become transfusion-free in the Fixed Dose Period will be summarized along with the 95% exact CI using the Clopper-Pearson method. Subjects who did not complete 12 weeks of treatment in the Fixed Dose Period will not be considered as transfusion-free. This analysis will be conducted based on the FAS and PPS.

7.6.2.4. Normal Hb concentrations

The frequency of subjects who achieve Hb concentrations in the normal range at least once in the Fixed Dose Period if the normal Hb sample is taken ≥8 weeks after the latest transfusion before the date of sample collection, will be summarized along with the 95% exact CI using the Clopper-Pearson method.

7.6.2.5. Transfusion trigger

Subjects should be transfused when the subject has reached his/her individual Transfusion Trigger (TT), as calculated by the investigator at screening and recorded in the eCRF.

When a subject's Hb concentration is below the upper margin of his/her individual TT but did not receive a transfusion, the reason (clinically asymptomatic, scheduling challenges, patient decision, waiting for additional assessments, other) will be collected in the transfusion trigger eCRF.

To assess transfusion compliance, the following summaries will be provided:

- Frequency of subjects who had missed transfusions after reaching TT, overall and by reason
- Frequency of subjects who received a transfusion without reaching TT, overall and by reason (adverse event, other)

7.6.3. Subgroup Analyses

Subgroup analyses to be performed for the primary endpoint based on the FAS are presented in Table 4.

Table 4: Subgroup Analyses for TRR

Subgroup	Categories
PKR genotype	missense/missense, missense/non-missense
Baseline individual TT	<8.5 g/dL, ≥8.5 g/dL
Historical transfusion episodes during the 52 weeks before IC standardized to 24 weeks	≤ 6, > 6
Number of RBC units transfused during the 52 weeks before IC standardized to 24 weeks	≤6 units, >6 units
Splenectomy at baseline	Yes, No

TRR rate will be summarized for each category within each subgroup along with the 95% exact CI using the Clopper-Pearson method.

Efficacy analyses in subgroups will be purely exploratory and are intended to evaluate the consistency of treatment effect. If there is a low number of subjects within a category (≤10% of the subjects in the FAS), the subgroup may not be summarized.

7.7. Safety Analyses

Summaries of safety data will be presented based on the safety analysis set.

7.7.1. Adverse Events

Treatment-emergent adverse events (TEAEs) are AEs with a first onset date during the on-treatment period or worsening from baseline. All summaries described below will be based on TEAEs, if not otherwise specified.

All AEs will be listed by subject and AEs with onset outside of the on-treatment period will be flagged in the listings. Unless otherwise specified, TEAEs will be summarized according to the latest version of MedDRA by SOC and/or PT, severity (based on CTCAE v4.03 grading), seriousness, and relation to study treatment in decreasing frequency based on the frequencies observed for all subjects.

Each subject will be counted only once within each SOC or PT. If a subject experiences multiple TEAEs under the same PT within a SOC for the same summary period, only the TEAE assessed as related or with the worst severity, as applicable, will be included in the summaries of relationship and severity. If a subject has TEAEs with missing and non-missing grades, the maximum of the non-missing grades will be displayed. No imputation of missing grades will be performed.

The following will be summarized:

- TEAEs by SOC and PT
- TEAEs by SOC, PT, and worst grade
- Most common TEAEs and Grade ≥3 TEAEs by PT; these will include TEAEs (any grade) reported in ≥10% of all subjects or Grade ≥3 TEAEs reported in ≥5%

of all subjects. These thresholds may be changed based on the observed data without an amendment to this SAP.

- Treatment-related TEAEs, by SOC and PT
- Treatment-related TEAEs, by SOC, PT, and worst grade
- Grade ≥3 TEAEs, by SOC and PT
- Treatment-related Grade ≥3 TEAEs, by SOC and PT
- Serious TEAEs, by SOC and PT
- Treatment-related Serious TEAEs, by SOC and PT
- TEAEs leading to discontinuation of study drug, by SOC and PT
- TEAEs leading to interruption of study drug, by SOC and PT
- TEAEs leading to dose reduction, by SOC and PT
- TEAEs leading to death, by SOC and PT
- Treatment-related TEAEs leading to death, by SOC and PT

In addition, the following will be summarized by prescribed dose at TEAE onset:

- First occurrence of TEAEs by PT
- First occurrence of serious TEAEs by PT
- TEAEs by PT
- Serious TEAEs by PT

7.7.1.1. Adverse Events of Special Interest

Transaminase increase is an AESI for mitapivat and will be reported by the investigator in the AESI eCRF page if there is a transaminase increase of >2.5×baseline or an increase in AST or ALT to Grade ≥2 in severity, whichever is lower.

