



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

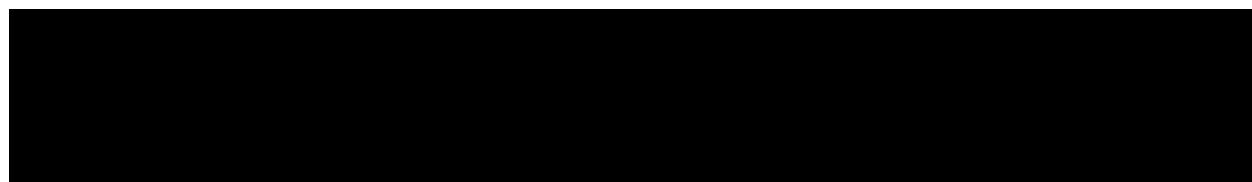
**NCT Number:** NCT04770753

**Document Date:** Protocol Amendment 3.0: 05-Aug-2024

**CLINICAL STUDY PROTOCOL****A Phase 3, Double-blind, Randomized, Placebo-Controlled, Multicenter Study Evaluating the Efficacy and Safety of Mitapivat in Subjects With Non–Transfusion-Dependent Alpha- or Beta-Thalassemia (ENERGIZE)****Study AG348-C-017**

<b>Study Sponsor:</b>	Agios Pharmaceuticals, Inc. 88 Sidney Street Cambridge, MA 02139-4169 USA Phone: 617-649-8600 Facsimile: 617-649-8618
<b>Medical Director:</b>	 Medical Director Clinical Development, Agios Pharmaceuticals, Inc.
<b>IND Number:</b>	
<b>NCT Number</b>	NCT04770753
<b>EudraCT Number:</b>	2021-000211-23
<b>Approval Date:</b>	Original Protocol (27 January 2021) Amendment 1 (21 July 2021) Amendment 2 (28 September 2022) Amendment 3 (05 August 2024)

This study will be conducted according to the protocol and in compliance with Good Clinical Practices, the International Council for Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use Guidelines, applicable local regulatory requirements, and the spirit of the ethical principles stated in the Declaration of Helsinki.



**SIGNATURE PAGE**

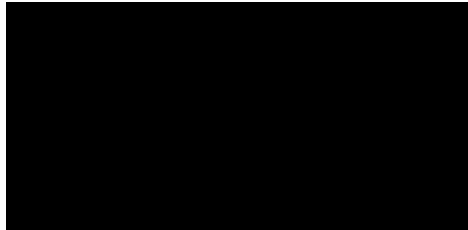
I hereby approve this clinical study protocol on behalf of Agios Pharmaceuticals, Inc. and attest that it complies with all applicable regulations and guidelines.

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

**Approval Date:** 05 August 2024

**Sponsor Signatory:**

██████████  
Vice President,  
Clinical and Translational  
Development



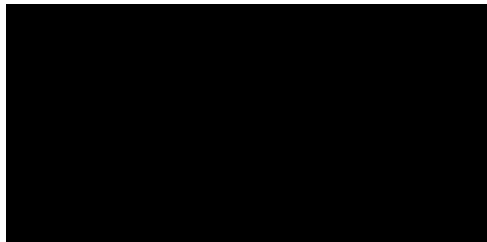
---

Name and Title (Printed)

---

Sign/Date (dd/mmm/yyyy)

██████████  
Senior Vice President,  
Biometrics



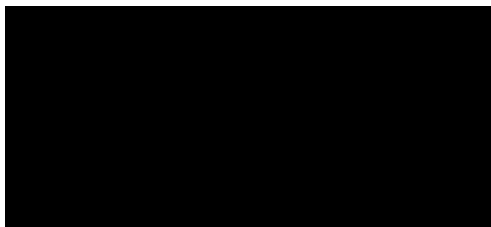
---

Name and Title (Printed)

---

Sign/Date (dd/mmm/yyyy)

██████████  
Vice President,  
DMPK/Clinical Pharmacology



---

Name and Title (Printed)

---

Sign/Date (dd/mmm/yyyy)

## INVESTIGATOR'S AGREEMENT

I understand that all documentation provided to me by Agios Pharmaceuticals, Inc. (Agios/the Sponsor) or its designated representative(s) concerning this study that has not been published previously will be kept in strict confidence. This documentation includes the study protocol, Investigator's Brochure, case report forms, and other scientific data.

This study will not commence without the prior written approval of a properly constituted Institutional Review Board (IRB)/Independent Ethics Committee (IEC). No changes will be made to the study protocol without the prior written approval of Agios and the IRB/IEC, except where necessary to eliminate an immediate hazard to the subject.

I have read, understood, and agree to conduct this study as outlined in the protocol, in compliance with the recommendations in the International Conference on Harmonisation Guideline on Good Clinical Practice, and in accordance all applicable regulatory requirements, laws, and regulations.

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

**Approval Date:** 05 August 2024

**Investigator Signatory:**

---

Name and Title  
(Printed)

---

Signature

---

Date  
(dd Month yyyy)

## PROTOCOL AMENDMENT SUMMARY OF CHANGES

### Assessment of Protocol Amendment 3, Dated 05 August 2024:

Protocol Amendment 3, dated 05 August 2024, is considered to be substantial.

### Purpose and Rationale for the Protocol Amendment:

The protocol was amended to clarify the permitted concomitant therapy, to add study drug modification and stopping rules for alanine aminotransferase (ALT) increases, and to add a section on liver-related adverse events or laboratory abnormalities because of a new important potential risk of hepatocellular injury for patients with thalassemia treated with mitapivat.

A detailed summary of the protocol amendment changes is provided in [Appendix 3](#).

## TABLE OF CONTENTS

TITLE PAGE .....	1
SIGNATURE PAGE .....	2
INVESTIGATOR’S AGREEMENT .....	3
PROTOCOL AMENDMENT SUMMARY OF CHANGES .....	4
1. PROTOCOL SUMMARY .....	11
1.1. Synopsis .....	11
1.2. Schedules of Assessments .....	19
2. INTRODUCTION .....	27
2.1. Background .....	27
2.1.1. Thalassemia .....	27
2.1.1.1. Non–Transfusion-Dependent Thalassemia .....	28
2.1.1.2. Non–Transfusion-Dependent Thalassemia Treatment .....	28
2.1.2. Mitapivat .....	29
2.1.2.1. Pharmacokinetics and Pharmacodynamics Experience .....	29
2.1.2.2. Nonclinical Experience .....	30
2.1.2.3. Clinical Experience .....	30
2.2. Study Rationale .....	32
2.3. Benefit-Risk Assessment .....	33
3. OBJECTIVES AND ENDPOINTS .....	34
4. STUDY DESIGN .....	36
4.1. Overall Design .....	36
4.1.1. Screening Period .....	36
4.1.2. Double-blind Period .....	36
4.1.3. Open-label Extension Period .....	37
4.1.4. Duration of Treatment and Safety Follow-up .....	37
4.2. Study Design Rationale .....	37
4.3. Dose Justification .....	38
4.4. End of Study Definition .....	39
5. STUDY POPULATION .....	40
5.1. Inclusion Criteria .....	40
5.2. Exclusion Criteria .....	40

5.3.	Lifestyle Considerations .....	42
5.4.	Screen Failures.....	42
6.	STUDY DRUG.....	43
6.1.	Study Drug Administered .....	43
6.2.	Preparation, Handling, Storage, and Accountability .....	44
6.3.	Randomization and Blinding .....	44
6.3.1.	Randomization.....	44
6.3.2.	Blinding .....	45
6.3.3.	Unblinding .....	45
6.4.	Study Drug Compliance .....	45
6.5.	Overdose .....	45
6.6.	Prior and Concomitant Therapy.....	46
6.6.1.	Prior Therapy .....	46
6.6.2.	Permitted Concomitant Therapy.....	46
6.6.3.	Prohibited Concomitant Therapy.....	46
6.6.3.1.	Concomitant Therapy to be Used With Caution or Replaced .....	47
6.6.3.2.	Rescue Medicine.....	47
6.7.	Dose Modifications.....	47
6.7.1.	Management of Adverse Events and Clinical Laboratory Test Result Changes.....	48
6.7.1.1.	Dose Modifications for Excessive Hb Response.....	48
6.7.1.2.	Dose Modifications for Study Drug–Related Adverse Events .....	48
7.	STUDY DRUG DISCONTINUATION AND SUBJECT WITHDRAWAL FROM STUDY .....	51
7.1.	Study Drug Discontinuation .....	51
7.2.	Subject Withdrawal From the Study.....	51
7.3.	Lost to Follow-up .....	52
8.	STUDY ASSESSMENTS AND PROCEDURES.....	53
8.1.	Informed Consent .....	53
8.2.	Screening and Eligibility .....	53
8.3.	Demographic Data .....	54
8.4.	Medical, Procedural, Surgical, and Disease History .....	54





