Official Title: A Phase 3, Double-blind, Randomized, Placebo-Controlled, Multicenter Study Evaluating the Efficacy and Safety of Mitapivat in Subjects With Transfusion-Dependent Alpha- or Beta-Thalassemia (ENERGIZE-T)

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STATISTICAL ANALYSIS PLAN

A Phase 3, Double-blind, Randomized, Placebo-Controlled, Multicenter Study Evaluating the Efficacy and Safety of Mitapivat in Subjects With Transfusion-Dependent Alpha- or Beta-Thalassemia (ENERGIZE-T)

AG348-C-018

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[redacted]

Prepared by:

[redacted] PhD

Study Statistician

Name and Title (Printed)	Signature	Date (DD MMM YYYY)

Approved by:

[redacted] MD

Medical Director, Clinical Development

Name and Title (Printed)	Signature	Date (DD MMM YYYY)

[redacted] PhD

Head of Clinical Pharmacology and DMPK

Name and Title (Printed)	Signature	Date (DD MMM YYYY)

[redacted] PhD

Head of Biometrics

Name and Title (Printed)	Signature	Date (DD MMM YYYY)

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

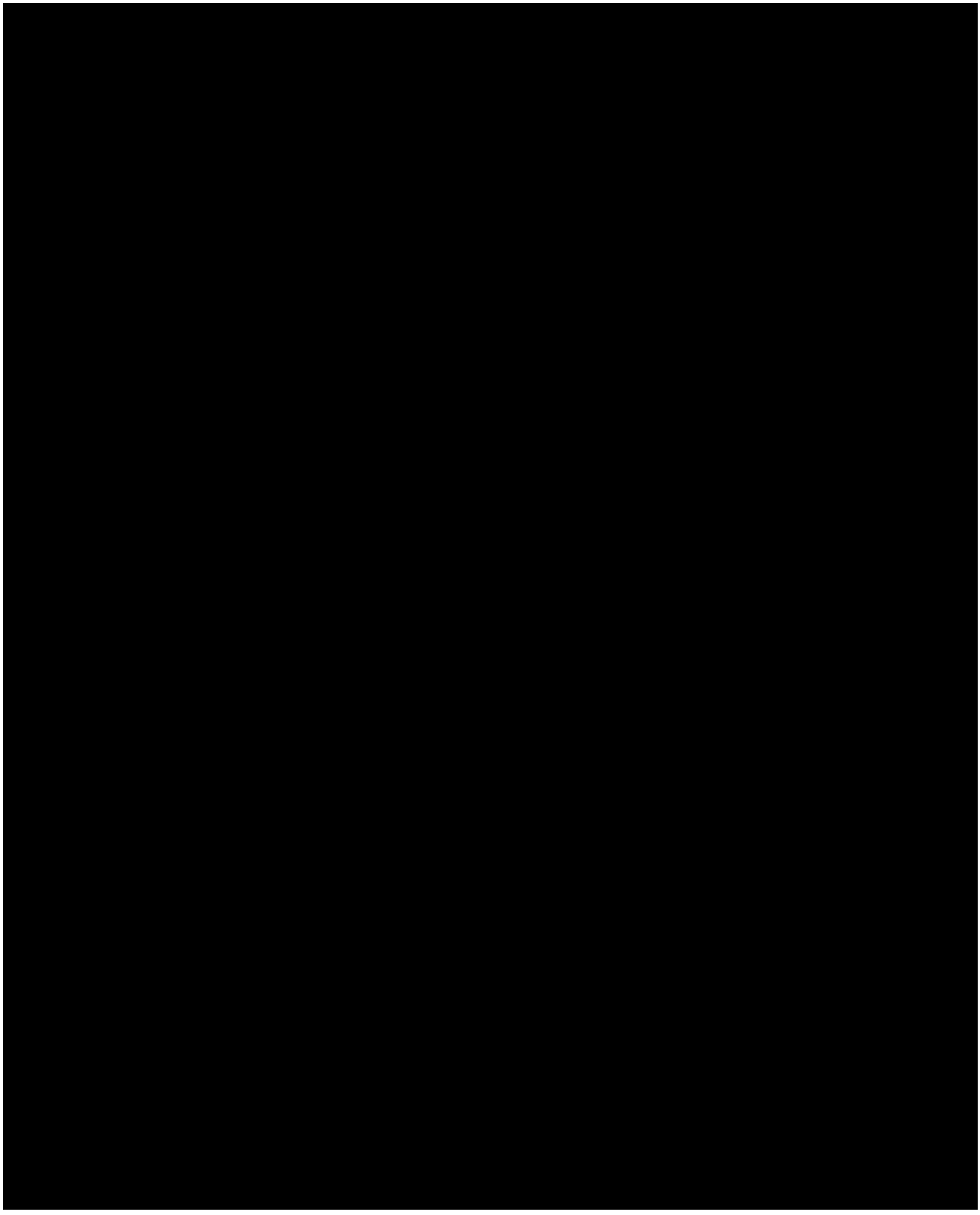
Abbreviation	Definition
2,3-DPG	2,3-Diphosphoglycerate
ACR	Albumin/creatinine ratio
AE	Adverse event
ALP	Alkaline Phosphatase
ALT	Alanine Aminotransferase
ANCOVA	Analysis of covariance
AST	Aspartate Aminotransferase
ATC	Anatomical Therapeutic Chemical
ATP	Adenosine Triphosphate
AUC	Area under the curve
BID	Twice daily
BMD	Bone mineral density
BMI	Body mass index
CI	Confidence Interval
C_{max}	Maximum observed concentration
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Events
CV	Coefficient of variation
DXA	Dual-energy x-ray absorptiometry
ECG	Electrocardiogram
eCRF	Electronic Case Report Form
eDISH	Evaluation of Drug-Induced Serious Hepatotoxicity
eGFR	Estimated glomerular filtration rate
EODB	End of double-blind
EOS	End of study
EOT	End of treatment
[redacted]	[redacted]
FAS	Full Analysis Set
Hb	Hemoglobin
[redacted]	[redacted]

ICF	Informed consent form
IDMC	Independent data monitoring committee
IQR	Interquartile range
IXRS	Interactive voice/web response system
[redacted]	
LLN	Lower limit of normal
LLQ	Lower limit of quantification
LS	Least squares
Max	Maximum
MedDRA	Medical Dictionary for Regulatory Activities
Min	Minimum
[redacted]	
OLE	Open-label extension
PD	Pharmacodynamic
[redacted]	
PK	Pharmacokinetic
PPS	Per-Protocol Set
PRO	Patient Reported Outcome
[redacted]	
PT	Preferred Term
QD	Once-daily
[redacted]	
RBC	Red blood cell
RSD	Relative standard deviation
SAE	Serious adverse event
SAP	Statistical analysis plan
SD	Standard deviation
SOC	System organ class
TDT	Transfusion-dependent thalassemia
TEAE	Treatment-emergent adverse event
T_{max}	Time of maximum observed concentration
[redacted]	

TRR	Transfusion reduction response
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-018 Amendment 2 (dated 28-September-2022).



2. INTRODUCTION

This SAP provides the detailed methodology for summary and statistical analyses of the data collected in Study AG348-C-018. This document may modify the plans outlined in the protocol; however, any major modifications of the primary endpoint definition or its analysis will also be reflected in a protocol amendment.

The primary clinical study report (CSR) will include all data for each subject up to the end of Double-blind (EODB) Period. The EODB period is defined as the end of the safety follow-up (for subjects who discontinue study treatment during the Double-blind Period) or before the start of study treatment in the Open-label Extension (OLE) Period (for subjects who continue onto the OLE Period after completing the Double-blind Period).

A CSR addendum will include data (associated with objectives that encompass OLE Period evaluations) through the End of Study (EOS). The EOS is defined as the time at which all subjects complete all study visits, are lost to follow-up, have withdrawn consent for further participation in the study, or when the Sponsor terminates the study. Study completion is the date of the last visit of the last subject in the study.

In the following sections, references to "EODB period date/EOS date" are meant to indicate that the EODB period subject-specific dates 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 addendum CSR.

3. OBJECTIVES AND ENDPOINTS

The study objectives and endpoints are provided in Table 2.

