Official Title: A Phase 2, Open-label, Multicenter Study to Determine the Efficacy, Safety, Pharmacokinetics, and Pharmacodynamics of AG-348 in Adult Subjects With Non-transfusion-dependent Thalassemia

NCT Number: NCT03692052

Document Date: SAP Version 1: 27 Jul 2020

STATISTICAL ANALYSIS PLAN

A Phase 2, Open-Label, Multicenter Study to Determine the Efficacy, Safety, Pharmacokinetics, and Pharmacodynamics of AG-348 in Adult Subjects with Non-Transfusion-Dependent Thalassemia

AG348-C-010

Version: 1.0

Date: 27-Jul-2020

Prepared by:

[redacted] PhD
Study Statistician

Name and Title (Printed)	Signature	Date (DD MMM YYYY)
	DocuSigned by: [redacted] Signer Name: [redacted] Signing Reason: I approve this document Signing Time: 27-Jul-2020 | 5:25 PM EDT 7B7C1628A1EA48B092DED4902239C01B	27-Jul-2020 | 5:26 PM EDT

Approved by:

[redacted], MD
[redacted]

Name and Title (Printed)	Signature	Date (DD MMM YYYY)
	DocuSigned by: [redacted] Signer Name: [redacted] Signing Reason: I approve this document Signing Time: 27-Jul-2020 | 9:18 PM EDT 45A8610618A3400E93605B3B3FD1E486	27-Jul-2020 | 9:18 PM EDT

[redacted], PhD
[redacted]

Name and Title (Printed)	Signature	Date (DD MMM YYYY)
	DocuSigned by: [redacted] Signer Name: [redacted] Signing Reason: I approve this document Signing Time: 27-Jul-2020 | 6:16 PM EDT 1733C50FC95F44F39DEF81FD0E9BEA2A	27-Jul-2020 | 6:16 PM EDT

TABLE OF CONTENTS, LIST OF TABLES, AND LIST OF FIGURES

LIST OF TABLES

LIST OF FIGURES

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
ATP	Adenosine triphosphate
BID	Twice daily
BMD	Bone mineral density
BMI	Body mass index
CI	Confidence Interval
CSR	Clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
DPG	Diphosphoglycerate
DXA	Dual-energy x-ray absorptiometry
ECG	Electrocardiogram
EOS	End of study
EOT	End of treatment
EPO	Erythropoietin
FAS	Full Analysis Set
GDF	Growth differentiation factor
Hb	Hemoglobin
HLT	High Level Term
LDH	Lactate dehydrogenase
LFT	Liver function test
LIC	Liver iron concentration
LOCF	Last observation carried forward
MedDRA	Medical Dictionary for Regulatory Activities
MRI	Magnetic resonance imaging
Max	Maximum value
Min	Minimum value

Abbreviation	Definition
NRBC	Nucleated red blood cell
NTBI	Non-transferrin bound iron
NTDT	Non-transfusion-dependent thalassemia
PD	Pharmacodynamic
PKR	Pyruvate kinase isoform R
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
WHO	World Health Organization

1. VERSION HISTORY

This statistical analysis plan (SAP) describes the analysis associated with protocol AG348-C-010 Amendment 3, Version 3.0 (dated 24 July 2019).

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

Version	Version Date	Summary of Changes
1.0	27-Jul-2020	Original version.

2. INTRODUCTION

This SAP provides the detailed methodology for summary and statistical analyses of the data collected in Study AG348-C-010 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 primary clinical study report (CSR) will include only data during the Core Period up to the data cutoff date, which is when all subjects have completed the Week 24 Visit if the subject enters the Extension Period, or have completed the Safety Follow-up Visit /discontinued the study (whichever is earlier) if the subject does not enter the Extension Period.

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

In the following sections, references to “data cutoff date/EOS date” are meant to indicate that the data cutoff date will be used for analyses to be reported in the primary CSR and the EOS date will be used for analyses to be reported in the final CSR.

3. STUDY OBJECTIVES AND ENDPOINTS

3.1. Objectives

3.1.1. Primary Objective

The primary objective of this study is to evaluate the efficacy of treatment with AG-348 in increasing hemoglobin (Hb) concentrations in subjects with non-transfusion-dependent thalassemia (NTDT).