Additional TEAEs of interest for mitapivat are as follows:

- AEs of endocrinological interest (identified based on the criteria outlined in the mitapivat program specified Safety Search Criteria)
- Insomnia (PTs under HLT of “Disturbances in Initiating and Maintaining Sleep” or identified based on the criteria outlined in the mitapivat program specified Safety Search Criteria)

The following will be summarized for AESIs and the additional TEAEs of interest:

- AESIs/TEAEs of interest by PT
- AESIs/TEAEs of interest by PT and worst grade
- Grade ≥3 AESIs/TEAEs of interest by PT
- AESIs/TEAEs of interest leading to discontinuation of study drug by PT

- Serious AESIs/TEAEs of interest by PT
- AESIs/TEAEs of interest leading to death by PT

In addition, the following will be summarized by prescribed dose at TEAE onset for the additional AE of interest "Insomnia":

- First occurrence of TEAEs by PT
- First occurrence of serious TEAEs by PT
- TEAEs by PT
- Serious TEAEs by PT

7.7.1.2. Adverse Events Associated with COVID-19

The selection of AEs associated with COVID-19 will be based on the MedDRA MSSO list of PTs. The following will be summarized:

- TEAEs associated with COVID-19, by SOC and PT
- Grade ≥3 TEAEs associated with COVID-19, by SOC and PT
- Serious TEAEs associated with COVID-19, by SOC and PT
- TEAEs associated with COVID-19 leading to discontinuation of study drug, by SOC and PT
- TEAEs associated with COVID-19 leading to interruption of study drug, by SOC and PT
- TEAEs associated with COVID-19 leading to dose reduction, by SOC and PT
- TEAEs associated with COVID-19 leading to death, by SOC and PT

7.7.2. Death

The frequency of subjects in the safety analysis set who died will be tabulated based on information from the EOS eCRF. Deaths will be summarized for the following categories:

- On-treatment death: Deaths within 28 days after the last dose of study treatment (ie, deaths during the on-treatment period)
- Post-treatment death: Deaths more than 28 days after the last dose of study treatment (ie, deaths after the end of the on-treatment period)
- Overall: All deaths

In addition, deaths related to COVID-19 will be summarized.

Deaths for all screened subjects will be provided in a by-subject listing.

7.7.3. Clinical Laboratory Data

Clinical laboratory test results will be expressed in SI units. Preferred unit (g/dL) will also be used for Hb in efficacy analysis.

For each laboratory test (chemistry, hematology, coagulation) performed in the study, a by-subject listing of laboratory test results will be presented with the corresponding CTCAE grades (if applicable), laboratory normal ranges, and flags for values below lower limit of normal (LLN) or above upper limit of normal (ULN).

Parameters with CTCAE grades available:

Clinical laboratory test results will be graded according to CTCAE v4.03 as applicable. Grading will be derived based on the numerical thresholds defined by the CTCAE criteria. Non-numerical qualifiers will not be taken into consideration in the derivation of CTCAE grading.

Laboratory test results classified according to CTCAE will be described using the worst grade. For parameters graded with 2 separate toxicity criteria, such as potassium (hypokalemia/hyperkalemia), the toxicities will be summarized separately. Low direction toxicity (eg, hypokalemia) grades at baseline and postbaseline will be set to 0 when the variables are derived for summarizing high direction toxicity (eg, hyperkalemia), and vice versa.

The frequency of subjects with laboratory toxicities during the on-treatment period will be tabulated as follows. The denominator used to calculate percentages for each laboratory test is the number of subjects evaluable for CTCAE grading for that parameter (ie, those subjects for whom a Grade of 0, 1, 2, 3 or 4 can be derived).