Table 2: Objectives and Endpoints

Primary Objective • To compare the effect of mitapivat versus placebo on transfusion burden in subjects with α- or β-transfusion-dependent thalassemia (TDT)	**Primary Endpoint** • Transfusion reduction response (TRR), defined as a ≥50% reduction in transfused red blood cell (RBC) units with a reduction of ≥2 units of transfused RBCs in any consecutive 12-week period through Week 48 compared with baseline
Key Secondary Objective • To compare the durability of the effect of mitapivat versus placebo on transfusion burden	**Key Secondary Endpoints** • ≥33% reduction in transfused RBC units from Week 13 through Week 48 compared with baseline (TRR3) • ≥50% reduction in transfused RBC units in any consecutive 24-week period through Week 48 compared with baseline (TRR2) • ≥50% reduction in transfused RBC units from Week 13 through Week 48 compared with baseline (TRR4)
Secondary Objectives • To evaluate the effect of mitapivat versus placebo on additional measures of transfusion burden • To evaluate the effect of mitapivat versus placebo on iron metabolism • To evaluate the safety of mitapivat • To evaluate the PK and PD effects of mitapivat	**Secondary Endpoints** • Change from baseline in transfused RBC units from Week 13 through Week 48 • Transfusion independence, defined as transfusion-free for ≥8 consecutive weeks through Week 48 • Change from baseline in iron, serum ferritin, total iron binding capacity, and transferrin saturation through Week 48 • Type, severity, and relationship of adverse events (AEs) and serious adverse events (SAEs) • Plasma or blood concentrations and PK parameters of mitapivat and PD parameters, including adenosine triphosphate (ATP) and 2,3-diphosphoglycerate (2,3-DPG)
[redacted]	

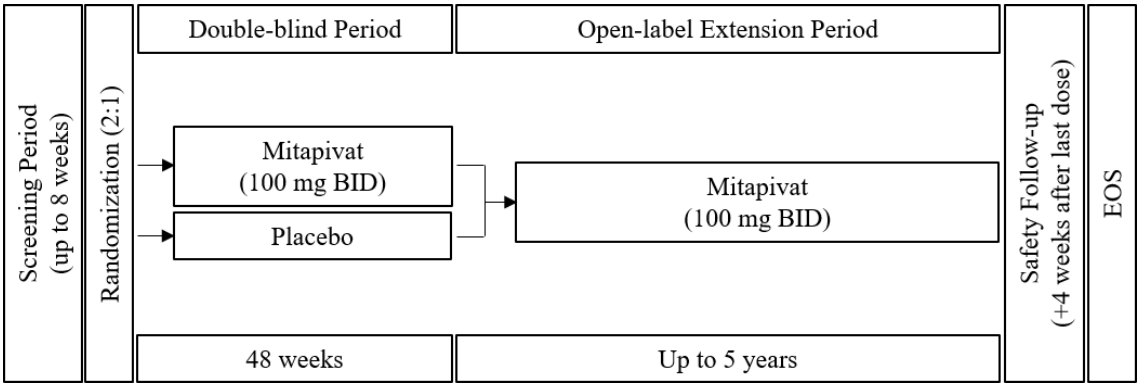
[redacted]

4. STUDY DESIGN

This is a Phase 3, double-blind, randomized, placebo-controlled, multicenter study evaluating the efficacy and safety of mitapivat versus placebo in adult subjects with α- or β-TDT followed by an OLE Period. Approximately 240 subjects are planned to be randomized in this study. This multicenter study will be conducted internationally.

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

Figure 1: Study Design



Abbreviations: BID = twice daily; EOS = End of Study.

5. ANALYSIS DATA SETS

The following analysis data sets will be evaluated and used for presentation of the data:

Population	Description
All Screened Subjects	All subjects who sign the informed consent form (ICF).
Full Analysis Set (FAS)	All subjects who are randomized. Subjects will be classified according to the randomized treatment arm according to the intent-to-treat (ITT) principle. The FAS is the primary analysis set for the evaluation of efficacy endpoints, unless otherwise specified.
Per-Protocol Set (PPS)	The PPS is a subset of the FAS. Subjects who meet any of the following criteria will be excluded from the PPS: • Do not receive at least 1 dose of the randomized treatment • Do not meet Inclusion Criterion #2 (ie, do not have documented thalassemia) • Do not meet Inclusion Criterion #3 (ie, do not meet criteria for transfusion-dependent) • Do not meet Inclusion Criterion #4 (ie, if taking hydroxyurea, the dose was not stable for ≥16 weeks before randomization)
Safety Analysis Set	All subjects who receive at least 1 dose of study treatment. Subjects will be classified according to the treatment received. If a subject randomized to placebo receives at least 1 dose of mitapivat in the Double-blind Period, then the subject will be classified to the mitapivat arm.
Open-label Extension (OLE) Analysis Set	All subjects who receive at least 1 dose of open-label mitapivat after the Double-blind Period
Pharmacokinetic (PK) Analysis Set	A subset of the safety analysis set including all subjects with at least 1 mitapivat plasma concentration measurement ≥LLQ
Pharmacodynamic (PD) Analysis Set	A subset of the safety analysis set including all subjects with at least 1 blood 2,3-DPG or ATP concentration measurement ≥LLQ
PK/PD Analysis Set	A subset of the PK analysis set including all subjects in the PK and PD analysis sets, with at least 1 time-matched ATP or 2,3-DPG concentration to a mitapivat plasma concentration.

Abbreviations: 2,3-DPG =2,3-diphosphoglycerate; ATP = adenosine triphosphate; ICF = informed consent form; ITT = intent-to-treat; LLQ = lower limit of quantification.

Table 3 summarizes the use of the analysis sets.

Table 3: Analysis Data Sets for Each Endpoint

Endpoints	FAS	PPS	Safety Analysis Set	OLE Analysis Set	PK Analysis Set	PD Analysis Set	PK/PD Analysis Set
Demographic and other baseline characteristics	✓			✓			
Disposition	✓			✓			
Major protocol deviations	✓			✓			
Exposure and concomitant therapies			✓	✓			
Efficacy [redacted]	✓	✓ (primary and key secondary only*)		✓			
Safety			✓	✓			
PK					✓		
PD						✓	
PK/PD							✓

* Key secondary endpoints are defined in Section 7.6.2.
Abbreviations: FAS = full analysis set; OLE = open-label extension; PD = pharmacodynamic; PK = pharmacokinetic; PPS = per-protocol set.

6. GENERAL STATISTICAL CONSIDERATIONS

6.1. Randomization, Blinding, and Unblinding

Eligible subjects will be randomized in a 2:1 ratio to mitapivat or matched placebo.

Randomization assignment will be implemented by an interactive voice/web response system (IXRS) and stratified by:

- Geographical region: North America and Europe; Asia-Pacific; rest of the world
- Thalassemia genotype:
  - subjects who do not have a β0 mutation at both alleles of the β-globin gene (non-[β0/β0]), including subjects with HbE/β-thalassemia and α-thalassemia/HbH disease;
  - subjects who have a β0 mutation at both alleles of the β-globin gene (β0/β0).

This study includes a Double-blind Period followed by an OLE Period. Study subjects, Investigators, clinical study center personnel, pharmacists, and the Sponsor will be blinded to the subject's treatment assignment. After completing the Double-blind Period, subjects will be provided the opportunity to receive mitapivat in the OLE Period. At the last study

visit of the Double-blind Period, subjects who continue in the OLE Period will be provided with active mitapivat; however, study subjects, Investigators, clinical study center personnel, and the Sponsor will continue to remain blinded to the randomized treatment assignment during the previous Double-blind Period until the study is unblinded for the analysis of the primary endpoint.

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:

$$H_{01}: p_{t1} - p_{c1} = 0 \text{ vs } H_{11}: p_{t1} - p_{c1} \neq 0$$

where p_{t1} and p_{c1} are the proportion of subjects achieving a TRR in the mitapivat arm and placebo arm, respectively.

Assuming a TRR response rate of 12.5% in the placebo arm, 240 subjects (160 subjects randomized to mitapivat and 80 subjects randomized to placebo) are needed to provide a 95% power to detect an increase in TRR response rate from 12.5% in the placebo arm to 33.7% in the mitapivat arm based on a 2-sided significance level of 0.05.

The conservative assumptions for the TRR rate were informed by the results of a Phase 3 clinical trial in patients with β-TDT where 12.5% was the upper bound of the 95% confidence interval (CI) for TRR rate in the placebo arm, and 33.7% was the lower bound of the 95% CI for the TRR rate for the active arm (Cappellini et al, 2020).