3.1.2. Secondary Objectives

The following secondary objectives will be assessed in subjects with NTDT:

- To evaluate the safety of AG-348
- To determine the effect of AG-348 on markers of hemolysis and erythropoietic activity
- To evaluate the pharmacokinetics of AG-348

3.1.3. Exploratory Objectives

The exploratory objectives of this study are as follows:

- To determine the effect of AG-348 in subjects with NTDT on the following:
  - Pharmacodynamic (PD) markers of thalassemia
  - Other markers of erythropoietic activity
  - Markers of iron metabolism and indicators of iron overload
  - Markers of oxidative stress and other related markers
  - Transfusion burden
  - Spleen size
- To evaluate the relationship between AG-348 pharmacokinetics and indicators of clinical activity in subjects with NTDT
- To evaluate the relationship between the dose of AG-348 and change in Hb concentrations in subjects with NTDT

3.2. Endpoints

3.2.1. Primary Endpoint

The primary endpoint of this study is the Hb response, defined as a ≥1.0 g/dL increase in Hb concentration from baseline at 1 or more assessments between Week 4 and Week 12 (inclusive). An individual subject's baseline Hb concentration is defined as the average of all of the subject's available Hb concentrations during the Screening Period up to the first dose of study drug.

3.2.2. Secondary Endpoints

The secondary endpoints of this study are as follows:

- The mean change from baseline in Hb concentrations over a continuous 12-week interval from Week 12 to Week 24
- The sustained Hb response, defined as a subject who has achieved an Hb response and has achieved a ≥1.0 g/dL increase in Hb concentration at 2 or more evaluable Hb assessments out of the 4 scheduled assessments between the Week 12 Visit and Week 24 Visit (ie, Weeks 12, 16, 20, and 24)
- The delayed Hb response, defined as a subject who has not achieved an Hb response, but has achieved a ≥1.0 g/dL increase in Hb concentration at 1 or more Hb assessments after Week 12 (ie, Weeks 16, 20, and 24)
- Change from baseline in Hb concentration over an additional 2 years in the Extension Period
- Time to first ≥1.0 g/dL increase in Hb concentration
- Change from baseline in markers of hemolysis: reticulocyte, bilirubin, LDH, and haptoglobin

- Change from baseline in markers of erythropoietic activity: nucleated RBC (NRBC), EPO, and soluble transferrin receptor
- Safety endpoints of this study are as follows:
  - The type, incidence, severity, and relationship to treatment with AG-348 of adverse events (AEs) and serious adverse events (SAEs), AEs of special interest (AESIs), AEs leading to study drug dose reduction, study drug interruption, and study drug discontinuation
  - Changes over time in clinical laboratory tests (serum chemistry, liver function tests [LFTs], LDH, hematology, coagulation, lipids, sex steroids, and urinalysis), physical examination (PE) findings, bone mineral density (BMD) of the hip and lumbar spine, vital signs, and 12-lead electrocardiogram (ECGs) findings
- Pharmacokinetic endpoints of this study are as follows:
  - Pharmacokinetic endpoints include drug concentrations over time and pharmacokinetic parameters of AG-348, including AUC, Cmax, and others as applicable

3.2.3. Exploratory Endpoints

The exploratory endpoints of this study are as follows:

- Change from baseline in α-, β-, and gamma (γ)-hemoglobin absolute levels and/or ratios
- Change from baseline in other markers of erythropoietic activity: growth differentiation factor (GDF)-15, GDF-11, non-transferrin bound iron (NTBI), and erythroferrone
- Change from baseline in markers of iron metabolism and indicators of iron overload
- Change from baseline in markers of oxidative stress: urinary 8-isoprostane, methylmalonic acid, total homocysteine, and other RBC metabolite measurements
- Proportion of subjects requiring transfusions and the total number of RBC units transfused
- Change from baseline in spleen size as assessed by magnetic resonance imaging (MRI)
- Change in Hb concentrations in relation to the dose of AG-348
- Pharmacokinetic/PD endpoints of this study are as follows:
  - Change from baseline in adenosine triphosphate (ATP), 2,3-DPG concentrations, PKR activity, PKR protein levels, and PKR flux assay results
  - Exposure-response (or pharmacokinetic-PD) relationship between relevant pharmacokinetic parameters and endpoints that are indicators of clinical activity