- The summary of laboratory parameters by CTCAE grade will include the number and percentage of subjects with Grade 1, 2, 3, 4; Grade 3-4; and Any Grade (Grades 1-4) during the on-treatment period. The highest CTCAE grade during the on-treatment period is considered the worst grade.
- The shift table will summarize baseline CTCAE grade versus worst CTCAE grade during the on-treatment period. The highest CTCAE grade during the on-treatment period is considered the worst grade.
- Newly occurring or worsening laboratory abnormalities (Any Grade, Grade 3-4) during the on-treatment period will also be summarized.

Parameters with CTCAE grades not available:

Results of laboratory tests that are not part of CTCAE will be presented according to the following categories: below the LLN, within normal limits, and above the ULN according to the laboratory normal ranges.

Shift tables will display the frequency of subjects with shifts from baseline missing, <LLN, normal, or >ULN to each of <LLN, normal or >ULN during the on-treatment period.

7.7.3.1. Hematology

For **WBC differential counts** [total neutrophil, lymphocyte, monocyte, eosinophil, and basophil counts], the absolute value will be used when reported. When only percentages are available (relevant primarily for neutrophils and lymphocytes, because the CTCAE grading is based on the absolute counts), the absolute value is derived as follows:

Derived differential absolute count=(WBC count)×(Differential % value/100)

If the range for the differential absolute count is not available (ie, the range is only available for the percentage) then Grade 1 will be attributed as follows:

- Lymphocyte count decreased:
  - Derived absolute count does not meet Grade 2-4 criteria, and
  - % value <% LLN value, and
  - Derived absolute count ≥800/mm^3
- Neutrophil count decreased:
  - Derived absolute count does not meet Grade 2-4 criteria, and
  - % value<% LLN value, and
  - Derived absolute count ≥1,500/mm^3

7.7.3.2. Chemistry

Liver function tests: Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total bilirubin are used to assess possible drug-induced liver toxicity. The ratios of test result to ULN will be calculated and categorized for these parameters during the on-treatment period.

The summary of liver function tests will include the following categories. The frequency of subjects with each of the following during the on-treatment period will be summarized:

- ALT >3×ULN, ALT >5×ULN, ALT >10×ULN, ALT >20×ULN
- AST >3×ULN, AST >5×ULN, AST >10×ULN, AST >20×ULN
- (ALT or AST) >3×ULN, (ALT or AST) >5×ULN, (ALT or AST) >10×ULN, (ALT or AST) >20×ULN
- total bilirubin >2×ULN
- Concurrent ALT >3×ULN and total bilirubin >2×ULN
- Concurrent AST >3×ULN and total bilirubin >2×ULN
- Concurrent (ALT or AST) >3×ULN and total bilirubin >2×ULN
- Concurrent (ALT or AST) >3×ULN and total bilirubin >2×ULN and ALP ≥2×ULN
- Concurrent (ALT or AST) >3×ULN and total bilirubin >2×ULN and (ALP <2×ULN or missing)

Concurrent measurements are those occurring on the same date.

Categories will be cumulative, ie, a subject with an AST >10×ULN will also appear in the categories >5×ULN and >3×ULN. Liver function test elevation and possible Hy's Law cases will be summarized using frequency counts and percentages.

An evaluation of Drug-Induced Serious Hepatotoxicity (eDISH) plot will be created, by graphically displaying:

- Peak serum ALT (/ULN) vs peak total bilirubin (/ULN) including reference lines at ALT=3×ULN and total bilirubin=2×ULN
- Peak serum AST (/ULN) vs peak total bilirubin (/ULN) including reference lines at AST=3×ULN and total bilirubin=2×ULN

In addition, the following individual longitudinal plots and by-subject listings will be provided:

- Individual longitudinal plot of ALT including subjects with at least one ALT during the on-treatment period >2.5×baseline or worsening to CTCAE Grade ≥2 during the on-treatment period
- Individual longitudinal plot of AST including subjects with at least one AST during the on-treatment period >2.5×baseline or worsening to CTCAE Grade ≥2 during the on-treatment period
- Listing of all total bilirubin, ALT, AST, and ALP values for subjects with a postbaseline total bilirubin >2×ULN, ALT >3×ULN, or AST >3×ULN
- Listing of all total bilirubin, indirect bilirubin, ALT, AST and ALP values for subjects with a postbaseline ALT >ULN or AST >ULN