9.3.1.	Reporting of Serious Adverse Events.....	64
9.4.	Other Safety-Related Issues.....	65
9.4.1.	Overdose or Dose Administration Error.....	65
9.4.2.	Pregnancy .....	65
9.4.2.1.	Pregnancy in Female Subjects .....	66
9.4.2.2.	Pregnancy Follow-up.....	66
<b>10. STATISTICAL CONSIDERATIONS .....</b>		
10.	STATISTICAL CONSIDERATIONS .....	67
10.1.	Statistical Hypotheses .....	67
10.2.	Sample Size Determination .....	67
10.3.	Populations for Analyses .....	69
10.4.	Statistical Analyses .....	69
10.4.1.	General Considerations.....	69
10.4.1.1.	Baseline Definitions.....	69
10.4.1.2.	On-Treatment Period .....	70
10.4.1.3.	Pooling of Data Across Clinical Study Centers.....	70
10.4.1.4.	Continuous and Categorical Variables .....	71
10.4.1.5.	Unscheduled Visits .....	71
10.4.2.	Primary Endpoint.....	71
10.4.2.1.	Hemoglobin Response .....	71
10.4.2.2.	Sensitivity Analyses.....	71
10.4.2.3.	Subgroup Analyses .....	72
10.4.3.	Secondary Efficacy Endpoints.....	72
10.4.3.1.	Key Secondary Efficacy Endpoints .....	72
10.4.3.2.	Other Secondary Efficacy Endpoints.....	73
10.4.4.	Safety Endpoints.....	74
10.4.4.1.	Adverse Events .....	74
10.4.4.2.	Clinical Laboratory Assessments .....	74
10.4.4.3.	Dual-Energy X-ray Absorptiometry Scans.....	75
10.4.4.4.	Vital Signs and Physical Examinations .....	75
<b>10.5. Interim Analyses .....</b>		
10.5.	Interim Analyses .....	75
10.6.	Independent Data Monitoring Committee .....	76

11.	REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS.....	77
11.1.	Regulatory and Ethical Considerations .....	77
11.1.1.	Temporary Modifications Allowed During Public Health Emergencies .....	77
11.1.1.1.	Allowable Temporary Modifications .....	78
11.2.	Informed Consent Process .....	79
11.3.	Data Protection .....	80
11.4.	Dissemination of Clinical Study Data .....	80
11.5.	Quality Compliance .....	81
11.6.	Source Documents .....	81
11.7.	Study Termination and Clinical Study Center Closure .....	82
11.8.	Publication Policy .....	82
12.	REFERENCES .....	83
13.	APPENDICES .....	87
APPENDIX 1.	LIST OF ABBREVIATIONS.....	88
APPENDIX 2.	DEFINITION OF WOMEN OF CHILDBEARING POTENTIAL AND CONTRACEPTION GUIDANCE .....	91

[REDACTED]

## LIST OF TABLES

Table 1:	Schedule of Assessments .....	19
Table 2:	Schedule of Assessments (Continued).....	24
Table 3:	Objectives and Endpoints .....	34
Table 4:	Study Drug Dose and Recommended Dose Taper Regimen.....	43
Table 5:	Study Drug.....	43
Table 6:	Dose Modifications for Excessive Hemoglobin Response.....	48
Table 7:	Dose Modifications for Study Drug–Related Adverse Events Except for Events of Excessive Hemoglobin Response [REDACTED] .....	48
[REDACTED]	[REDACTED]	
Table 9:	Clinical Laboratory Efficacy Assessments .....	55
Table 10:	Clinical Laboratory Safety Assessments .....	56
Table 11:	Adverse Event Severity .....	63
Table 12:	Causal Attribution Guidance .....	64

## LIST OF FIGURES

Figure 1:	Study Design.....	36
-----------	-------------------	----

## 1. PROTOCOL SUMMARY

### 1.1. Synopsis

**Name of Sponsor/Company:** Agios Pharmaceuticals, Inc.

**Name of Investigational Product:** mitapivat (also known as mitapivat sulfate, AG-348, PYRUKYND)

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

**Study Center(s):** This multicenter study will be conducted internationally.

**Phase of Development:** 3

**Objectives and Endpoints:**

<b>Primary Objective</b> <ul style="list-style-type: none"> <li>To compare the effect of mitapivat versus placebo on anemia in subjects with <math>\alpha</math>- or <math>\beta</math>- non–transfusion-dependent thalassemia (NTDT)</li> </ul>	<b>Primary Endpoint</b> <ul style="list-style-type: none"> <li>Hemoglobin (Hb) response, defined as a <math>\geq 1.0</math> g/dL increase in average Hb concentration from Week 12 through Week 24 compared with baseline</li> </ul>
<b>Key Secondary Objectives</b> <ul style="list-style-type: none"> <li>To compare the effect of mitapivat versus placebo on fatigue</li> <li>To compare the effect of mitapivat versus placebo on additional measures of anemia</li> </ul>	<b>Key Secondary Endpoints</b> <ul style="list-style-type: none"> <li>Change from baseline in average Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-Fatigue) subscale score from Week 12 through Week 24</li> <li>Change from baseline in average Hb concentration from Week 12 through Week 24</li> </ul>
<b>Secondary Objectives</b> <ul style="list-style-type: none"> <li>To evaluate the effect of mitapivat versus placebo on anemia and markers of hemolysis and erythropoiesis</li> </ul>	<b>Secondary Endpoints</b> <ul style="list-style-type: none"> <li>Hb 1.5+ response, defined as a <math>\geq 1.5</math> g/dL increase in average Hb concentration from Week 12 through Week 24 compared with baseline</li> <li>Change from baseline in indirect bilirubin, lactate dehydrogenase, and haptoglobin at Week 24</li> <li>Change from baseline in reticulocytes and erythropoietin at Week 24</li> </ul>

<ul style="list-style-type: none"> <li>To evaluate the effect of mitapivat versus placebo on additional measures of fatigue</li> </ul>	<ul style="list-style-type: none"> <li>Improvement in the Patient Global Impression of Severity (PGIS)-Fatigue by at least 1 category at Weeks 12, 16, 20, and 24 compared with baseline, or “no change” if no or mild fatigue at baseline</li> <li>Improvement in the Patient Global Impression of Change (PGIC)-Fatigue at Weeks 12, 16, 20, and 24, or “no change” if no or mild fatigue at baseline</li> </ul>
<ul style="list-style-type: none"> <li>To evaluate the effect of mitapivat versus placebo on physical activity</li> <li>To evaluate the effect of mitapivat versus placebo on iron metabolism</li> <li>To evaluate the safety of mitapivat</li> <li>To evaluate the pharmacokinetic and pharmacodynamic effects of mitapivat</li> </ul>	<ul style="list-style-type: none"> <li>Change from baseline in the 6-minute walk test (6MWT) distance at Week 24</li> <li>Change from baseline in markers of iron metabolism, including serum ferritin and transferrin saturation, at Week 24</li> <li>Type, severity, and relationship of adverse events (AEs) and serious adverse events</li> <li>Plasma or blood concentrations and pharmacokinetic parameters of mitapivat and pharmacodynamic parameters, including adenosine triphosphate (ATP) and 2,3-diphosphoglycerate (2,3-DPG)</li> </ul>

**Study Design:** Study AG348-C-017 is a Phase 3, double-blind, randomized, placebo-controlled, multicenter study evaluating the efficacy and safety of mitapivat versus placebo in adult subjects with  $\alpha$ - or  $\beta$ -NTDT followed by an Open-label Extension Period. Subject eligibility will be determined during the (up to 6 weeks) Screening Period.

The primary objective of the study is to compare the effect of mitapivat versus placebo on anemia (ie, Hb response), supported by patient-reported outcome (FACIT-Fatigue) and performance outcome (6MWT) measures evaluating how subjects feel and function, and hemolytic and erythropoietic parameters and iron metabolism. Other secondary objectives include the evaluation of pharmacokinetic and pharmacodynamic parameters and safety. Safety will be evaluated by the incidence, severity, and type of AEs, and by evaluation of vital signs, physical examination findings, clinical laboratory results, and bone mineral density scans.

Transfusions and other supportive care therapies (including iron chelation therapy) are permitted as clinically indicated to manage symptoms and prevent secondary complications. Dose modifications are permitted for excessive Hb response and study drug-related AEs.

During the 24-week Double-blind Period, study visits will occur approximately every 4 weeks for efficacy and safety assessments. Subjects will then be provided the opportunity to receive mitapivat for an additional 5 years in the Open-label Extension Period. During the Open-label Extension Period, subjects will continue to undergo safety and clinical activity assessments; however, the frequency of study visits will be reduced. Subjects will have a Safety Follow-up visit approximately 4 weeks after their last dose of study drug.

**Number of Subjects Planned:** Approximately 171 subjects are planned to be randomized in this study.

**Eligibility Criteria:*****Inclusion Criteria:***

1.  $\geq 18$  years of age at the time of providing informed consent.
2. Documented diagnosis of thalassemia ( $\beta$ -thalassemia with or without  $\alpha$ -globin gene mutations, HbE/ $\beta$ -thalassemia, or  $\alpha$ -thalassemia/HbH disease) based on Hb electrophoresis, Hb high-performance liquid chromatography, and/or DNA analysis from the subject's medical record. If this information is not available from the subject's medical record, the test(s) can be performed by a local laboratory during the Screening Period. If a local laboratory is unable to perform the test(s), results from the comprehensive  $\alpha$ - and  $\beta$ -globin genotyping performed by the study central laboratory can be used.
3. Hb concentration  $\leq 10.0$  g/dL (100.0 g/L), based on an average of at least 2 Hb concentration measurements (separated by  $\geq 7$  days) collected during the Screening Period.
4. Non-transfusion dependent, defined as:  $\leq 5$  red blood cell (RBC) units during the 24-week period before randomization **and** no RBC transfusions  $\leq 8$  weeks before providing informed consent **and** no RBC transfusions during the Screening Period.
5. If taking hydroxyurea, the hydroxyurea dose must be stable for  $\geq 16$  weeks before randomization.
6. Women of childbearing potential (WOCBP) must be abstinent of sexual activities that may result in pregnancy as part of their usual lifestyle or agree to use 2 forms of contraception, 1 of which must be considered highly effective, from the time of providing informed consent, throughout the study, and for 28 days after the last dose of study drug. The second form of contraception can be an acceptable barrier method.
7. Written informed consent before any study-related procedures are conducted and willing to comply with all study procedures for the duration of the study.