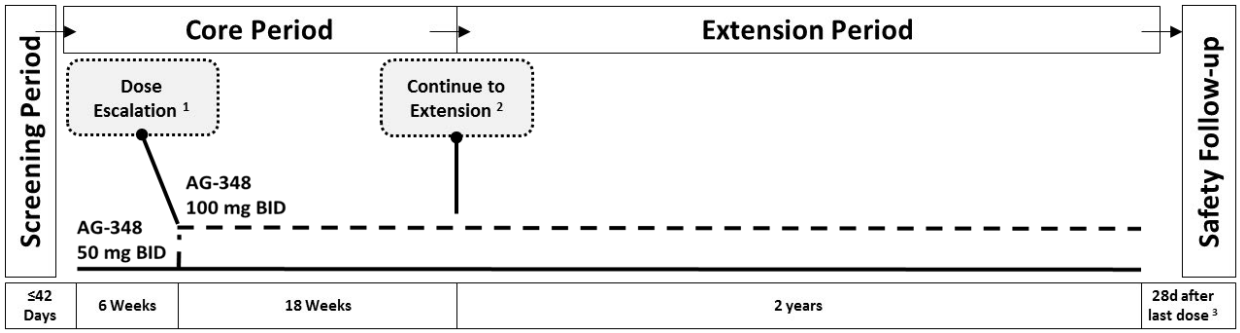
4. STUDY DESIGN

AG348-C-010 is a Phase 2, open-label, multicenter study evaluating the efficacy, safety, pharmacokinetics, and pharmacodynamics of treatment with AG-348 (mitapivat) in adult subjects with NTDT. This study will consist of a 24-week Core Period followed by a 2-year Extension Period. Approximately 17 subjects with NTDT will be enrolled.

All eligible subjects will receive an initial mitapivat dose of 50 mg BID and may undergo an intrasubject dose escalation to 100 mg BID based on an evaluation of the subject's safety and Hb concentrations. Subjects who complete the 24-week Core Period and achieve an Hb response or delayed Hb response with an acceptable safety profile may continue mitapivat treatment in the Extension Period.

An overview of the study design is in Figure 1.

Figure 1: Study Schema



Abbreviations: BID = twice daily; d = days.
[1] Subjects may undergo an intrasubject dose escalation at the Week 6 Visit based on an evaluation of safety and Hb concentration.
[2] Subjects who complete the 24-week Core Period and achieve an Hb response or a delayed Hb response with an acceptable safety profile may continue study treatment for an additional 2 years in the Extension Period.
[3] The Safety Follow-up Visit will occur 28 days (±4 days) after the subject's last dose of study drug (including taper doses).

5. ANALYSIS SETS

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

- The Full Analysis Set (FAS) will include all subjects who have received 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.

Table 2 summarizes the use of the analysis sets.

Table 2: Analysis Sets for Each Endpoint

Endpoints	Full Analysis Set	Safety Analysis Set
Demographic and other baseline characteristics	✓	
Disposition	✓	
Major protocol deviations	✓	
Exposure and concomitant therapies		✓
Efficacy	✓	
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:

$$H_0: \lambda_t = 0.3 \quad \text{vs} \quad H_1: \lambda_t > 0.3$$

where λ_t is the Hb response rate in the mitapivat arm.

With a total of 17 subjects, the study will have 80% power to reject H_0 at a 1-sided α=0.05 when the true response rate is 0.6.

The sample size calculation was based on an exact test implemented using nQuery 8.5.

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 the primary endpoint of Hb response is <0.05 and the Hb response rate is >0.3 at the time of the data cutoff for the primary CSR.

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 data cutoff date/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

For efficacy and exploratory laboratory parameters [Hb, hemolysis markers (indirect bilirubin, LDH, haptoglobin), reticulocyte, iron markers, and erythropoietic markers], baseline is defined as the average of all screening assessments within 45 (42+3) days before the start of study treatment. Assessments collected within 8 weeks (56 days) after a transfusion will be excluded from the baseline derivation.