In addition, a shift table from baseline to the worst CTCAE grade of ALT and AST during the on-treatment period will be provided. For each subject:

- If the worst CTCAE grade of ALT is worse than that of AST during the on-treatment period, the baseline CTCAE grade of ALT will be used
- If the worst CTCAE grade of AST is worse than that of ALT during the on-treatment period, the baseline CTCAE grade of AST will be used
- If AST and ALT have the same worst CTCAE grade during the on-treatment period, the lower baseline CTCAE grade of ALT and AST will be used.

For **calcium**, CTCAE grading is based on corrected calcium and ionized calcium. Corrected Calcium is calculated from albumin and calcium as follows:

Corrected calcium (mmol/L)=measured total calcium (mmol/L)+0.02×[40−serum albumin (g/L)]

7.7.3.3. Sex Steroid Tests

For sex steroid test results, shift tables will display the frequency of subjects with shifts from baseline missing, < LLN, normal, > ULN to each of < LLN, normal or > ULN during the on-treatment period.

In addition, individual longitudinal plots will be provided for each sex hormone by sex.

7.7.3.4. Pregnancy Test

Pregnancy test results will be presented in a by-subject listing.

7.7.4. Vital Signs and Physical Measurements

All physical measurements and vital sign assessments (height, weight, BMI, systolic blood pressure, diastolic blood pressure, pulse rate, temperature) will be presented in a by-subject listing.

7.7.5. Electrocardiograms

All ECG summaries and listings will be based on the central reading results.

ECG summaries will include all ECG assessments from the on-treatment period.

Selecting Primary QT Interval Correction for Heart Rate

The analysis of QT interval data is complicated by the fact that the QT interval is highly correlated with heart rate. Because of this correlation, formulas are routinely used to obtain a corrected QT interval, denoted QTc, which is independent of heart rate. This QTc is intended to represent the QT interval at a standardized heart rate. Several correction formulas have been proposed in the literature. For this analysis several of those methods of correction will be used, as described below. The QT interval corrected for heart rate by the Bazett's formula, QTcB, is defined as

$$QTcB = \frac{QT}{\sqrt{RR}}$$

and the QT interval corrected for heart rate by the Fridericia's formula, QTcF, is defined as

$$QTcF = \frac{QT}{\sqrt[3]{RR}},$$

where RR represents the RR interval of the ECG, in seconds.

Although Bazett's correction is the historical standard, it does not perform well when heart rate fluctuates. Fridericia's formula may perform better under these conditions.

ECG Summaries

The following analyses will be performed for each applicable ECG parameter (RR, PR, QRS, QT, and QTc) during the on-treatment period. The denominator used to calculate percentages for each category is the number of subjects evaluable for the category.

- Pearson correlation between QT and RR interval, QTc (QTcF, QTcB) and RR interval using individual (non-averaged) baseline assessments
- Frequency of subjects with notable ECG values, defined as those in the following categories:
  - QT/QTc interval increase from baseline >30 ms, >60 ms

- QT/QTc interval >450 ms, >480 ms, >500 ms
- PR interval >200 ms
- QRS duration >120 ms

All ECG assessments and qualitative ECG abnormalities will be presented in by-subject listings.

7.7.6. DXA Scans

DXA scan results including bone mineral density (BMD), T-scores, Z-scores during the on-treatment period will be summarized by location (total femur and adjusted spine), and visit. For T-scores, shift from baseline to Week 24 in the Fixed Dose Period by category (≤-2.5, >-2.5 to <-1.0, ≥-1.0) will be provided.

All DXA scan results will be presented in a by-subject listing.

7.7.7. Menstrual Cycle Diary

Menstrual cycle diary data collected from women of childbearing potential during the on-treatment period will be summarized by regular contraceptive status (oral contraceptives or depot injection). The following summaries will be included:

- Total number of menstrual cycles reported
- Total number of abnormal menstrual cycles in the following categories: heavier, lighter, longer, shorter, sooner and later than usual.