Additionally, the following statistical hypotheses will be tested to address the key secondary objectives:

$$H_{02}: p_{t2} - p_{c2} = 0 \text{ vs } H_{12}: p_{t2} - p_{c2} \neq 0$$

$$H_{03}: p_{t3} - p_{c3} = 0 \text{ vs } H_{13}: p_{t3} - p_{c3} \neq 0$$

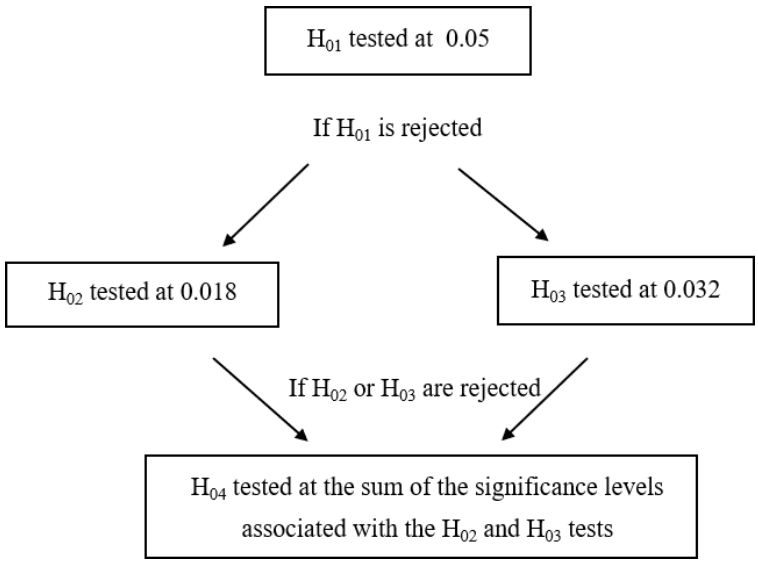
$$H_{04}: p_{t4} - p_{c4} = 0 \text{ vs } H_{14}: p_{t4} - p_{c4} \neq 0$$

where

- p_{t2} and p_{c2} are the proportion of subjects achieving a TRR2 in the mitapivat arm and placebo arm, respectively
- p_{t3} and p_{c3} are the proportion of subjects achieving a TRR3 in the mitapivat arm and placebo arm, respectively
- p_{t4} and p_{c4} are the proportion of subjects achieving a TRR4 in the mitapivat arm and placebo arm, respectively

Overall type I error will be maintained at or below a 2-sided significance level of 0.05 based on a graphical gatekeeping approach as illustrated in Figure 2.

Figure 2: Statistical Testing Strategy



The primary endpoint will be tested at a 2-sided α-level of 0.05. Then, the first 2 key secondary endpoints will be tested at an α-level of 0.032 and 0.018, respectively, if the null hypothesis for the primary endpoint is rejected. The last key secondary endpoint will be tested at an α-level of 0.018 (if only H_{02} is rejected), 0.032 (if only H_{03} is rejected), or 0.05 (if both H_{02} and H_{03} are rejected).

6.2.2. Decision Rules

The study will have demonstrated that mitapivat is statistically significantly superior to placebo for TRR if the 2-sided p-value for the test of the difference in the TRR rate is ≤0.05 and the TRR rate is higher for the mitapivat arm than for the placebo arm.

To protect the integrity of the study and to preserve the type I error at or below 2-sided α=0.05, the testing strategy outlined in Section 6.2.1 will be followed and statistical significance will be achieved for mitapivat compared to placebo for each key secondary endpoint that can be tested per this strategy at the associated significance level.

6.3. Definitions

6.3.1. Study Drug and Study Treatment

In the Double-blind Period, both study drug and study treatment are defined as mitapivat or matched placebo.

In the OLE Period, both study drug and study treatment are defined as mitapivat.

6.3.2. Start and End Dates of Study Treatment

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

The end of study treatment is the latest date/time of administration of a non-zero dose of the study drug on or before the EODB period 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, disease 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, baseline characteristics, 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

6.3.4.1. Double-blind Period

Throughout the SAP for analyses based on the Double-blind Period, 'reference date' will be used to refer to randomization date for subjects randomized and not dosed, or the start of study treatment for subjects randomized and dosed.

Efficacy [redacted] Evaluations

The 24-week baseline transfusion burden is defined as the total number of RBC units transfused during the 24-week period (168 days) before the 'reference date'. Baseline transfusion burden will be standardized to match the postbaseline assessment duration specified for each endpoint associated with reduction of transfusion burden from baseline.

For iron metabolism (Section 7.6.3.2), [redacted] baseline is defined as the last assessment before Transfusion 0 (the last RBC transfusion that is administered before randomization) to minimize the impact of transfusion on these endpoints. Baseline will be derived based on central laboratory data; if no central laboratory data are available, then local laboratory data will be used to derive the baseline.

[redacted]

Demographic and Other Baseline Characteristics

For summaries of demographic and other baseline characteristics based on the FAS, baseline is defined as the last assessment before the 'reference date'.

Safety Evaluations

For clinical laboratory assessments the following applies:

- 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.
  - 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 before the start of study treatment.

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

PD Evaluations

The baseline is defined as the pre-dose whole blood concentration of 2,3-DPG and ATP on study day 1. If this concentration is <LLQ, baseline will be set to missing.

If, per protocol, an assessment 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 are missing or not collected, it will be assumed that the assessment is performed before the first dose of study treatment. Unscheduled assessments will be used in the determination of baseline; however, an unscheduled assessment on study day 1 for which the time relative to start of study treatment cannot be determined, will be considered to have been obtained after administration of study treatment.

If no assessment meets the definition of baseline for an evaluation, the baseline will be set to missing.

6.3.4.2. Open-label Extension Period

For subjects randomized to mitapivat, the baseline for all assessments will be that derived in the Double-blind Period (Section 6.3.4.1).

For subjects randomized to placebo, the baseline is derived following the same rules as for the Double-blind Period but using start of mitapivat in the OLE Period instead of randomization date or ‘reference date’.

Age at baseline will be calculated based on the date of informed consent for the study as described in Section 6.4.2.

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 (including dose taper period).

Within the on-treatment period, there are two distinct treatment periods:

- Double-blind on-treatment period starts on the date of the start of study treatment and ends
  - 28 days after the end of study treatment, for subjects who discontinue study treatment during the Double-blind Period of the study
  - Before the start of the OLE treatment period, for subjects who continue onto the OLE Period of the study

- OLE on-treatment period applies only to subjects who complete the Double-blind Period of the study, and starts with the first dose of open-label mitapivat in the OLE Period and ends 28 days after the end of study treatment.

For subjects who receive mitapivat in the OLE Period, assessments or events occurring on the date of first dose of mitapivat in the OLE Period will be included in the analysis of the data from the double-blind on-treatment period, except for AEs and concomitant medications reported for subjects in the placebo arm which will be included only in the analysis of the data from the OLE on-treatment period.

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

6.4. General Methods

6.4.1. Data Handling After End of Double-blind Period

Data after the EODB period may not undergo the cleaning process and will not be displayed in any listings or used for summary statistics, statistical analyses, or imputations for the primary CSR.

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)=(date of informed consent−date of birth+1)/365.25

  If only day of birth is missing: Age (years): (year/month of informed consent−year/month of birth)

  If day and month of birth are missing: Age (years): (year of informed consent−year of birth)

  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, randomization date, 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 (including arithmetic mean and geometric 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 (including percent changes from baseline for whole blood concentration of 2,3-DPG and ATP, relative standard deviation [RSD %], arithmetic coefficient of variation [CV%], and geometric coefficient of variation [CV% geometric mean]) 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.
- PK parameters will be reported to 3 significant figures, except for T_{max} and T_{last} which will be reported to 2 significant digits.

Note that some PK parameters are observed values (eg, C_{max}), while other PK parameters are derived from the intensive PK profiles (eg, $AUC_{0\text{-}last}$). For all the PK parameters, regardless of whether they are observed or derived, the individual PK parameter values are considered "raw data" when implementing the above reporting rules.

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 randomized 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. PK concentrations and parameters will be further summarized using arithmetic mean together with the CV% and 90% CI, geometric mean together with CV% geometric mean and 90% CI. PD concentration and parameters will be further summarized using arithmetic mean together with the CV% and 95% CI, geometric mean together with the CV% and 95% CI, and RSD% (applicable to PD concentration only).