***Exclusion Criteria:***

1. Pregnant, breastfeeding, or parturient.
2. Documented history of homozygous or heterozygous HbS or HbC.
3. Prior exposure to gene therapy or prior bone marrow or stem cell transplantation.
4. Currently receiving treatment with luspatercept; the last dose must have been administered  $\geq 18$  weeks before randomization.
5. Currently receiving treatment with hematopoietic stimulating agents; the last dose must have been administered  $\geq 18$  weeks before randomization.
6. History of malignancy (active or treated)  $\leq 5$  years before providing informed consent, except for nonmelanomatous skin cancer in situ, cervical carcinoma in situ, or breast carcinoma in situ.
7. History of active and/or uncontrolled cardiac or pulmonary disease  $\leq 6$  months before providing informed consent, including but not limited to:
  - a. New York Heart Association Class III or IV heart failure or clinically significant dysrhythmia
  - b. Myocardial infarction or unstable angina pectoris; hemorrhagic, embolic, or thrombotic stroke; deep venous thrombosis; or pulmonary or arterial embolism

- c. Heart rate–corrected QT interval using Fridericia’s method  $\geq 450$  milliseconds (males) or  $\geq 470$  milliseconds (females), except for right or left bundle branch block
  - d. Severe pulmonary fibrosis as defined by severe hypoxia, evidence of right-sided heart failure, and radiographic pulmonary fibrosis  $> 50\%$
  - e. Severe pulmonary hypertension as defined by severe symptoms associated with hypoxia, right-sided heart failure, and oxygen indicated
8. Hepatobiliary disorders, including but not limited to:
- a. Liver disease with histopathological evidence of cirrhosis or severe fibrosis
  - b. Clinically symptomatic cholelithiasis or cholecystitis (prior cholecystectomy is not exclusionary)
  - c. History of drug-induced cholestatic hepatitis
  - d. Aspartate aminotransferase  $> 2.5 \times$  upper limit of normal (ULN); unless due to hemolysis and hepatic iron deposition) and alanine aminotransferase  $> 2.5 \times$  ULN (unless due to hepatic iron deposition)
9. Estimated glomerular filtration rate  $< 45$  mL/min/1.73 m<sup>2</sup> by Chronic Kidney Disease Epidemiology Collaboration creatinine equation.
10. Nonfasting triglycerides  $> 440$  mg/dL (5 mmol/L)
11. Active infection requiring systemic antimicrobial therapy at the time of providing informed consent. If antimicrobial therapy is required during the Screening Period, screening procedures should not be performed while antimicrobial therapy is being administered, and the last dose of antimicrobial therapy must be administered  $\geq 7$  days before randomization.
12. Positive test for hepatitis C virus (HCV) antibody (Ab) with evidence of active HCV infection, or positive test for hepatitis B surface antigen.
13. Positive test for HIV-1 Ab or HIV-2 Ab.
14. History of major surgery (including splenectomy)  $\leq 16$  weeks before providing informed consent and/or a major surgical procedure planned during the study.
15. Current enrollment or past participation ( $\leq 12$  weeks before administration of the first dose of study drug or a time frame equivalent to 5 half-lives of the investigational study drug, whichever is longer) in any other clinical study involving an investigational treatment or device.
16. Receiving strong cytochrome P450 (CYP)3A4/5 inhibitors that have not been stopped for  $\geq 5$  days or a time frame equivalent to 5 half-lives (whichever is longer), or strong CYP3A4 inducers that have not been stopped for  $\geq 4$  weeks or a time frame equivalent to 5 half-lives (whichever is longer), before randomization.
17. Receiving anabolic steroids, that have not been stopped for at least 4 weeks before randomization. Testosterone replacement therapy to treat hypogonadism is allowed; the testosterone dose and preparation must be stable for  $\geq 10$  weeks before randomization.
18. Known allergy, or other contraindication, to mitapivat or its excipients (microcrystalline cellulose, croscarmellose sodium, sodium stearyl fumarate, mannitol, and magnesium stearate, Opadry® II Blue [hypromellose, titanium dioxide, lactose monohydrate, triacetin, and FD&C Blue #2]).

19. Any medical, hematological, psychological, or behavioral condition(s) or prior or current therapy that, in the opinion of the Investigator, may confer an unacceptable risk to participating in the study and/or could confound the interpretation of the study data. Also excluded are:

- Subjects who are institutionalized by regulatory or court order
- Subjects with any condition(s) that could create undue influence (including but not limited to incarceration, involuntary psychiatric confinement, and financial or familial affiliation with the Investigator or Sponsor)

**Investigational Product, Dosage, and Mode of Administration:** Subjects will receive 100 mg twice-daily mitapivat for oral administration.

**Duration of Treatment and End of Study:** The duration of participation of a subject in the study is up to 5.8 years. The duration of treatment is up to 5.6 years. The end of the study 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.

**Reference Therapy, Dosage, and Mode of Administration:** Subjects will receive matched placebo for oral administration.

#### **Statistical Methods:**

Sample size determination and statistical methodology for the primary analysis of key efficacy endpoints, and analyses of safety and pharmacokinetic/pharmacodynamic endpoints are described below. Further details will be provided in the statistical analysis plan (SAP), which will be finalized before database lock and unblinding.

**Randomization and Blinding:** Eligible subjects will be randomly assigned in a 2:1 ratio to receive study drug (mitapivat or placebo). Randomization will be stratified by baseline Hb concentration ( $\leq 9.0$  g/dL or 9.1-10.0 g/dL) and by thalassemia genotype. Study subjects, Investigators, clinical study center personnel, pharmacists, and the Sponsor will be blinded to the subject's treatment assignment. During the Double-blind Period, an unblinded Independent Data Monitoring Committee will be responsible for ongoing monitoring of the safety of subjects.

**Statistical Testing Strategy:** 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 with an Hb response in the mitapivat arm and placebo arm, respectively.

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

$$H_{02}: \mu_{t1} - \mu_{c1} = 0 \text{ vs } H_{12}: \mu_{t1} - \mu_{c1} \neq 0;$$

$$H_{03}: \mu_{t2} - \mu_{c2} = 0 \text{ vs } H_{13}: \mu_{t2} - \mu_{c2} \neq 0;$$

where  $\mu_{t1}$  and  $\mu_{c1}$  are the mean change from baseline in average FACIT-Fatigue subscale score from Week 12 through Week 24 in the mitapivat arm and placebo arm respectively; and  $\mu_{t2}$  and  $\mu_{c2}$  are the mean change from baseline in average Hb concentration from Week 12 through Week 24 in the mitapivat arm and placebo arm, respectively.



Overall type I error will be maintained at or below 2-sided significance level of 0.05 by a fixed-sequence testing procedure.  $H_{01}$  will be tested first; if  $H_{01}$  is rejected, then  $H_{02}$  will be tested; if  $H_{02}$  is rejected, then  $H_{03}$  will be tested. All tests will be conducted at the 2-sided 0.05 significance level.

**Sample Size Determination:** Assuming an Hb response rate of 10% in the placebo arm, 171 subjects (114 subjects randomized to mitapivat and 57 subjects randomized to placebo) are needed to provide a 95% power to detect an increase in Hb response rate from 10% in the placebo arm to 35% in the mitapivat arm based on a 2-sided significance level of 0.05.

The sample size of 171 subjects will also provide 80% power at a 2-sided significance level of 0.05 to detect a 5.5 difference in the change from baseline in average FACIT-Fatigue subscale score from Week 12 through Week 24 between the mitapivat arm and the placebo arm. These calculations assume a change from baseline in average FACIT-Fatigue subscale score from Week 12 through Week 24 in the mitapivat arm of 4.5 and in the placebo arm of -1; and a common standard deviation of 12.

#### Populations for Analyses:

All Screened Subjects	All subjects who sign the informed consent form.
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 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 do not receive at least 1 dose of the randomized treatment will be excluded from the PPS. Other criteria leading to exclusion of subjects from the PPS will be prespecified in the SAP.
Safety Analysis Set	All subjects who receive at least 1 dose of the study drug. 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.
Pharmacodynamic Analysis Set	A subset of the Safety Analysis Set including all subjects with at least 1 blood 2,3-DPG or ATP concentration measurement above the lower limit of quantitation (LLQ).
Pharmacokinetic Analysis Set	A subset of the Safety Analysis Set including all subjects with at least 1 plasma mitapivat concentration measurement above LLQ.

**Primary Endpoint:** The primary endpoint of Hb response is defined as a  $\geq 1.0$  g/dL increase in average Hb concentration from Week 12 through Week 24 compared with baseline.

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

Hemoglobin concentrations assessed within 8 weeks after an RBC transfusion will be excluded from the analysis of the primary endpoint. Subjects who do not have at least 2 on-treatment Hb concentration assessments from Week 12 to Week 24 will be considered nonresponders.