Safety Evaluations and Baseline Characteristics

For alanine aminotransferase (ALT) and aspartate aminotransferase (AST), baseline is defined as the average of all screening assessments collected within 45 (42+3) days before the start of study treatment.

For other laboratory assessments:

- Before 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.
- The baseline will then be the last value on or before the start of study treatment.

Triplicate ECGs are collected in the 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.

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, baseline characteristic, 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, baseline characteristic, or safety), the baseline will be set to missing.

6.3.5. On-Treatment Period

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 **Core Dosing Period** is also defined as follows:

- Starts on the date of the start of study treatment
- Ends 28 days after the end of study treatment, if the subject does not enter the Extension Period
- Ends on the last dose date of the Core Period, recorded in the Core Period EOT eCRF, if the subjects enter the Extension Period

6.4. General Methods

6.4.1. Data Handling After Cutoff Date

For the primary CSR, data after the cutoff date may not undergo the cleaning process and will not be displayed in any listings or used for summary statistics, statistical analyses or imputations.

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.
- Standard deviation (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 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 nominal visit will only be provided for DXA scans results and liver iron concentrations (LIC) by MRI. Data collected at unscheduled and scheduled postbaseline visits will be mapped to scheduled visits using analysis visit windows, and then values at scheduled postbaseline visits will be derived based on the rules described below.

For efficacy and exploratory endpoints [Hb, hemolysis markers (indirect bilirubin, LDH, and haptoglobin), reticulocyte, iron markers and erythropoietic markers], data collected at unscheduled and scheduled postbaseline visits will be mapped to scheduled visits using analysis visit windows, and then values at scheduled postbaseline visits will be derived based on the rules described below. Descriptive statistics by nominal visit and longitudinal plots during the on-treatment period for efficacy endpoints such as Hb concentration will be provided using the derived values at scheduled visits.

Analysis Visit Windows

For the evaluation of Hb, hemolysis markers (indirect bilirubin, LDH, and haptoglobin), reticulocytes, iron markers, and erythropoietic markers, 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 except for the last scheduled visit. The end day of the last visit window on or before the data cutoff date is the min(cutoff date/EOS date, end of on-treatment period).

For DXA scan results and LIC by MRI, the analysis visit window for the first scheduled assessment at Week 24 Visit will start on study day 86. The derivation for the end day for Week 24 Visit and the visit window for Week 72 and Week 120 will follow the same rule of deriving "analysis visit(n)" specified above.

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 data cutoff date/EOS date, the AE will be considered as ongoing at the data cutoff date/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:

- 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 data cutoff date/EOS date 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 data cutoff date/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 (data cutoff date/EOS date, death date) and Month < Month of min (EOT date, death date)

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

7. STATISTICAL ANALYSES

All summaries will be presented by thalassemia type (α-thalassemia, β-thalassemia) and overall (combined across thalassemia type).

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 the 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.

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 a by-subject listing.

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 [both continuously and by categories (<8.5 g/dL, ≥8.5 g/dL)]
- Baseline Laboratory: reticulocyte, LDH, indirect bilirubin, erythropoietin, haptoglobin, soluble transferrin receptor, transferrin saturation, ferritin
- Iron overload status (Yes, No; Yes if collected in the medical/surgical history eCRF with the preferred term of 'IRON OVERLOAD')

- Transfusion 24 weeks prior to Day 1 (Yes, No; if Yes, RBC units transfused)
- DXA scan by location (femoral total and adjusted spine): Bone mineral density (BMD) and corresponding T-scores and Z-scores. Frequency of subjects with T-scores in 3 categories (≤−2.5, >−2.5-<−1, ≥−1.0)
- Prior splenectomy status (Yes, No; Yes if collected in the medical/surgical history eCRF with the preferred term of 'SPLENECTOMY')
- Total volume of spleen
- Liver iron concentration (LIC) and categories (≤3, >3-≤7, >7-≤15, >15)
- Prior chelation status (Yes, No; Yes if a subject has received chelation therapy within 52 weeks (364 days) before start of study treatment)
- UGT1A1 Type [(TA)6/(TA)6, (TA)6/(TA)7, (TA)7/(TA)7]
- PKLR mutation (present, absent, and unknown)
- Prior Hydroxyurea status (Yes, No, as collected in the prior and concomitant medication eCRF with drug preferred term of 'HYDROXYUREA' started before start of study treatment)

Disease characteristics will be provided in by-subject listings.