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. PK and PD data collected at unscheduled visits will be listed and may not be summarized. Descriptive statistics (mean, SD, median, quartiles, minimum, and maximum) by nominal visit or time point for safety endpoints such as clinical laboratory measurements and vital signs will include only data from 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, vital signs, and clinical laboratory measurements will include data from both scheduled and unscheduled visits.

For the evaluation of transfusion burden, iron metabolism (Section 7.6.3.2), [redacted] and bone mineral density (BMD) by dual-energy x-ray absorptiometry scan (DXA, Section 7.7.5), 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 will be provided using the derived values at scheduled visits.

Analysis Visit Windows

For the evaluation of iron metabolism (Section 7.6.3.2), [redacted] and BMD by DXA scan (Section 7.7.5), the analysis visit windows will be derived based on the target study day for the scheduled visits as follows. Note that based on the schedule of assessments, a Week 4 Visit, for example, will have a target study day of 1+(4×7)=29.

For the Double-blind Period:

- Visit windows will be implemented for scheduled visits after study day 1 (ie, starting at Week 4 through the end of the Double-blind Period on Week 48).
- 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 Week < 48. The end day of visit window for Week 48 is the end of the Double-blind Period
  - Consistent with the rules above, for DXA scan results and [redacted] which are only scheduled at Week 48, the analysis

visit window for Week 48 will start on study day 2. The end day of visit window for Week 48 is the end of the Double-blind Period

For the OLE Period:

- Visit windows will be implemented for all scheduled visits after the first dose of mitapivat in the OLE Period. Week 312 is the last scheduled visit in the OLE Period.
- 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 is the day after the first dose of mitapivat in the OLE Period.
  - End day of visit window = [(target day for analysis visit(n) + target day for analysis visit(n+1))/2]–1 for Week < 312. The end day of visit window for Week 312 is the EOS date.

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

For laboratory parameters [transfusion burden, iron metabolism (Section 7.6.3.2), [redacted], if multiple assessments are identified within a visit window for a parameter, then

- Central laboratory assessment(s) (scheduled or unscheduled) within the visit window will be used
- If no central laboratory value is within the visit window, local laboratory assessment(s) within the visit window will be used

For the evaluation of transfusion burden, iron metabolism (Section 7.6.3.2), [redacted], and BMD by DXA scan (Section 7.7.5), if multiple assessments are identified within a visit window for a parameter and after selecting the assessments for laboratory parameters as described above, 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 laboratory parameters
  - the later assessment will be used for [redacted] and BMD by DXA scan

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 the death date is available and the imputed end date is after the death date, the death 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 EODB period date/EOS date, the AE will be considered as ongoing at the EODB period 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 (separately for each period):

- If the last date of study drug is completely missing and there is no End of Treatment Disposition electronic case report form (eCRF) page for the study drug AND there is no death date, the subject should be considered to be ongoing and the EODB period 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 EODB period 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(end of treatment [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

6.5.4. Death Date

Missing or partial death dates will not be imputed.

6.5.5. Pharmacokinetic Data

6.5.5.1. Handling of Missing Concentrations

Missing concentrations before the first dose of study treatment will be set to 0 in the analysis. A concentration is deemed missing if the PK sample is not collected, or the PK sample is collected but the concentration is not reportable.

In addition:

- If ≤2 samples have concentrations ≥LLQ, none of the PK parameters will be derived.
- If pre-dose concentrations are missing at a steady-state visit, PK parameters associated with that visit will not be derived with the exception of C_{max} and T_{max}.

Since the protocol allows a ±30 minutes window for the 7-hour PK sample collection, if the end time of the predefined area under the concentration-time curve falls outside the range of the available data then:

- the $AUC_{0\text{-}last}$ will be used to represent $AUC_{0\text{-}7,}$ if T_{last} ≥6.5 h.
- $AUC_{0\text{-}7}$ will not be reported, if T_{last} <6.5 h.

6.5.5.2. Incomplete Dosing Information

Samples associated with incomplete dosing information (ie, missing dosing date, dosing time, actual dose received, and/or dosing schedule) will be excluded from the analysis. The missing dosing information will not be imputed.

6.5.5.3. Missing Sampling Dates or Times

Samples associated with missing sample dates and/or times will be excluded from the analysis. The missing sampling dates and/or times will not be imputed.

6.5.5.4. Incomplete Concentration-time Data

Subjects who have incomplete concentration-time profiles (ie, missing concentrations at more than one scheduled time point) may be excluded from the PK analysis set. The decision will be made by the unblinded clinical pharmacologist before the study is unblinded for the analysis of the primary endpoint.

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 randomization, 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 by treatment arm. 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 by treatment arm based on the FAS or (for the summaries associated with the OLE Period only) the OLE analysis set:

- Frequency of subjects in each randomization strata and combination of randomization strata (per IXRS)
- Frequency of subjects randomized in each geographic region, country, and site
- Frequency of subjects randomized and not treated, overall and by reason for discontinuation
- Frequency of subjects who completed the Double-blind Period
- Frequency of subjects with study drug ongoing in the Double-blind Period
- Frequency of subjects who discontinued study drug in the Double-blind Period, overall and by the reason for discontinuation of study drug
- Frequency of subjects who completed the OLE Period
- Frequency of subjects with study drug ongoing during the OLE Period
- Frequency of subjects who discontinued study drug in the OLE Period, overall and by the reason for discontinuation of study drug
- Frequency of subjects who completed the safety follow-up period
- Frequency of subjects ongoing in the safety follow-up period
- Frequency of subjects who discontinued the safety follow-up period, overall and by the reason for discontinuation

- Frequency of subjects who completed the study
- Frequency of subjects ongoing in the study
- Frequency of subjects who discontinued the study, overall and by reason for discontinuation; the reason for discontinuation will be derived as the reason associated with the earliest epoch that was discontinued.

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.

In addition, cross-tabulation of subjects randomized (mitapivat, matched placebo) vs subjects who have received at least 1 dose of study drug (mitapivat, matched placebo) in the Double-blind Period will be performed.

Disposition for all screened subjects and randomization data 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 by treatment arm based on the FAS or (for the summaries associated with the OLE Period only) the OLE analysis set. These will include:

- Subjects randomized despite not satisfying the eligibility criteria
- Subjects who develop withdrawal criteria while on the study but are not withdrawn
- Subjects who receive a study drug different from that assigned at randomization
- Subjects who are randomized under the wrong stratification factor(s)
- 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 by treatment arm and overall based on the FAS or (for the summaries associated with the OLE Period only) the OLE analysis set, 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), unknown
  - Race: American Indian or Alaska Native, Asian, Black or African American, Native Hawaiian or other Pacific Islander, White, multiracial, unknown, not reported
  - Ethnic origin: Hispanic or Latino, not Hispanic or Latino, not reported
  - Age (years): summary statistics
  - Age categories:
    - <65 years, ≥65 years
    - <35 years, ≥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

In addition to randomization stratification factors (Section 7.1) and baseline for disease-related clinical laboratory assessments (Section 7.6.3.2, [redacted], the following baseline characteristics of the underlying disease will be summarized based on the data entered in the eCRF:

- Pretransfusion Hb threshold. A pretransfusion Hb threshold will be determined for each subject based on transfusion history. The pretransfusion Hb threshold is defined as the mean of all documented pretransfusion Hb concentration values recorded for the RBC transfusions administered during the 24-week period before randomization
- 24-week baseline transfusion burden as defined in Section 6.3.4.1 (≤12 RBC units, >12 RBC units)
- Splenectomy status (Yes, No; if Yes, age of splenectomy)
- Prior cholecystectomy status (Yes, No; if Yes, age of cholecystectomy)
- Prior Iron chelation status (Yes, No); the status is "Yes" if a subject has received chelation therapy within 1 year (365 days) before randomization
- Prior hydroxyurea status (Yes, No)
- DXA scan results by location (hip and spine): BMD and their corresponding T-scores and Z-scores. Frequency of subjects with worst T-score in 3 categories (≤-2.5, >-2.5-<-1, ≥-1.0) by location and across the two locations.

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

7.3.3. Medical and Surgical 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 and surgical history will be provided in by-subject listings.

7.3.4. Prior Therapies

The following summaries will be presented by treatment arm and overall 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 procedures are defined as procedures (from the "Prior 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 medications and prior procedures will be provided in by-subject listings.