**Key Secondary Endpoints:****Change from baseline in average FACIT-Fatigue subscale score from Week 12 through Week 24**

The FACIT-Fatigue subscale will be scored according to published scoring guidelines. The change from baseline in average FACIT-Fatigue subscale score from Week 12 through Week 24 will be compared between the mitapivat arm and the placebo arm using an analysis of covariance (ANCOVA) model. The model will include the score change from baseline as the dependent variable, treatment arm as the independent variable, and covariates for randomization stratification factors and baseline FACIT-Fatigue subscale score. Missing data will not be imputed for the primary analysis of FACIT-Fatigue. FACIT-Fatigue subscale scores within 8 weeks after an RBC transfusion will be excluded from the analysis.

Descriptive statistics will be used to summarize the individual items and change from baseline in the average subscale scores at each scheduled assessment time point by treatment arm.

Additionally, anchor-based analyses may be used to establish the threshold for clinically meaningful change in the FACIT-Fatigue using PGIS and PGIC ratings as a basis for the anchors.

**Change from baseline in average Hb concentration from Week 12 through Week 24**

The change from baseline in average Hb concentration from Week 12 through Week 24 will be compared between the mitapivat arm and the placebo arm using an ANCOVA model. The model will include the score change from baseline as the dependent variable, treatment arm as the independent variable, and covariates for randomization stratification factors and baseline Hb concentration. The least squares mean of the change from baseline in average Hb concentration (average from Week 12 to Week 24) will be presented for each treatment arm.

Hemoglobin concentrations assessed within 8 weeks after an RBC transfusion will be excluded from the analysis.

**Pharmacokinetic and Pharmacodynamic Endpoints**

Descriptive statistics of pharmacokinetic (arithmetic and geometric means, standard deviation, percent coefficient of variation [CV%], CV% geometric mean, minimum, median, and maximum) and pharmacodynamic parameters, including ATP and 2,3-DPG, will be summarized as appropriate.

**Safety Endpoints**

Summaries of safety data from the Double-blind Period will be presented by treatment arm based on the Safety Analysis Set. Summaries of safety data from the Open-label Extension Period will be presented by treatment arm and overall based on the Safety Analysis Set.

The on-treatment period begins on the date of the start of study drug and ends 28 days after the end of study drug administration (including the recommended dose taper).

Treatment-emergent adverse events are defined as AEs with a first onset date during the on-treatment period or worsening from baseline. Treatment-emergent adverse events will be summarized according to the latest version of Medical Dictionary for Regulatory Activities terminology by System Organ Class and/or Preferred Term, severity (based on the National

Cancer Institute [NCI] Common Terminology Criteria for Adverse Events [CTCAE] Version 4.03 grading), seriousness, and relationship to study drug, unless otherwise specified.

Clinical laboratory test results will be graded according to the NCI CTCAE Version 4.03, as applicable, and will be summarized based on the worst CTCAE grade.

## 1.2. Schedules of Assessments

**Table 1: Schedule of Assessments**

Study Visit Week:	Screening Period	On-Treatment Period <sup>1</sup>																
		Double-blind Period							Open-label Extension Period									
Study Visit Week:	--	1	4 <sup>2</sup>	8 <sup>2</sup>	12	16 <sup>2</sup>	20	24	28	32 <sup>2</sup>	36	48	60 <sup>2</sup>	72	84 <sup>2</sup>	96	108 <sup>2</sup>	120
Study Visit Day:	-42 to -1	1	29	57	85	113	141	169	197	225	253	337	421	505	589	673	757	841
Visit Window (Days):	--	--	±4	±4	±4	±4	±4	±4	±4	±8	±8	±14	±14	±14	±14	±14	±14	±14
<b>Procedures:</b>																		
Informed consent	X																	
Demographics	X																	
Eligibility	X	X																
Documentation of thalassemia diagnosis <sup>3</sup>	X																	
Comprehensive α- and β-globin genotyping <sup>4</sup>	X																	
Medical, procedural, surgical, and disease history	X																	
Transfusion history <sup>5</sup>	X																	
Prior medications <sup>6</sup>	X																	
Hepatitis and HIV screening <sup>7</sup>	X																	
Pregnancy testing (WOCBP only) <sup>8</sup>	X	X																
Randomization <sup>9</sup>		X																

**Table 1: Schedule of Assessments**

Study Visit Week:	Screening Period	On-Treatment Period <sup>1</sup>																
		Double-blind Period							Open-label Extension Period									
	--	1	4 <sup>2</sup>	8 <sup>2</sup>	12	16 <sup>2</sup>	20	24	28	32 <sup>2</sup>	36	48	60 <sup>2</sup>	72	84 <sup>2</sup>	96	108 <sup>2</sup>	120
Study Visit Day:	-42 to -1	1	29	57	85	113	141	169	197	225	253	337	421	505	589	673	757	841
Visit Window (Days):	--	--	±4	±4	±4	±4	±4	±4	±4	±8	±8	±14	±14	±14	±14	±14	±14	±14
<b>Procedures:</b>																		
Physical examination		X						X										
Vital signs, weight, and height <sup>12</sup>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
12-lead ECG <sup>13</sup>	X																	
Hematology <sup>14</sup>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical chemistry <sup>15</sup>	X	X			X			X			X	X	X	X	X	X	X	X
Liver tests and LDH <sup>16</sup>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Lipid panel <sup>17</sup>	X							X				X				X		
Coagulation tests <sup>18</sup>		X																
Sex hormones <sup>19</sup>		X			X			X			X	X		X		X		X
Erythropoietin and haptoglobin		X			X			X			X	X						
Iron panel <sup>20</sup>		X						X				X		X		X		X

**Table 1: Schedule of Assessments**

Study Visit Week:	Screening Period	On-Treatment Period <sup>1</sup>																
		Double-blind Period							Open-label Extension Period									
	--	1	4 <sup>2</sup>	8 <sup>2</sup>	12	16 <sup>2</sup>	20	24	28	32 <sup>2</sup>	36	48	60 <sup>2</sup>	72	84 <sup>2</sup>	96	108 <sup>2</sup>	120
Study Visit Day:	-42 to -1	1	29	57	85	113	141	169	197	225	253	337	421	505	589	673	757	841
Visit Window (Days):	--	--	±4	±4	±4	±4	±4	±4	±4	±8	±8	±14	±14	±14	±14	±14	±14	±14
<b>Procedures:</b>																		
Blood sample for pharmacodynamic analysis		X <sup>22</sup>			X <sup>23</sup>		X <sup>24</sup>											
Blood sample for pharmacokinetic analysis					X <sup>23</sup>		X <sup>24</sup>											
FACIT-Fatigue		X	X	X	X	X	X	X	X	X	X	X		X				X
PGIS- and PGIC-Fatigue		X <sup>26</sup>	X	X	X	X	X	X	X	X	X	X		X				X
6MWT		X			X			X			X	X		X				X
Study drug administration <sup>28</sup>		X (continuous)																
AEs and SAEs <sup>29</sup>		X (continuous)																
Concomitant medications, procedures, and surgeries <sup>30</sup>		X (continuous)																

**Table 1: Schedule of Assessments**

Study Visit Week:	Screening Period	On-Treatment Period <sup>1</sup>																
		Double-blind Period							Open-label Extension Period									
Study Visit Week:	--	1	4 <sup>2</sup>	8 <sup>2</sup>	12	16 <sup>2</sup>	20	24	28	32 <sup>2</sup>	36	48	60 <sup>2</sup>	72	84 <sup>2</sup>	96	108 <sup>2</sup>	120
Study Visit Day:	-42 to -1	1	29	57	85	113	141	169	197	225	253	337	421	505	589	673	757	841
Visit Window (Days):	--	--	±4	±4	±4	±4	±4	±4	±4	±8	±8	±14	±14	±14	±14	±14	±14	±14
<b>Procedures:</b>																		
On-treatment transfusion record <sup>31</sup>		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Abbreviations: 6MWT = 6-minute walk test; Ab = antibody; AE = adverse event; ALP = alkaline phosphatase; ALT = alanine aminotransferase; aPTT = activated partial thromboplastin time; AST = aspartate aminotransferase; BMD = bone mineral density; BUN = blood urea nitrogen; CO<sub>2</sub> = carbon dioxide; [REDACTED];

[REDACTED] ECG = electrocardiogram; [REDACTED]; EOS = End of Study; [REDACTED];

FACIT-Fatigue = Functional Assessment of Chronic Illness Therapy-Fatigue; [REDACTED]; FSH = follicle stimulating hormone;

Hb = hemoglobin; [REDACTED]; HBsAg = hepatitis B surface antigen; hCG = human chorionic gonadotropin; HCT = hematocrit; HCV = hepatitis C virus;

HDL-C = high-density lipoprotein cholesterol; HPLC = high-performance liquid chromatography; HRQOL = health-related quality of life; INR = international normalized ratio;

LCMS = liquid chromatography-mass spectrometry; LDH = lactate dehydrogenase; LDL-C = low-density lipoprotein cholesterol; MCH = mean corpuscular hemoglobin;

MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; [REDACTED]; PGIC = Patient Global Impression of Change;

PGIS = Patient Global Impression of Severity; PRO = patient-reported outcome; [REDACTED]; RBC = red blood cell;

RDW-CV = red cell distribution width coefficient of variation; SAE = serious adverse event; Thal = thalassemia; WBC = white blood cell; WOCBP = women of childbearing potential.