7.3.3. Medical History

Medical and surgical 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 and surgical history of splenectomy 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 transfusions are collected from the Transfusion History eCRF.

Transfusion history will be provided in a by-subject listing.

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

Frequency of subjects who remain on 50 mg BID and frequency of subjects who escalated to 100 mg BID during the Core Dosing Period will be summarized.

Duration of exposure to study drug will be summarized as a continuous variable as well as in categories

- For the primary CSR: >0-≤6, >6-≤12, >12-≤24, >24 weeks
- For the final CSR additional categories will be summarized: >24- ≤36, >36-≤60, >60-≤84, >84-≤120, >120 weeks

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 dose/50. 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.

Concomitant transfusions will be provided in a by-subject listing.

7.6. Efficacy Analyses

Efficacy analyses will be based on the FAS unless otherwise specified.

7.6.1. Primary Endpoint

7.6.1.1. Definition

Hb response is defined as a ≥1.0 g/dL increase in Hb concentration from baseline at 1 or more assessments between Week 4 and Week 12 (inclusive). For subjects who discontinue study treatment before Week 4, Hb increase at the last available Hb assessment will be imputed [ie, last observation carried forward (LOCF)] and will be used to evaluate the subject's Hb responder status.

Hb concentrations within 8 weeks after RBC transfusion will be excluded. Details for derivation of Hb at baseline and postbaseline visits are provided in Sections 6.3.4 and 6.4.5.

7.6.1.2. Primary Analysis

The primary analysis will be performed at the time of data cutoff for the primary CSR. In the primary analysis, the frequency of subjects with an Hb response will be summarized based on the FAS along with 1-sided p-value of binomial exact test and the 2-sided 90% 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).

Hb concentrations and their change from baseline will be summarized, by visit during the Core Dosing Period, as a continuous variable. Box plot of Hb change from baseline at each visit will be presented.

By-subject longitudinal plots for Hb concentrations will be presented over time (at each scheduled visit) with prescribed dose. Baseline characteristics of the subject including baseline Hb concentration, age, sex, race, and thalassemia type will also be included in the plots. Similar by-subject longitudinal plots will be presented for Hb changes from baseline.

7.6.1.3. Sensitivity Analysis

To evaluate the impact of early discontinuation and LOCF in the primary analysis, sensitivity analyses will be conducted without imputation, including only subjects who have at least 1 assessment between the Week 4 and the Week 12 Visits (inclusive).

The frequency of subjects with an Hb response will be summarized along with the 2-sided 90% exact CI using the Clopper-Pearson method.

7.6.2. Secondary Efficacy Endpoints

7.6.2.1. Average Change from Baseline in Hb Concentrations over a Continuous 12-week Interval from Week 12 to Week 24

Average change from baseline in Hb concentrations from Week 12 to Week 24 will be calculated for each subject using all Hb concentrations collected within Week 12 to Week 24 windows and will be summarized as a continuous variable.

In addition, average change from baseline in Hb concentrations from Week 4 to Week 6, Week 8 to Week 12, Week 16 to Week 24, Week 4 to Week 24 and Week 4 to Week 12 for all subjects in FAS as well as Hb responders will be summarized descriptively.

7.6.2.2. Sustained Hb Response

Sustained Hb response is defined for subjects who have achieved an Hb response and also a ≥1.0 g/dL increase in Hb concentration at 2 or more evaluable Hb assessments out of the 4 scheduled assessments between the Week 12 Visit and the Week 24 Visit.

The frequency of subjects with a sustained Hb response will be summarized along with the 2-sided 90% exact CI using the Clopper-Pearson method.

7.6.2.3. Delayed Hb Response

The delayed Hb response is defined for subjects who do not achieve an Hb response (primary endpoint; ie, between Week 4 and Week 12) but achieve a ≥1.0 g/dL increase in Hb concentration at 1 or more assessments after Week 12 (ie, Weeks 16, 20, 24).

The frequency of subjects with a delayed Hb response will be summarized along with the 2-sided 90% exact CI using the Clopper-Pearson method.