7.4. Exposure to Study Drug and Compliance

The following summaries will be presented by treatment arm based on the safety analysis set for the Double-blind Period using "Prescribed Dose", "First Dose of Study Medication (Double-blind Period)" , and "Study Drug Dispensation and Return" eCRFs; and the OLE analysis set for the OLE Period using the "Prescribed Dose", "First Dose of Study Medication (Open-label Extension Period)", and "Study Drug Dispensation and Return" eCRFs.

Subjects will receive 100 mg BID mitapivat or matched placebo for oral administration. Subjects who discontinue study drug should undergo the recommended dose taper. Study drug dose and recommended dose taper are provided in Table 4.

Table 4: Study Drug Dose and Recommended Dose Taper Regimen

Level	Dose	Schedule
Full dose	100 mg	BID
Taper dose	100 mg	QD for 7 days, then discontinue study drug

Abbreviations: BID = twice daily; QD = once daily

7.4.1. Treatment Duration and Exposure

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

- Duration of exposure (days)=(last dose date−first dose date+1)

  Duration of exposure (weeks)=(last dose date−first dose date+1)/7

  Duration of exposure (years)=(last dose date−first dose date+1)/365.25

- Double-blind Period: the categories will be >0-6, >6-12, >12-24, >24-36, >36-48, and >48 weeks
- OLE Period: the categories will be >0-0.5, >0.5-1, >1-2, >2-3, >3-4, >4-≤5, and >5 years.

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

  Total number of tablets intended = sum of number of tablets intended over all prescription periods.

  Number of tablets intended during each prescription period = prescribed dosing frequency × number of tablets intended for each administration × duration of the prescription. Prescribed dosing frequency takes value of 1 and 2 for QD and BID, respectively

Percentage of tablets taken will be summarized by treatment arm. 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
- The reason for dose reduction
- The frequency of subjects with at least 1 interruption of study drug
- The reason for interruption of study drug

Dose reduction is defined as a prescribed non-zero daily dose that is lower than the planned dose (200 mg daily). An interruption of study drug is defined as a prescribed daily dose of 0 mg.

The reason for an interruption of study drug or dose reduction will be derived based on the “Reason for prescribed dose change” in the “Prescribed Dose” eCRF.

7.5. Concomitant Therapies

The following summaries will be presented by treatment arm based on the safety analysis set for the Double-blind Period and the OLE analysis set for the OLE Period.

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 medications and concomitant procedures will be provided in by-subject listings.

7.6. Efficacy Analyses

The following analyses will be based on the FAS using the IXRS randomization stratification factors, unless otherwise specified. The efficacy analyses will include all data from the Double-blind Period. For subjects who discontinue treatment or become unblinded prior to completion of the 48-week Double-blind Period, their efficacy data prior to start of the OLE Period will be included in this evaluation based on the ITT principle.

7.6.1. Primary Endpoint

The primary endpoint is the transfusion reduction response (TRR), defined as a ≥50% reduction in transfused RBC units with a reduction of ≥2 units of transfused RBCs in any consecutive 12-week period through Week 48 compared with the baseline transfusion burden (defined in Section 6.3.4.1) standardized to 12 weeks.

- Baseline transfusion burden standardized to 12 weeks (units/12-weeks) = total number of RBC units transfused during the 24-week period before the 'reference date'×12/24
- Reduction from baseline in transfusion burden for a 12-week period = Baseline transfusion burden standardized to 12 weeks – Number of transfused RBC units in the 12-week period
- Percent reduction from baseline in transfusion burden for a 12-week period =100×(Baseline transfusion burden standardized to 12 weeks – Number of transfused RBC units in the 12-week period)/ Baseline transfusion burden standardized to 12 weeks

Subjects who are withdrawn from the study before Week 12 (Day 85) will be considered nonresponders. A subject will meet the primary endpoint if, in any 12-week (84 days) consecutive period from the date of first dose in the Double-blind Period (Day 1) through Week 48 in the Double-blind Period both conditions below are met:

- Reduction from baseline in transfusion burden ≥2 RBC units
- Percent reduction from baseline in transfusion burden is ≥50%

The consecutive 12-week periods for the evaluation are Day 1 through Day 84, Day 2 through Day 85, and so on to cover all 84-day periods through Week 48 in the Double-blind Period.

7.6.1.1. Primary Analyses

The primary endpoint of TRR will be tested using the Mantel-Haenszel stratum weighted method adjusting for randomization stratification factors. The proportion of subjects who achieved a TRR will be summarized for each treatment arm. The adjusted difference in TRR rate between the mitapivat arm and placebo arm, along with the 95% CI and the 2-sided p-value, will be provided.

7.6.1.2. Sensitivity Analyses

The following sensitivity analyses will be performed:

- TRR will be analyzed using the methodology described for the primary analysis but based on PPS.
- TRR will be analyzed using the methodology described for the primary analysis but including only those subjects who completed 48 weeks of study treatment and who did not receive any concomitant medications (prior to completion of 48 weeks of study treatment) that could affect Hb concentrations. The list of associated concomitant medications will be finalized prior to the database lock and unblinding of the study for the primary analysis
- TRR will be analyzed using the methodology described for the primary analysis with the following changes: if a subject's Hb concentration met the individual transfusion threshold but the subject did not receive an RBC transfusion for a reason other than not clinically indicated then the transfusion will be considered missing; in this sensitivity analysis such subjects will be considered as having received an RBC transfusion on the day that the Hb concentration met the individual transfusion threshold, and the number of RBC units will be imputed as the number of RBC units received at the last transfusion before the Hb assessment associated with the missed transfusion

7.6.2. Key Secondary Endpoints

The key secondary endpoints are

- TRR2, defined as ≥50% reduction in transfused RBC units in any consecutive 24-week period through Week 48 compared with the 24-week baseline transfusion burden (defined in Section 6.3.4.1). The consecutive 24-week

periods for the evaluation are Day 1 through Day 168, Day 2 through Day 169, and so on to cover all 168-day periods through Week 48 in the Double-blind Period.

- TRR3, defined as ≥33% reduction in transfused RBC units from Week 13 through Week 48 compared with the baseline transfusion burden standardized to 36 weeks.
- TRR4, defined as ≥50% reduction in transfused RBC units from Week 13 through Week 48 compared with the baseline transfusion burden standardized to 36 weeks.

  Baseline transfusion burden standardized to 36 weeks (units/36-weeks) = 24-week baseline transfusion burden (defined in Section 6.3.4.1)×36/24

  Transfusion burden from Week 13 through Week 48 standardized to 36 weeks (units/36 weeks) = Number of transfused RBC units from Day 85 through Week 48 in the Double-blind Period ×36/(Number of days from Day 85 through Week 48 in the Double-blind Period/7)

  Percent reduction from baseline =100×(Baseline transfusion burden standardized to 36 weeks – Transfusion burden from Week 13 through Week 48 standardized to 36 weeks)/ Baseline transfusion burden standardized to 36 weeks

These endpoints (TRR2, TRR3, and TRR4) will be tested using the Mantel-Haenszel stratum weighted method adjusting for randomization stratification factors. The response rate based on the different definitions of response will be summarized for each treatment arm. The adjusted differences in response rate between the mitapivat arm and placebo arm, along with the 95% CI and the 2-sided p-value, will be provided.

Subjects who are withdrawn from the study before Week 24 (Day 169) will be considered nonresponders in TRR2. Subjects who are withdrawn from the study before Week 48 will be considered nonresponders in TRR3 and TRR4.