**Notes: All HRQOL assessments must be conducted before all other study visit assessments, including the 6MWT (see Section 8.11). The 6MWT should be conducted before the remaining assessments at the study visit, including blood samples. All blood samples must be collected before study drug administration and before the RBC transfusion, if an RBC transfusion is scheduled on the same day (see Section 8.7).**

<sup>1</sup> At any time during the study, subjects who discontinue study drug should undergo the recommended dose taper and complete the assessments at the Safety Follow-up visit after administration of their last dose of study drug (ie, including the recommended dose taper). Subjects who withdraw from the study should be encouraged to first undergo the recommended dose taper (Table 4) and complete the Safety Follow-up visit (Table 2). Subjects who are discontinuing study drug should be monitored for signs of hemolysis and worsening of anemia, as clinically indicated.

<sup>2</sup> If allowed by local institutional guidelines, and at the discretion of the Investigator and the subject, subjects will have the option to complete study-specific assessments at home instead of in person at the clinical study center. These visits (Investigator Assessment and Home Health) will include a visit conducted by the Investigator (or designee) and a visit at home with a nurse or home health-care provider (see Section 8).

<sup>3</sup> Documentation of thalassemia diagnosis by Hb electrophoresis, Hb HPLC, and/or DNA analysis will be recorded from the subject's medical record. If this documentation is not available from the subject's medical record, the test(s) should be performed during the Screening Period (see Section 8.2).

<sup>4</sup> A blood sample will be collected for comprehensive  $\alpha$ - and  $\beta$ -globin genotyping. Results of this analysis are not required for eligibility.

<sup>5</sup> Transfusion history will be recorded for the 24-week period before randomization (see Section 8.7 for definition of an RBC unit and information to be recorded for each transfusion).

<sup>6</sup> Prior medications are defined as those administered any time  $\leq 4$  weeks before providing informed consent until administration of the first dose of study drug, unless otherwise specified. Prior use of iron chelation therapy for up to 1 year before administration of the first dose of study drug will be recorded.

- <sup>7</sup> A test for HBsAg and HCV Ab will be performed during the Screening Period. Positive results for HCV Ab testing require confirmation by a test for HCV-RNA. A test for HIV-1 Ab and HIV-2 Ab will also be performed during the Screening Period.
- <sup>8</sup> During the Screening Period, an FSH assessment will be performed for women in the absence of 12 months of amenorrhea to confirm non-reproductive potential (see [Appendix 2](#)), and a serum (hCG) pregnancy test will be performed for WOCBP. On Day 1, a serum or urine pregnancy test will be performed locally for WOCBP and confirmed negative before administration of the first dose of study drug. Thereafter, pregnancy testing should occur more frequently if clinically indicated (ie, if there is suspicion of pregnancy) or required by local regulations.
- <sup>9</sup> Randomization can occur up to 48 hours before administration of the first dose of study drug on Day 1.
- 
- 
- <sup>12</sup> Vital signs include systolic and diastolic blood pressure, pulse rate, and body temperature. Weight will also be recorded. Height will be recorded at screening only. Vital signs should be measured after 5 minutes of recumbency, and before blood sample collection.
- <sup>13</sup> A single, 12-lead ECG will be recorded and read at the clinical study center after 5 min of recumbency, and before blood sample collection.
- <sup>14</sup> Hematology includes a complete blood count (Hb, HCT, MCHC, MCH, MCV, RBC count, nucleated RBCs, RDW-CV, % reticulocyte count, absolute reticulocyte count, and platelet count) with WBC count and differential (neutrophils, basophils, eosinophils, lymphocytes, and monocytes). During the Screening Period, at least 2 Hb concentration measurements (separated by  $\geq 7$  days) will be collected.
- <sup>15</sup> Clinical chemistry includes albumin, BUN or urea, total calcium, CO<sub>2</sub> or bicarbonate, chloride, creatinine, ■■■■■■■■■■ magnesium, phosphorus, potassium, sodium, total protein, and uric acid.
- <sup>16</sup> Liver tests include ALP, ALT/SGPT, AST/SGOT, and bilirubin (direct/conjugated, indirect/unconjugated, and total). LDH will also be measured.
- <sup>17</sup> Lipid panel includes nonfasting HDL-C, LDL-C, total cholesterol, and triglycerides.
- <sup>18</sup> Coagulation tests include fibrinogen, aPTT, and INR.
- <sup>19</sup> Sex hormones include nonfasting estradiol, estrone, and testosterone (free and total). If possible, blood samples should be collected in the morning. A highly sensitive LCMS assay should be used to analyze samples.
- <sup>20</sup> Iron panel includes iron, serum ferritin, transferrin, transferrin saturation, and total iron binding capacity.
- 
- <sup>22</sup> A blood sample for pharmacodynamic analysis will be collected on Day 1  $\leq 60$  min predose.
- <sup>23</sup> Blood samples for pharmacokinetic and pharmacodynamic analysis will be collected at Week 12 ( $\leq 60$  min predose). If an RBC transfusion takes place during this visit, the blood sample for pharmacokinetic and pharmacodynamic analysis should be collected before the RBC transfusion.
- <sup>24</sup> Blood samples for pharmacokinetic and pharmacodynamic analysis will be collected at Week 20 ( $\leq 60$  min predose, and 0.5 hr [ $\pm 5$  min], 1 hr [ $\pm 5$  min], 3 hr [ $\pm 15$  min], 5 hr [ $\pm 15$  min], and 7 hr [ $\pm 30$  min] postdose). If an RBC transfusion takes place during this visit, the blood samples for pharmacokinetic and pharmacodynamic analysis should be collected before the RBC transfusion.
- 
- <sup>26</sup> PRO questionnaires for PGIC (Fatigue, ■■■■■■■■■■) are not conducted on Day 1.
- 
- <sup>28</sup> Study drug will be dispensed at every visit. Subjects must return study drug bottles (including empty bottles) and unused tablets at each visit to the clinical study center. The morning dose of study drug will be administered at the clinical study center during in-person visits to the clinical study center. During the Double-blind Period, subjects will be asked to record study drug dosing in a diary. The last dose of blinded study drug will be administered on the morning of the Week 24 visit. The first dose of open-label mitapivat will be administered on the evening of the Week 24 visit.
- <sup>29</sup> Adverse events and SAEs will be collected throughout the study.
- <sup>30</sup> Concomitant medications, procedures, and surgeries are defined as all medications administered and procedures conducted from the first dose of study drug through 4 weeks after administration of the last dose of study drug (ie, EOS visit).
- <sup>31</sup> See Section 8.7 for definition of an RBC unit and information to be recorded for each RBC transfusion.



**Table 2: Schedule of Assessments (Continued)**

Study Visit Week	Open-label Extension Period <sup>1</sup>														Safety Follow-up <sup>2</sup>
	132 <sup>3</sup>	144	156 <sup>3</sup>	168	180 <sup>3</sup>	192	204 <sup>3</sup>	216	228 <sup>3</sup>	240	252 <sup>3</sup>	264	276 <sup>3</sup>	288 <sup>4</sup>	28 Days After Last Dose/EOS
Study Visit Day	925	1,009	1,093	1,177	1,261	1,345	1,429	1,513	1,597	1,681	1,765	1,849	1,933	2,017	
Study Visit Window (Days)	±14	±14	±14	±14	±14	±14	±14	±14	±14	±14	±14	±14	±14	±14	±4
<b>Procedures:</b>															
Pregnancy testing (WOCBP only) <sup>5</sup>															X
Physical examination														X	X
Vital signs and weight <sup>8</sup>		X		X		X		X		X		X		X	X
Hematology <sup>9</sup>		X		X		X		X		X		X		X	X
Clinical chemistry <sup>10</sup>		X		X		X		X		X		X		X	X
Liver tests and LDH <sup>11</sup>		X		X		X		X		X		X		X	X
Lipid panel <sup>12</sup>		X				X				X				X	X
Sex hormones <sup>13</sup>		X		X		X		X		X		X		X	X
Erythropoietin															X
Iron panel <sup>14</sup>		X		X		X		X		X		X		X	

Study Visit Week	Open-label Extension Period <sup>1</sup>														Safety Follow-up <sup>2</sup>
	132 <sup>3</sup>	144	156 <sup>3</sup>	168	180 <sup>3</sup>	192	204 <sup>3</sup>	216	228 <sup>3</sup>	240	252 <sup>3</sup>	264	276 <sup>3</sup>	288 <sup>4</sup>	28 Days After Last Dose/EOS
Study Visit Day	925	1,009	1,093	1,177	1,261	1,345	1,429	1,513	1,597	1,681	1,765	1,849	1,933	2,017	
Study Visit Window (Days)	±14	±14	±14	±14	±14	±14	±14	±14	±14	±14	±14	±14	±14	±14	±4
<b>Procedures:</b>															
FACIT-Fatigue				X				X						X	X
PGIS- and PGIC-Fatigue [REDACTED] [REDACTED]				X				X						X	X
[REDACTED]															
6MWT				X				X						X	
Study drug administration <sup>17</sup>	X (continuous)														X
AEs and SAEs <sup>18</sup>	X (continuous)														X
Concomitant medications, procedures, and surgeries <sup>19</sup>	X (continuous)														X
On-treatment transfusion record <sup>20</sup>	X	X	X	X	X	X	X	X	X	X	X	X	X	X	

Abbreviations: 6MWT = 6-minute walk test; AE = adverse event; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BMD = bone mineral density; BUN = blood urea nitrogen; CO<sub>2</sub> = carbon dioxide; [REDACTED]; [REDACTED]; EOS = End of Study; FACIT-Fatigue = Functional Assessment of Chronic Illness Therapy-Fatigue; Hb = hemoglobin; [REDACTED]; HCT = hematocrit; HDL-C = high-density lipoprotein cholesterol; HRQOL = health-related quality of life; LCMS = liquid chromatography-mass spectrometry; LDH = lactate dehydrogenase; LDL-C = low-density lipoprotein cholesterol; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; [REDACTED]; [REDACTED]; PGIC = Patient Global Impression of Change; PGIS = Patient Global Impression of Severity; [REDACTED]; RBC = red blood cell; RDW-CV = red cell distribution width coefficient of variation; SAE = serious adverse event; Thal = thalassemia; WBC = white blood cell; WOCBP = women of childbearing potential.