7.6.2.4. Time to First ≥1.0 g/dL Increase in Hb Concentration from Baseline

Time to first ≥1.0 g/dL increase in Hb concentration from baseline in weeks will be summarized as a continuous variable as well as in categories (<6, 6-<12, ≥12 weeks) for Hb responders.

7.6.2.5. Change from Baseline in Hemoglobin Concentration in the Extension Period

Not applicable for the primary CSR.

For the final CSR, change from baseline in Hb concentration during the on-treatment period will be summarized by visit and plotted over time with box plot.

7.6.2.6. Markers of Hemolysis

Markers of hemolysis include reticulocyte, indirect bilirubin, LDH and haptoglobin. Values and their changes from baseline will be summarized descriptively as continuous variables.

By-subject longitudinal plots for markers of hemolysis will be presented over time (at each scheduled visit) with prescribed dose. Baseline characteristics of the subject including baseline Hb concentration, age, sex, race, and thalassemia type will also be included in the plots.

Longitudinal plots of mean value (+/- SD) at each visit will be presented.

When indirect bilirubin is missing but total and direct bilirubin are present, indirect bilirubin will be calculated as:

Indirect bilirubin=total bilirubin-direct bilirubin.

7.6.2.7. Markers of Erythropoietic Activity

Markers of erythropoietic activity include NRBC, EPO and soluble transferrin receptor. Values and their changes from baseline will be summarized descriptively as continuous variables.

By-subject longitudinal plots for markers of erythropoietic activity will be presented over time (at each scheduled visit) with prescribed dose. Baseline characteristics of the subject including baseline Hb concentration, age, sex, race, and thalassemia type will also be included in the plots.

Longitudinal plots of mean value (+/- SD) at each visit will be presented.

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 treated.

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 treated 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

ECG summaries will include all ECG assessments from the on-treatment period. QTcB and QTcF interval will be derived based on RR and QT interval (see below), if not collected in the eCRF. The average of the replicate measurements should be determined after the derivation of the individual parameter at each time point.

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 and can be derived as RR (sec)=60/heart rate (bpm).

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 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 will be presented in a by-subject listing.

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 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 Analysis

All exploratory endpoints will be summarized based on the FAS.

7.8.1. Alpha(α)-, Beta(β)-, and Gamma(γ)-hemoglobin Absolute Levels and/or Ratios

All α-, β-, and gamma (γ)-hemoglobin values and α/β ratio, if available, and their changes from baseline will be summarized over time as continuous variables and provided in a by-subject listing.

7.8.2. Other Markers of Erythropoietic Activity

Other markers of erythropoietic activity include GDF-15, GDF-11, NTBI, and erythroferrone. Values and change from baseline will be summarized over time and provided in a by-subject listing.

7.8.3. Markers of Iron Metabolism and Indicators of Iron Overload

Markers of iron metabolism include iron, ferritin, TIBC, transferrin saturation and hepcidin. Values and change from baseline will be summarized over time and provided in a by-subject listing.

By-subject longitudinal plots for iron markers will be presented over time (at each scheduled visit) with prescribed dose. Baseline characteristics of the subject including baseline Hb concentration, age, sex, race, and thalassemia type will also be included in the plots.

7.8.4. Markers of Oxidative Stress

Markers of oxidative stress include urinary 8-isoprostane, methylmalonic acid, total homocysteine, and other RBC metabolite measurements. Values and changes from baseline will be summarized over time and provided in a by-subject listing.

7.8.5. Transfusions

Frequency of subjects with transfusions will be summarized. Transfusion information will be listed for all subjects who have had transfusions during the study.

7.8.6. Spleen Size and Liver Iron Concentration

MRIs are performed at screening and Week 24 during the Core Period and every 48 weeks during the Extension Period. Change from baseline in spleen size and LIC will be summarized at each visit and provided in a by-subject listing.

7.8.7. Change in Hb Concentrations in Relation to the Dose of Mitapivat

By-subject longitudinal plots for Hb concentrations will be plotted over time and include the subject's concurrent prescribed mitapivat dose of 50 mg or 100 mg.

7.9. Interim Analysis

No formal interim analysis is 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.