The following sensitivity analyses will be performed for each of these endpoints:

- TRR2, TRR3, TRR4 will be analyzed using the methodology described for the primary analysis but based on PPS.
- TRR2, TRR3, TRR4 will be analyzed using the methodology described for the primary analysis but including only those subjects who completed 48 weeks of study treatment and who did not receive any concomitant medications (prior to completion of 48 weeks of study treatment) that could affect Hb concentrations. The list of associated concomitant medications will be finalized prior to the database lock and unblinding of the study for the primary analysis
- TRR2, TRR3, TRR4 will be analyzed using the methodology described for the primary analysis with the following changes: if a subject's Hb concentration met the individual transfusion threshold but the subject did not receive an RBC transfusion for a reason other than not clinically indicated then the transfusion will be considered missing; in this sensitivity analysis such subjects will be

considered as having received an RBC transfusion on the day that the Hb concentration met the individual transfusion threshold, and the number of RBC units will be imputed as the number of RBC units received at the last transfusion before the Hb assessment associated with the missed transfusion

7.6.3. Additional Secondary Efficacy Endpoints

7.6.3.1. Additional Measures of Transfusion Burden

Change from Baseline in Transfused RBC Units from Week 13 Through Week 48

The change from baseline in transfused RBC units from Week 13 through Week 48 is derived as for the TRR3 and TRR4 endpoints, namely

- Baseline transfusion burden standardized to 36 weeks (units/36-weeks) = 24-week baseline transfusion burden (defined in Section 6.3.4.1)×36/24
- Transfusion burden from Week 13 through Week 48 standardized to 36 weeks (units/36 weeks) = Number of transfused RBC units from Day 85 through Week 48 in the Double-blind Period ×36/(Number of days from Day 85 through Week 48 in the Double-blind Period/7)
- Percent reduction from baseline =100×(Baseline transfusion burden standardized to 36 weeks – Transfusion burden from Week 13 through Week 48 standardized to 36 weeks)/ Baseline transfusion burden standardized to 36 weeks
- Reduction from baseline = Baseline transfusion burden standardized to 36 weeks – Transfusion burden from Week 13 through Week 48 standardized to 36 weeks

and will be summarized by treatment arm.

Reduction (%) in RBC transfusion burden from Week 13 to Week 48 from the baseline transfusion burden standardized to 36 weeks will be analyzed using an analysis of covariance (ANCOVA) with treatment arm as the independent variable, and covariates for randomization stratification factors and baseline transfusion burden standardized to 36 weeks. Treatment effect will be evaluated as a contrast of mitapivat versus placebo. The least square (LS) means will be presented by treatment arm and the LS mean for the treatment difference between mitapivat and placebo will be presented with the associated 95% CI.

The frequency of subjects within the following percentage reduction categories will also be provided: <0, 0 to <20, 20 to <33, 33 to <50, ≥33, and ≥50%.

Transfusion Independence

Transfusion independence is defined as transfusion-free for ≥8 consecutive weeks through Week 48 in the Double-blind Period. The frequency of subjects achieving transfusion independence will be summarized by treatment arm. The adjusted difference in the proportion of subjects who achieve transfusion independence between the mitapivat arm and the placebo arm using a Mantel-Haenszel stratum weighted method adjusting for randomization stratification factors, along with the 95% CI, will be provided.

For subjects who achieved transfusion independence, the duration of transfusion independence will be calculated as the number of days in the longest transfusion-free period starting on or after the 'reference date' (as defined in Section 6.3.4.1) through Week 48 in the Double-blind Period (evaluation period). Each transfusion-free period will be derived as follows:

- A transfusion-free period starts on the day after an RBC transfusion and ends the day before the next RBC transfusion
- For subjects who do not receive any RBC transfusions during the evaluation period, the start of the transfusion-free period is the 'reference date'
- For subjects who either do not receive any RBC transfusions during the evaluation period or receive an RBC transfusion during the evaluation period with no subsequent RBC transfusions during the evaluation period, the end of the transfusion-free period is censored at the end of the evaluation period

7.6.3.2. Iron Metabolism

Iron metabolism will be assessed based on iron, serum ferritin, total iron binding capacity, and transferrin saturation.

Values and changes from baseline will be summarized based on descriptive statistics, by treatment arm and study visit, through Week 48 of the Double-blind Period. Change from baseline will be compared between treatment arms following the same methodology as described in Section 7.6.3.1 for reduction from baseline in RBC transfusion burden.

By-subject longitudinal plots will be presented with values at baseline and postbaseline, transfusions, and prescribed dose over time. The plots will further include TRR status, treatment arm, age, sex, race, randomization stratification factors, 24-week baseline transfusion burden, and hydroxyurea status.

7.6.4. Subgroup Analyses

Subgroup analyses to be performed for the primary and key secondary endpoints based on the FAS are presented in Table 5.

Table 5: Subgroup Analyses for the Primary and the Key Secondary Endpoints

Subgroup	Categories
Randomization stratification factor (per IXRS): Geographical region	North America and Europe; Asia-Pacific; rest of world
Randomization stratification factor (per IXRS): Thalassemia genotype	Non-(β^0/β^0); (β^0/β^0)
Age at screening	<35 years; ≥35 years
Sex	Male; Female
Race	Asian; Black or African American; White; other (other includes American Indian or Alaska Native, Native Hawaiian or other Pacific Islander, multiracial, unknown, and not reported)
24-week baseline transfusion burden	≤12 RBC units; >12 RBC units

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 categories will be pooled (if 3 or more categories are pre-specified for the subgroup) or the subgroup will not be analyzed (if only 2 pre-specified categories in the subgroup).

For each category within each subgroup the following unstratified analyses will be performed and presented in a forest plot, separately for the primary endpoint and each of the key secondary endpoints. TRR, TRR2, TRR3 and TRR4 will be summarized for each treatment arm (number of responders and response rate) along with the 95% exact CI using the Clopper-Pearson method. The difference in response rates between the mitapivat arm and the placebo arm will be summarized together with the 95% CI based on the exact Clopper-Pearson confidence limits.

7.7. Safety Analyses

Safety data from the double-blind on-treatment period (Section 6.3.5) will be summarized based on the safety analysis set, by treatment arm.

Safety data from the OLE on-treatment period alone (Section 6.3.5) will be summarized separately based on the OLE analysis set, by treatment arm and overall.

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 the mitapivat arm.

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 subjects in either treatment arm or Grade ≥3 TEAEs reported in ≥5% of subjects in either treatment arm. 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

7.7.1.1. 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 who died, along with the cause of death, will be summarized for the following categories:

- On-treatment death: Deaths during the on-treatment period
- Post-treatment death: 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. For chemistry and hematology laboratory tests, the actual values and the changes from baseline will be summarized by study visit.

For each laboratory test 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 **white blood cell (WBC) differential counts** [total neutrophil (including bands), 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 by treatment arm:

- 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, with different symbols for different treatment arms, 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

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 Hormone Tests

For sex hormone 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 Tests

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

7.7.4. Vital Signs and Physical Measurements

For all physical measurements and vital sign assessments (height, weight, BMI, systolic blood pressure, diastolic blood pressure, pulse rate, temperature) the actual values and the changes from baseline will be summarized by treatment arm and study visit. Change from baseline does not apply to height.

Vital signs and physical measurements will be presented in a by-subject listing.

7.7.5. Dual-Energy X-ray Absorptiometry Scans

Results from bone mineral density on DXA scan (BMD and their T-scores and Z-scores) will be summarized by treatment arm and study visit, for each location.

In addition, shift tables will summarize the frequency of subjects with shifts from baseline T-scores missing, ≤-2.5, >-2.5-<-1, ≥-1.0 to worst T-score ≤-2.5, >-2.5-<-1, ≥-1.0 during the on-treatment period, by location and across the two locations.

7.7.6. Electrocardiograms

ECGs are collected during the screening period only and will be presented in a by-subject listing.

7.8. Pharmacokinetic Analyses

Only data from subjects in the mitapivat arm will be included in summary tables and plots. If a subject randomized to placebo receives at least 1 dose of mitapivat and has a PK concentration ≥LLQ, the data will be listed but will be excluded from the summary tables and plots.

All listings, summaries and plots will be presented by treatment arm based on the PK analysis set.

Concentrations <LLQ will be set to 0 for summary tables and plots with the following exceptions:

- Concentrations <LLQ between 2 quantifiable concentrations will be set to missing.
- Concentrations <LLQ following the last quantifiable concentration in a profile will be set to missing.

The following data will be excluded from summary tables and plots, unless otherwise specified:

- Pre-dose samples collected post-dose
- Post-dose samples collected pre-dose
- Concentrations >5% of C_{max}, for sample collected before the first dose of mitapivat
- Multiple samples collected at the same actual time point

- Samples not collected within the protocol-specified sampling windows will be excluded from the analysis but will be included in by-subject concentration-time plots.

In addition, concentrations ≥LLQ following 2 or more consecutive concentrations <LLQ and samples not collected within the scheduled visit window will be flagged in listings. The decision as to whether these will be included or excluded in whole or in part from the analyses will be made by the unblinded clinical pharmacologist before the study is unblinded for the analysis of the primary endpoint.

7.8.1. Concentration-Time Data

PK concentrations will be summarized by treatment arm, study visit and nominal time point. All the concentration-time data will be listed.