**Notes:** All HRQOL assessments must be conducted before all other study visit assessments, including the 6MWT (see Section 8.11). The 6MWT should be conducted before the remaining assessments at the study visit, including blood samples. All blood samples must be collected before study drug administration and before the RBC transfusion, if an RBC transfusion is scheduled on the same day (see Section 8.7).

- <sup>1</sup> At any time during the study, subjects who discontinue study drug should undergo the recommended dose taper and complete the assessments at the Safety Follow-up visit after administration of their last dose of study drug (ie, including the recommended dose taper). Subjects who withdraw from the study should be encouraged to first undergo the recommended dose taper (Table 4) and complete the Safety Follow-up visit. Subjects who are discontinuing study drug should be monitored for signs of hemolysis and worsening of anemia, as clinically indicated.
- <sup>2</sup> The Safety Follow-up visit is to be completed after administration of the last of any dose of study drug (ie, including the recommended dose taper).
- <sup>3</sup> If allowed by local institutional guidelines, and at the discretion of the Investigator and the subject, subjects will have the option to complete study-specific assessments at home instead of in person at the clinical study center. These visits (Investigator Assessment only) will be conducted by the Investigator (or designee) (see Section 8).
- <sup>4</sup> After completing the study visit at Week 288, subjects should begin the dose taper.
- <sup>5</sup> Pregnancy testing should occur more frequently as clinically indicated (ie, if there is suspicion of pregnancy) or if required by local regulations. A serum or urine pregnancy test will be performed locally for WOCBP at the Safety Follow-up visit.
- [REDACTED]
- <sup>8</sup> Vital signs include systolic and diastolic blood pressure, pulse rate, and body temperature. Weight will also be recorded. Vital signs should be measured after 5 minutes of recumbency, and before blood sample collection.
- <sup>9</sup> Hematology includes a complete blood count (Hb, HCT, MCHC, MCH, MCV, RBC count, nucleated RBCs, RDW-CV, % reticulocyte count, absolute reticulocyte count, and platelet count) with WBC count and differential (neutrophils, basophils, eosinophils, lymphocytes, and monocytes).
- <sup>10</sup> Clinical chemistry includes albumin, BUN or urea, total calcium, CO<sub>2</sub> or bicarbonate, chloride, creatinine, [REDACTED] magnesium, phosphorus, potassium, sodium, total protein, and uric acid.
- <sup>11</sup> Liver tests include ALP, ALT/SGPT, AST/SGOT, and bilirubin (direct/conjugated, indirect/unconjugated, and total). LDH will also be measured.
- <sup>12</sup> Lipid panel includes nonfasting HDL-C, LDL-C, total cholesterol, and triglycerides.
- <sup>13</sup> Sex hormones include nonfasting estradiol, estrone, and testosterone (free and total). If possible, blood samples should be collected in the morning. A highly sensitive LCMS assay should be used to analyze samples.
- <sup>14</sup> Iron panel includes iron, serum ferritin, transferrin, transferrin saturation, and total iron binding capacity.
- [REDACTED]
- [REDACTED]
- <sup>17</sup> Study drug will be dispensed at every visit. Subjects must return study drug bottles (including empty bottles) and unused tablets at each visit to the clinical study center. The morning dose of study drug will be administered at the clinical study center during in-person visits to the clinical study center.
- <sup>18</sup> Adverse events and SAEs will be collected throughout the study.
- <sup>19</sup> Concomitant medications, procedures, and surgeries are defined as all medications administered and procedures conducted from the first dose of study drug through 4 weeks after administration of the last dose of study drug (ie, EOS visit).
- <sup>20</sup> See Section 8.7 for definition of an RBC unit and for information to be recorded for each RBC transfusion.

## 2. INTRODUCTION

### 2.1. Background

#### 2.1.1. Thalassemia

The thalassemias are a group of disorders in which the normal ratio of  $\alpha$ - to  $\beta$ -globin production is disrupted due to a disease-causing variant in one or more of the globin genes. Inherited hemoglobin (Hb) disorders can be divided into 2 main groups. The first group includes the  $\alpha$ - and  $\beta$ -thalassemias, which result from the defective synthesis of the  $\alpha$ - or  $\beta$ -globin chains of adult hemoglobin (HbA). The second group includes structural Hb variants, such as sickle cell hemoglobin (HbS), HbC, and HbE (Musallam, Rivella, et al, 2013; Taher et al, 2017).

Alpha-globin aggregates (found in  $\beta$ -thalassemia) readily precipitate, which disrupts the red blood cell (RBC) membrane and results in oxidative stress. Beta-globin tetramers (found in  $\alpha$ -thalassemia/HbH disease) are generally more soluble but are still unstable and can form precipitates as well (Cappellini et al, 2014).

In thalassemia, the imbalance in globin chain synthesis imposes metabolic stress on the RBCs, specifically in the form of excess generation of reactive oxygen species and an increased demand on adenosine triphosphate (ATP)-dependent proteolytic mechanisms to clear excess globin chains. In thalassemic RBCs, ATP supply appears to be insufficient to maintain RBC membrane fitness and clearance of globin precipitates, leading to early and increased death of RBC precursors in the bone marrow and in extramedullary sites (Chakraborty et al, 2012; Shaeffer, 1988; Ting et al, 1994).

These pathophysiological changes lead to the hallmarks of the disease: ineffective erythropoiesis, peripheral hemolysis, and subsequent anemia. Clinical implications of the  $\alpha$ - and  $\beta$ -globin imbalance include lack of sufficient RBCs and Hb for effective oxygen transport, and ineffective erythropoiesis and hemolysis, which can lead to splenomegaly, bone marrow expansion (extramedullary hematopoiesis), concomitant bone deformities, and iron overload (Cappellini et al, 2014).

Alpha- and  $\beta$ -thalassemias are genetically heterogeneous and vary by phenotype and severity (Galanello, 2012; Galanello and Cao, 2011; Thein, 2013; Vichinsky, 2012; Vichinsky, 2016). Clinical management with RBC transfusions is an essential factor in classifying thalassemias as either transfusion-dependent thalassemia (TDT) or non-transfusion-dependent thalassemia (NTDT). Patients with TDT need life-long regular transfusions for survival. Patients with NTDT do not need life-long regular transfusions for survival; however, they may require transfusions during times of erythroid stress such as infection, pregnancy, surgery, or aplastic crisis, and may transition to requiring regular transfusions and becoming transfusion-dependent later in life (Musallam et al, 2020; Musallam, Rivella, et al, 2013; Taher, Radwan, et al, 2015). While TDT and NTDT are widely used clinical classifications of thalassemia that capture the different transfusional needs, the underlying molecular and pathophysiological basis are similar across the spectrum of the disease (Galanello, 2012; Galanello and Cao, 2011; Thein, 2013; Vichinsky, 2012; Vichinsky, 2016). As this clinical study (AG348-C-017) is focused on a population of subjects with NTDT, the following sections focus more specifically on disease characteristics and available treatments for patients with NTDT.

### 2.1.1.1. Non–Transfusion-Dependent Thalassemia

Patients with NTDT are typically diagnosed because of chronic anemia, with Hb concentrations between 7 g/dL and 10 g/dL. Although these patients do not require regular transfusions for survival and have been regarded historically as having less severe disease than patients with TDT, patients with NTDT still suffer from untreated hemolytic anemia, and it is now recognized that they develop severe comorbidities as a result of untreated disease (Taher et al, 2017).

Comorbidities in NTDT include iron overload (even without transfusions), cerebral ischemia, pulmonary hypertension, right-sided heart failure, hepatic fibrosis, cirrhosis, gall stones, osteoporosis, folic acid deficiency, infections, and venous thrombosis (Musallam, Angastiniotis, et al, 2013; Taher et al, 2011; Taher, Musallam, et al, 2015). A higher Hb concentration has been shown to be significantly associated with decreased morbidity; there is strong correlation between each 1g/dL change in Hb concentration and the odds of morbidity development even upon adjusting for confounding factors of age, sex, and splenectomy status (Musallam et al, 2020; Taher, Musallam, et al, 2015). In patients with NTDT, lower Hb concentration is also associated with reduced health-related quality of life (HRQOL) (Amid et al, 2016; Mihailescu et al, 2020).