Menstrual cycle diary data will be presented in a by-subject listing with regular contraceptive status flagged.

7.8. Exploratory Analyses

Unless otherwise specified, summaries for exploratory endpoints will be presented based on the FAS.

7.8.1. Markers of Hemolysis

Markers of hemolysis include bilirubin, LDH, and haptoglobin.

By-subject longitudinal plots will be presented with markers of hemolysis at baseline and postbaseline, transfusions, and prescribed dose over time. The plots will further include optimized dose, age, sex, race, PKLR gene mutation category, baseline individual TT, prior splenectomy status, baseline chelation status, and postbaseline chelation status.

Markers of hemolysis will be presented in a by-subject listing

7.8.2. Erythropoietic Activity Markers

Erythropoietic markers include erythropoietin (EPO), soluble transferrin receptor, erythroferrone, and reticulocyte.

By-subject longitudinal plots will be presented with erythropoietic markers at baseline and postbaseline, transfusions, and prescribed dose over time. The plots will further include

optimized dose, age, sex, race, PKLR gene mutation category, baseline individual TT, prior splenectomy status, baseline chelation status, and postbaseline chelation status.

Erythropoietic markers will be presented in a by-subject listing.

7.8.3. Iron Markers

Iron markers include serum iron, serum ferritin, total iron-binding capacity (TIBC), transferrin saturation, and hepcidin.

By-subject longitudinal plots will be presented with iron markers at baseline and postbaseline, transfusions, and prescribed dose over time. The plots will further include optimized dose, age, sex, race, PKLR gene mutation category, baseline individual TT, prior splenectomy status, baseline chelation status, and postbaseline chelation status.

Iron markers including non-transferrin bound iron (NTBI), and C-reactive protein (CRP) will be provided in listings.

7.8.4. Liver Iron Concentration (LIC) by Magnetic Resonance Imaging (MRI)

Liver MRI results will be listed and summarized by visit.

7.8.5. HRQoL

PRO data are collected with an electronic device outside of the clinic, hence the reported nominal visit will not be used. The analysis visit windows detailed in Section 6.4.5 will be used. When there are multiple assessments within the same visit window, the one closest to the target study day will be used; when there are more than 2 assessment of the same distance to the target day, the last assessment will be used.

Given the nature of the PROs and their limitations in this open label study, no additional modelling will be conducted.

7.8.5.1. PKDD

PKDD is a self-administered, daily, 7-item PRO measure of the core signs and symptoms of PKD in adults. The PKDD daily scores will be calculated based on the subject's response to the PKDD questionnaire.

The PKDD scores up to the end of the Fixed Dose Period will summarized descriptively at each visit.

7.8.5.2. PKDIA

The PKDIA is a 12-item PRO measure of the common impacts of PK deficiency on activities of daily living. Subjects rate how PK deficiency has impacted aspects of daily living in the past 7 days, including impacts on relationships; perceived appearance; work performance; and leisure, social, mental, and physical activities.

The PKDIA score at each visit will be calculated and provided by the instrument developer (Endpoint Outcomes) based on the subject's response to the PKDIA questionnaire.

The following summaries will be provided for PKDIA scores up to the end of the Fixed Dose Period at each visit:

- Summaries of scores for each item. For questions 9a, 11a and 12, the frequency of subjects with answers in each category will be summarized. For questions 9b and 11b, scores from patients who answered "Yes" will be summarized. For all the other questions, responses will be treated as continuous variables and summarized descriptively.
- Descriptive summaries for PKDIA score and change from baseline

7.8.5.3. EQ-5D-5L

For EQ-5D-5L results, the following descriptive summaries will be provided at each visit, including:

- Index score and change from baseline.
- VAS score and change from baseline.
- Frequency of subjects in each level for each of the 5 dimensions.

7.8.5.4. PGIS

Frequency of subjects with answers in each category for each question will be summarized.

7.9. Interim Analyses

There is no interim analysis planned for this study.

8. REFERENCES

Clopper, C., & Pearson, E. S. (1934). The use of confidence or fiducial limits illustrated in the case of the binomial. *Biometrika, 26*(4), 404-413.