Arithmetic mean (±SD) of PK concentrations associated with the intensive PK sampling collection visit (Week 36), will be plotted vs nominal sampling time on both linear and log-linear scales.

By-subject PK concentrations associated with the intensive PK sampling collection visit will be plotted vs actual sampling time on both linear and log-linear scales (Week 36) by treatment arm.

By-subject PK concentrations associated with sparse PK sampling collection visits (pre-dose on Day 1, Week 12, and Week 24) will be plotted vs visit using box plots. The lower and upper boundaries of the box correspond to the interquartile range (IQR). The solid line represents the median value. The whiskers represent the minimum or maximum values within 1.5×IQR.

7.8.2. Pharmacokinetic Parameters

Concentration-time data associated with the intensive PK sampling collection visit (Week 36) will be used to derive the PK parameters defined in Table 6 using noncompartmental analysis methodology (Gabrielsson J and Weiner D, 2016) in Phoenix™ WinNonlin® version 8.2 or later. The derivations will be based on the actual sampling time points calculated relative to the actual time of the most recent administration of mitapivat.

PK parameters will be listed and summarized by treatment arm and study visit. $AUC_{0\text{-last}}$ and T_{last} will be reported in the listings, but not included in the table summary if $T_{last} < 6.5$h or $T_{last} > 7.5$h.

Table 6: Pharmacokinetic Parameters of Mitapivat in Plasma

Parameter	Description
$AUC_{0\text{-last}}$	Area under the concentration-time curve from time "0" to T_{last} on dosing day, calculated using the linear-log trapezoidal rule
T_{last}	Time of the last quantifiable concentration
C_{max}	Maximum observed concentration.
T_{max}	Time of maximum observed concentration
C_{last}	Last quantifiable concentration after a single dose or within the dosing interval (tau) for

	multiple doses

7.9. Pharmacodynamic Analyses

All listings, summaries and plots for PD data will be presented by treatment arm based on the PD analysis set. Concentrations <LLQ will be set to missing.

The following data will be excluded from summary tables and plots, unless otherwise specified:

- Pre-dose blood samples collected post-dose
- Post-dose blood samples collected pre-dose
- Multiple samples collected at the same actual time point
- Samples not collected within the protocol-specified sampling windows

7.9.1. Whole Blood Concentrations of 2,3-DPG and ATP

Whole blood concentrations of 2,3-DPG and ATP, and their change from baseline and percent change from baseline will be summarized by treatment arm and study visit. Individual data will be listed.

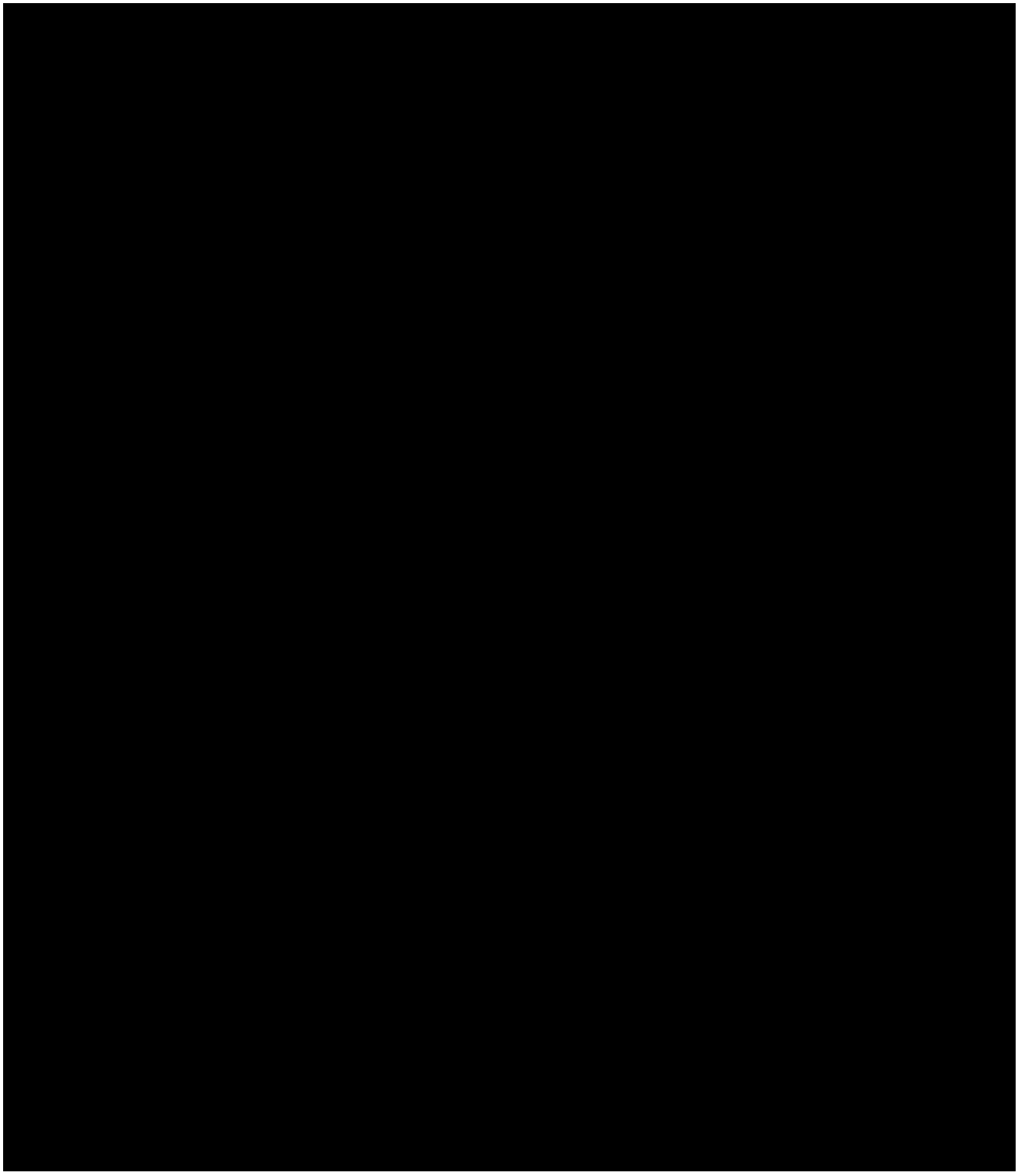
Whole blood concentrations of 2,3-DPG and ATP measured on Week 36 will be used to derive the PD parameters defined in Table 7. The PD parameters will be listed and summarized by treatment arm.