### 2.1.1.2. Non–Transfusion-Dependent Thalassemia Treatment

Available therapies for patients with NTDT are primarily supportive, addressing either symptoms (eg, RBC transfusions or splenectomy for anemia) or complications of the underlying disease or its treatment (eg, chelation therapy for iron overload) (Cappellini et al, 2014). Regular transfusions are associated with iron overload; however, in the absence of RBC transfusions as a result of chronic hemolysis, ineffective erythropoiesis, and hypoxia lead to dysregulated increased intestinal iron absorption (Taher et al, 2011).

Iron chelation therapies may be administered to reduce iron overload; however, these therapies have limitations in efficacy and safety, and access and adherence to treatment are not uniform (Cappellini, Porter, et al, 2018; Taher et al, 2018). Splenectomy increases Hb concentration and can lead to improved growth and development (Karimi et al, 2014); however, patients are at increased risk of thrombotic and vascular events after splenectomy (Cappellini et al, 2000; Taher, Musallam, et al, 2015). Although supportive care measures have resulted in an improved prognosis and longer survival, high morbidity and mortality persist (Borgna-Pignatti et al, 2004; Modell et al, 2000). Access to services and supportive care is challenging, particularly in resource-constrained countries (Cappellini, Viprakasit, et al, 2018; Taher et al, 2018; Vichinsky, 2012; Weatherall, 2011).

Current approved therapies for thalassemia are limited and are restricted to patients with  $\beta$ -TDT. Hematopoietic stem cell transplantation (HSCT) and gene therapy are potentially curative treatments (Angelucci et al, 2014; Schuessler-Lenz et al, 2020). Zynteglo<sup>®</sup> (autologous CD34+ cells encoding  $\beta$ A-T87Q-globin) was approved in the European Union in 2019 for patients 12 years and older with  $\beta$ -thalassemia (non- $\beta^0/\beta^0$  genotype) who require regular RBC transfusions and for whom HSCT is appropriate, but a donor is not available (Zynteglo Summary of Product Characteristics, 2019). Reblozyl<sup>®</sup> (luspatercept), an activin receptor fusion protein, was approved in the United States (US) in 2019 and the European Union in 2020 to treat anemia in adults with  $\beta$ -thalassemia who require regular RBC transfusions (Reblozyl (luspatercept-aamt) USPI, 2019).

An unmet medical need exists for patients with  $\alpha$ - or  $\beta$ -thalassemia who do not require regular RBC transfusions. Mitapivat provides the option of oral administration with a favorable safety profile, and has the potential to treat anemia, improve iron homeostasis, and serve a broad portion of the population of patients with thalassemia.

### 2.1.2. Mitapivat

Mitapivat (also known as mitapivat sulfate, AG-348, and PYRUKYND) is a first-in-class, orally bioavailable, potent, allosteric activator of wild-type RBC-specific form of pyruvate kinase (PKR) and a range of PKR-mutant enzymes (Kung et al, 2017). The RBC-specific form of pyruvate kinase is 1 of 4 pyruvate kinase isoenzymes expressed in human tissues from 2 separate genes, liver-specific form of pyruvate kinase (PKL) and pyruvate kinase muscle isozyme (PKM). Both PKR and PKL are splice isoforms of the *PKLR* gene, while PKM1 and PKM2 are both expressed from the *PKM* gene. Mitapivat is an allosteric activator of the PKR, PKL, and PKM2 isoenzymes, with similar potency against each.

Mitapivat acts by allosterically binding to the PKR tetramer and enhancing its affinity for phosphoenolpyruvate (PEP), thereby increasing the conversion of PEP + adenosine diphosphate to pyruvate + ATP.

Mitapivat (PYRUKYND) was approved by the US Food and Drug Administration (FDA) on 17 February 2022 for the treatment of hemolytic anemia in adults with pyruvate kinase deficiency (Pyrukynd (mitapivat) USPI, 2022).

Additional information is in the current edition of the Investigator's Brochure (IB).

#### 2.1.2.1. Pharmacokinetics and Pharmacodynamics Experience

The pharmacokinetics and pharmacodynamic effects of mitapivat have been characterized in healthy subjects. Results from single- and multiple-ascending dose studies and a drug-drug interaction study are summarized.

In a single-ascending dose study, mitapivat doses ranging from 30 to 2,500 mg were evaluated. Mitapivat was rapidly absorbed as indicated by the short time to maximum (peak) concentration ( $t_{max}$ ) (ranged from 0.77 to 2.5 hours with doses from 30 to 1,400 mg). Dose-normalized area under the plasma concentration  $\times$  time curve (AUC) remained constant over the dose range studied, suggesting that mitapivat total exposure increased in a dose-proportional manner. The mean half-life ( $t_{1/2}$ ) ranged from 17.8 to 20.4 hours when samples were collected through 72 hours and from 50.3 to 79.3 hours when samples were collected through 120 hours. This terminal elimination phase contributed little to the overall exposure of mitapivat, as indicated by the small difference between AUC from time 0 to 12 hours and AUC from time 0 to infinity ( $AUC_{0-\infty}$ ), suggesting a shorter effective  $t_{1/2}$  of approximately 3 to 6 hours.

After single-ascending doses of mitapivat, a dose-dependent decrease in 2,3-diphosphoglycerate (2,3-DPG) concentrations was observed starting at 3 hours postdose, reaching a minimum at 24 hours postdose, and then returning to values similar to baseline by 72 to 120 hours postdose. This decrease was accompanied by a minimal increase in ATP concentrations observed at 24 to 120 hours postdose.

The effect of food on the pharmacokinetics of mitapivat (700 mg) showed that food did not alter the pharmacokinetics of mitapivat. As a result, mitapivat can be taken with or without food.

In a multiple-ascending dose study, mitapivat doses ranging from 15 to 700 mg twice daily (BID) and 120 mg once daily (QD) were evaluated. At doses of 120 mg BID or higher, mitapivat exposure, as measured by both maximum (peak) concentration ( $C_{\max}$ ) and AUC, was lower on Day 14 compared with Day 1. This observation is attributed to the cytochrome P450 (CYP) 3A autoinduction effect of mitapivat.

Changes in 2,3-DPG concentrations after multiple-ascending doses were similar to those observed after single-ascending doses, with concentrations approaching baseline approximately 72 hours after the final dose of mitapivat. The effect of mitapivat on decreasing 2,3-DPG blood concentrations reached 90% of maximal response between 120 mg and 360 mg BID. There were no increases in ATP concentrations for 12 hours after the first dose; however, mean ATP concentrations increased from baseline, reaching steady state by Day 10. Adenosine triphosphate concentrations just before the last dose on Day 14 were similar to predose concentrations on Day 10. Mean ATP concentrations remained above baseline values for 120 hours after the last dose on Day 14. The effect of mitapivat on increasing ATP concentrations also reached 90% of maximal response between 120 and 360 mg BID.

In a drug-drug interaction study, the pharmacokinetics of mitapivat was evaluated in the presence and absence of itraconazole, a strong CYP3A and P-glycoprotein (P-gp) inhibitor, and in the presence and absence of rifampin, a strong CYP3A inducer. Systemic mitapivat exposure in the presence of itraconazole was higher compared with mitapivat alone; geometric mean AUC from time 0 to time  $t$  ( $AUC_{0-t}$ ),  $AUC_{0-\infty}$ , and  $C_{\max}$  ratios in the presence and absence of itraconazole were 4.7, 4.9, and 1.7, respectively. Systemic mitapivat exposure in the presence of rifampin was lower compared with mitapivat alone; geometric mean  $AUC_{0-t}$ ,  $AUC_{0-\infty}$ , and  $C_{\max}$  ratios in the presence and absence of rifampin were 0.09, 0.09, and 0.23, respectively. Similar decreases were observed in physiologically-based pharmacokinetic modelling simulations with multiple doses of mitapivat and concurrent itraconazole administration.

Additional information is in the current edition of the IB.

#### **2.1.2.2. Nonclinical Experience**

Information on the use of mitapivat in nonclinical studies is in the current edition of the IB.

#### **2.1.2.3. Clinical Experience**

##### **2.1.2.3.1. Pyruvate Kinase Deficiency**

Mitapivat has been evaluated in 4 studies in adult subjects with pyruvate kinase deficiency, another hemolytic anemia. A Phase 2, open-label, safety and efficacy study (AG348-C-003 [DrivePK]) is ongoing. A Phase 3, double-blind, randomized, placebo-controlled study evaluating efficacy and safety in subjects who are not regularly transfused (AG348-C-006 [ACTIVATE]) has completed. Additionally, 2 open-label studies are ongoing: a Phase 3 study evaluating efficacy and safety in subjects who are regularly transfused (AG348-C-007 [ACTIVATE-T]) and a Phase 3 extension study for subjects who were previously enrolled in mitapivat studies (AG348-C-011).

In Study AG348-C-003, data from the initial 24 weeks of treatment (Core Period) indicated that approximately 50% of subjects (N=26) who received mitapivat (doses ranged from 50 to 300 mg BID) achieved maximum increases in Hb concentration that were  $>1$  g/dL. Most increases were



rapid. The median time to the first observation of an Hb concentration increase  $>1$  g/dL above baseline was 10 days (range: 7 to 187 days). The Hb response was sustained in all 19 subjects who continued to receive mitapivat in the Extension Period. The median duration of exposure was 29 months (range: 22 to 35 months). Common adverse events (AEs), including headache and insomnia, occurred at the time of drug initiation and were transient; approximately 92% of the episodes of headache and 47% of the episodes of insomnia resolved within 7 days. The most common serious adverse events (SAEs), hemolytic anemia and pharyngitis, each occurred in 2 patients (4%) (Grace, Rose, et al, 2019). Additionally, AEs during the Extension Period were similar to those reported during the initial 24-week Core Period, indicating that safety was consistent with extended mitapivat administration (Grace, Layton, et al, 2019).