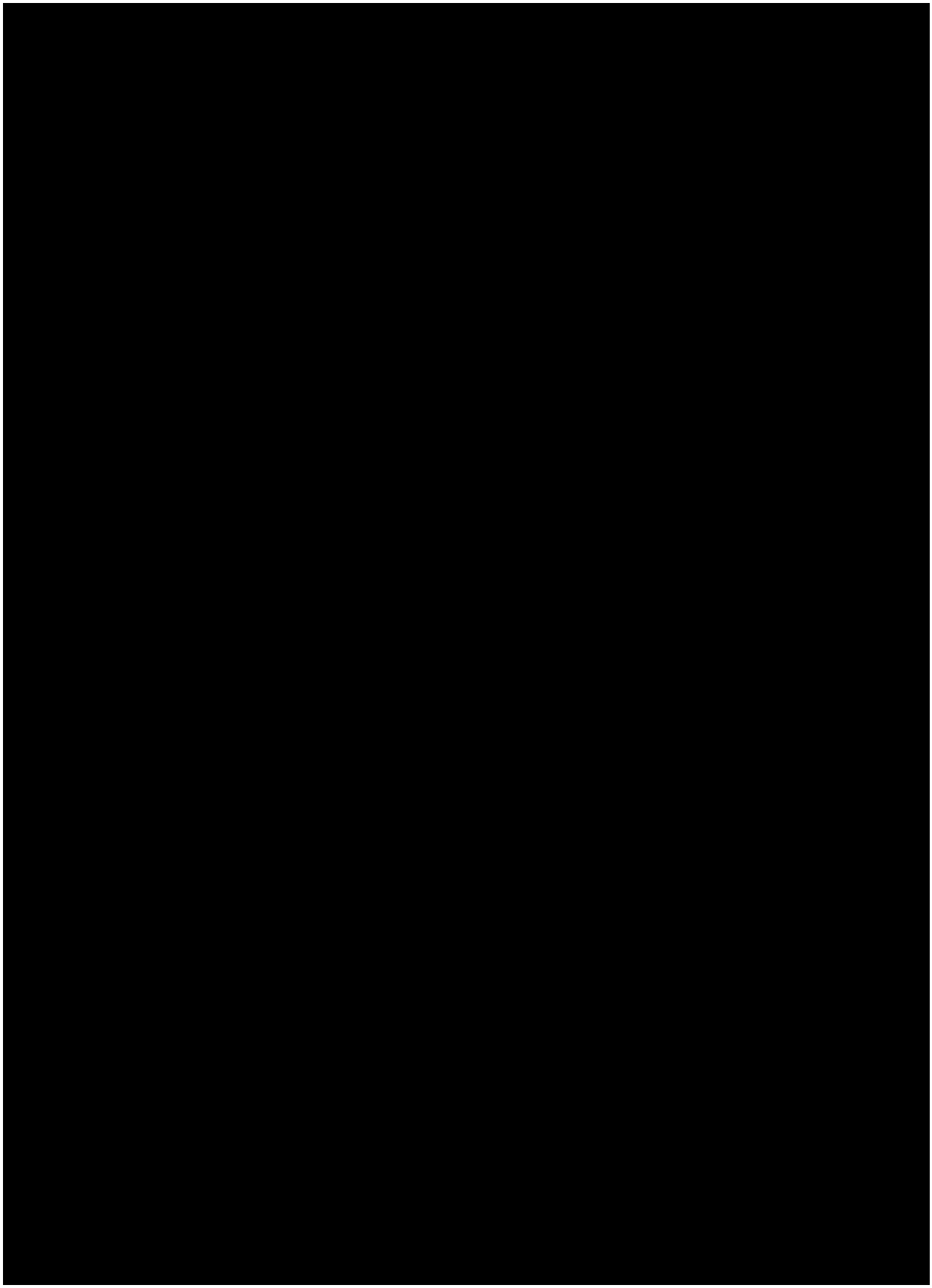
Table 7: Pharmacodynamic Parameters of Whole Blood Concentrations of 2,3-DPG and ATP

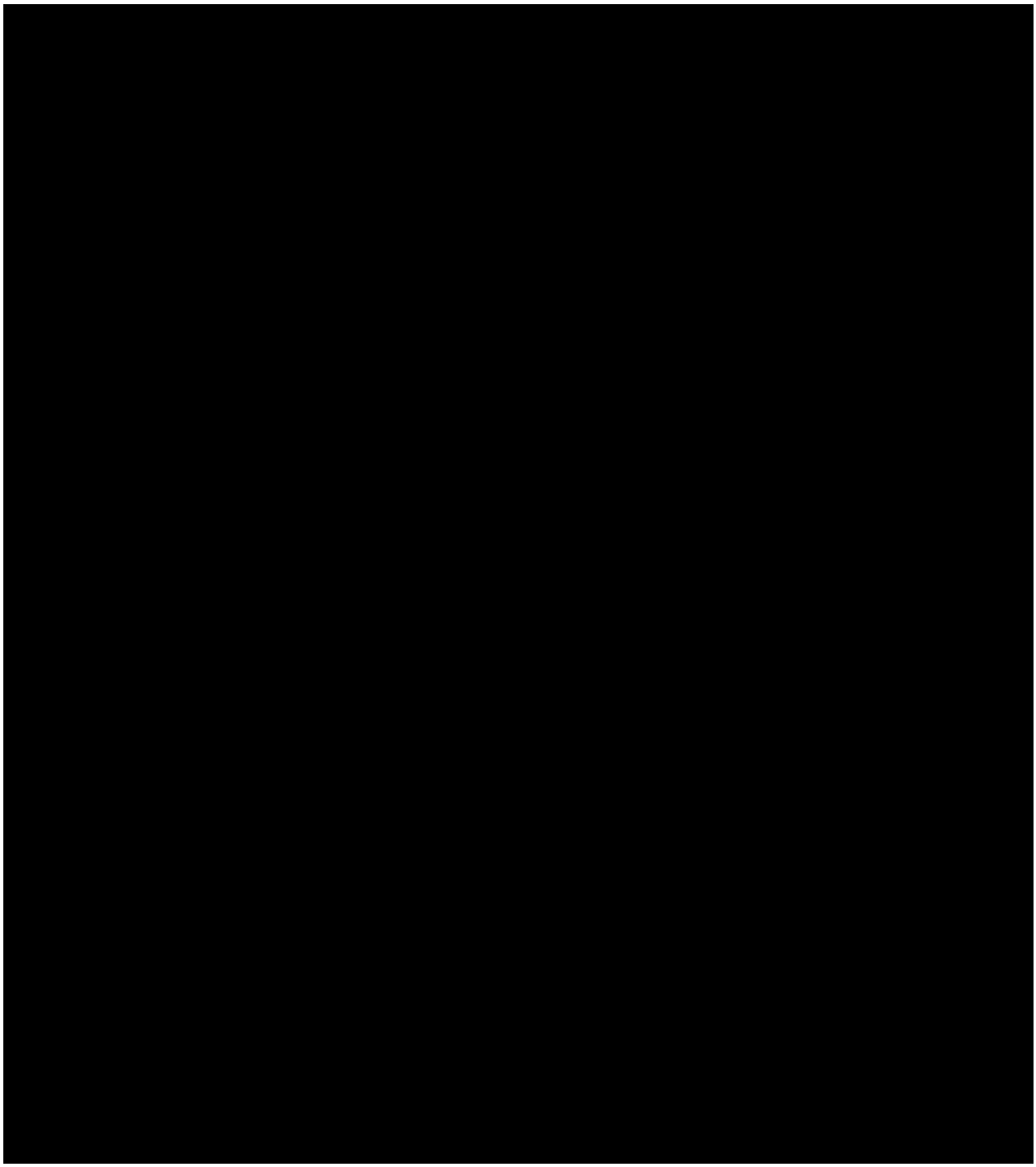
Parameters	Description
B	Baseline value as defined in Section 6.3.4
AUC_Above_$B_{0\text{-}7}$	Area of the response curve that is above the baseline value from time point zero (pre-dose) up to 7 hours (nominal time), using the linear trapezoid rule
AUC_Below_$B_{0\text{-}7}$	Area of the response curve that is below the baseline value from time point zero (pre-dose) up to 7 hours (nominal time), using the linear trapezoid rule.
AUC_Net_$B_{0\text{-}7}$	Net area of the response curve above and below the baseline value, calculated as AUC_Above_$B_{0\text{-}7}$ - AUC_Below_$B_{0\text{-}7}$
R_{max}	Maximum observed response value over 7 hours post-dose
BR_{max}	Maximum change from baseline value over 7 hours post-dose, calculated as R_{max} - B
$\%BR_{max}$	Maximum percent change from baseline value over 7 hours post-dose, calculated as (R_{max} - B) / B × 100
R_{min}	Minimum observed response value over 7 hours post-dose
BR_{min}	Minimum change from baseline value over 7 hours post-dose, calculated as R_{min} - B
$\%BR_{min}$	Minimum percent change from baseline value over 7 hours post-dose, calculated as (R_{min} - B) / B × 100
T_{max}	Time of observed R_{max}
T_{min}	Time of observed R_{min}

7.10. Pharmacokinetic and Pharmacodynamic Analyses

Longitudinal boxplots will be presented side-by-side for mitapivat plasma trough levels and for whole blood concentrations of ATP and 2,3-DPG vs time. The analysis will be based on the PK/PD analysis set including only time points associated with time-matched mitapivat plasma concentrations and ATP/2,3-DPG whole blood concentrations.







7.12. Interim Analyses

There is no planned interim analysis in this study. An Independent Data Monitoring Committee (IDMC) will be responsible for ongoing monitoring of the safety of subjects according to the IDMC Charter.

8. REFERENCES

Cappellini MD, Viprakasit V, Taher AT, et al. A phase 3 trial of luspatercept in patients with transfusion-dependent beta-thalassemia. *N Engl J Med.* 2020;382(13):1219-1231. doi:10.1056/NEJMoa1910182

Gabrielsson J, Weiner D. *Pharmacokinetic & Pharmacodynamic Data Analysis: Concepts and Applications.* 5th ed: Swedish Pharmaceutical Society; 2016.