In Study AG348-C-006, 80 subjects randomized 1:1 to mitapivat or placebo received escalating doses of mitapivat to a maximum of 50 mg BID. Recently, data from this study demonstrated that approximately 40% of the 40 subjects who received mitapivat achieved a sustained Hb concentration increase of  $\geq 1.5$  g/dL compared with placebo (2-sided p-value  $<0.0001$ ). Additionally, treatment with mitapivat demonstrated statistically significant improvements compared with placebo in key secondary endpoints including markers of hemolysis (indirect bilirubin, haptoglobin, and lactate dehydrogenase [LDH]) and markers of hematopoietic activity (reticulocyte percentages). The safety data from Study AG348-C-006 were consistent with data observed in Study AG348-C-003, and there were no AEs leading to study drug discontinuation in either the mitapivat or placebo arm. Safety data from Studies AG348-C-007 and AG348-C-011 have been consistent with those in Study AG348-C-006, and no new safety signals were observed.

#### **2.1.2.3.2. Non-Transfusion-Dependent Thalassemia (Study AG348-C-010)**

Study AG348-C-010 is an ongoing Phase 2, open-label study to determine the efficacy, safety, pharmacokinetics, and pharmacodynamics of mitapivat in adult subjects with  $\alpha$ - or  $\beta$ -NTDT (EudraCT 2018-002217-35; ClinicalTrials.gov NCT03692052). Subjects with a baseline Hb concentration  $\leq 10.0$  g/dL received an initial dose of 50 mg BID mitapivat, which was increased to 100 mg BID at Week 6 in the absence of drug-related AEs or excessive Hb response.

The study completed enrollment with 20 subjects (5 with  $\alpha$ -thalassemia and 15 with  $\beta$ -thalassemia) who received mitapivat, and the Core Period of the study (initial 24 weeks of treatment) has completed; data from the Core Period are presented here, unless otherwise noted. Nineteen subjects completed the Core Period and 1 subject was withdrawn from the study due to an unrelated SAE. Seventeen subjects were continuing to receive mitapivat in the Extension Phase of the study (1 subject withdrew consent from the study and 1 subject did not meet criteria for participation in the Extension Phase).

Of the 20 subjects who received mitapivat, the median age of subjects was 44 years and 15 subjects were female. The mean baseline Hb concentration was 7.94 g/dL (range: 5.13 to 9.80 g/dL). The mean baseline Hb concentrations in the  $\alpha$ - and  $\beta$ -thalassemia subgroups were 7.89 g/dL (range: 5.13 to 9.80 g/dL) and 8.12 g/dL (range: 6.67 to 9.20 g/dL), respectively.

A total of 16 subjects (80.0%; 5 with  $\alpha$ -thalassemia and 11 with  $\beta$ -thalassemia) met the primary endpoint of Hb response, defined as an increase in Hb concentration of  $\geq 1.0$  g/dL from baseline at  $\geq 1$  assessment between Week 4 and Week 12. The time to the first Hb response (ie,  $\geq 1.0$  g/dL from baseline) was observed as early as Week 2, with a median of 3.3 weeks (range: 2.1 to



12.1 weeks). The mean ( $\pm$ standard deviation [SD]) increase from baseline in Hb concentration over a continuous 12-week interval (Week 4 to Week 12) was  $1.15 \pm 0.07$  g/dL (range: -0.24 to 2.52 g/dL). Of the 16 subjects who met the primary endpoint, 13 subjects (65.0%) had a sustained Hb response; these subjects met the primary endpoint and had an increase in Hb concentration  $\geq 1.0$  g/dL from baseline at 2 of the 4 assessments from Week 12 to Week 24. Of the 4 subjects who did not meet the primary endpoint, 2 subjects demonstrated a delayed Hb response, defined as an increase in Hb concentration of  $\geq 1.0$  g/dL from baseline at  $\geq 1$  assessment from Week 16 to Week 24.

In the 19 subjects who completed 24 weeks of mitapivat treatment, the mean ( $\pm$ SD) increase from baseline in Hb concentration over a continuous 12-week interval (Week 12 to Week 24) was  $1.3 \pm 6.28$  g/dL (range: -0.03 to 2.56 g/dL). The average increase from baseline in Hb concentration was similar between subjects with  $\alpha$ -thalassemia and  $\beta$ -thalassemia. Fourteen of 20 subjects (70.0%; 4 with  $\alpha$ -thalassemia, 10 with  $\beta$ -thalassemia) had a  $\geq 1.0$  g/dL increase in average Hb concentration from Week 12 through Week 24 compared with baseline.

Among all 20 subjects, hemolytic and erythropoietic markers relevant to the pathophysiology of the disease showed trends for improvement as early as Week 2. Baseline mean indirect bilirubin, LDH, and erythropoietin was  $31.49 \pm 27.536$   $\mu$ mol/L,  $269.35 \pm 113.055$  U/L, and  $870.53 \pm 2,659.950$  IU/L, respectively. Over a continuous 12-week interval (Week 12 to Week 24), the mean ( $\pm$ SD) change from baseline in indirect bilirubin, LDH, and erythropoietin was  $-17.72 \pm 18.861$   $\mu$ mol/L,  $-40.68 \pm 123.699$  U/L, and  $-627.00 \pm 2,404.460$  IU/L, respectively.

The safety profile of mitapivat in subjects with NTDT is consistent with that in healthy subjects and in subjects with pyruvate kinase deficiency, another hemolytic anemia with similar clinical manifestations. No new safety signals were identified when mitapivat was administered in subjects with thalassemia. There were no differences in the type and frequency of AEs between subjects with  $\alpha$ - and  $\beta$ -thalassemia. During the Core Period, 17 (85.0%) of the 20 subjects who received at least 1 dose of mitapivat had at least 1 AE. Commonly reported AEs (occurring in  $\geq 20\%$  or  $\geq 4$  subjects) included cough, dizziness, dyspepsia, fatigue, headache, initial insomnia, nasal congestion, and upper respiratory tract infection. One subject experienced a National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) Grade 1 adverse event of special interest of aspartate aminotransaminase increased. One subject had a related nonserious Grade 3 event of initial insomnia that led to a (permanent) dose reduction to 50 mg BID, and 1 subject had an unrelated SAE of renal impairment that led to study drug discontinuation. Overall, when compared with 50 mg BID, 100 mg BID dosing did not result in additional AEs despite the higher dose and longer duration of follow-up.

## 2.2. Study Rationale

Data from the ongoing Phase 2 study (AG348-C-010) demonstrate that mitapivat has a manageable safety profile, improves anemia as measured by an increase in Hb concentration and a reduction in hemolytic parameters, and results in favorable trends in erythropoietic parameters relevant to thalassemia (see Section 2.1.2.3.2).

Study AG348-C-017 is a Phase 3, double-blind, randomized, placebo-controlled, multicenter study designed to demonstrate the clinical efficacy and safety of mitapivat in subjects with  $\alpha$ - or  $\beta$ -NTDT. The primary objective of the study is to compare the effect of mitapivat versus placebo on anemia (ie, Hb response), supported by patient-reported outcome (PRO) and performance

outcome (PerfO) measures evaluating how subjects feel and function, and additional hemolytic and erythropoietic parameters relevant to the pathophysiology of the disease. Additionally, safety and pharmacokinetic and pharmacodynamic parameters will be evaluated.

Although recently approved therapies offer new options for patients with  $\beta$ -TDT, there are no approved treatments for patients with  $\alpha$ -thalassemia or for patients with NTDT, indicating the unmet medical need for these patients. Mitapivat has the potential to treat anemia and improve how patients feel and function in a broader portion of the population of patients with thalassemia, while providing the option of oral administration.

The study design rationale and dose justification are provided in Section 4.2 and Section 4.3, respectively.

### **2.3. Benefit-Risk Assessment**

The Sponsor has conducted a risk assessment for concomitant use of a COVID-19 vaccine with mitapivat with specific consideration for the trial population, and determined that the COVID-19 vaccine given to a study subject is considered a simple concomitant medication with no interaction that requires advice on timing of the vaccine or other aspects that need to be mitigated.

The COVID-19 public health emergency is expected to be ongoing when this study is initiated. To ensure subject safety, maintain compliance with Good Clinical Practice (GCP), and minimize risks to study integrity while conducting this study during a pandemic, this protocol includes the allowance for temporary modifications to protocol-specified procedures (see Section 11.1.1). These modifications are consistent with ensuring subject safety and continued access to study drug while minimizing risk to study subjects, Investigators, and clinical study center personnel. The importance of conducting this study despite an ongoing pandemic was assessed, and this study is considered a critical evaluation of a study drug for a serious unmet medical need in patients with thalassemia (Section 2.1.1.1). COVID-19 is not expected to affect the disease of thalassemia. Therefore, the COVID-19 public health emergency is not considered to negatively affect the risk of subject participation or the assessment of study objectives. Overall, with the potential allowance for temporary modifications in place, the benefits of conducting this study despite the challenges due to COVID-19 outweigh the potential risks to patients with thalassemia. Throughout the study, this assessment will be reevaluated by the Sponsor and Investigator.

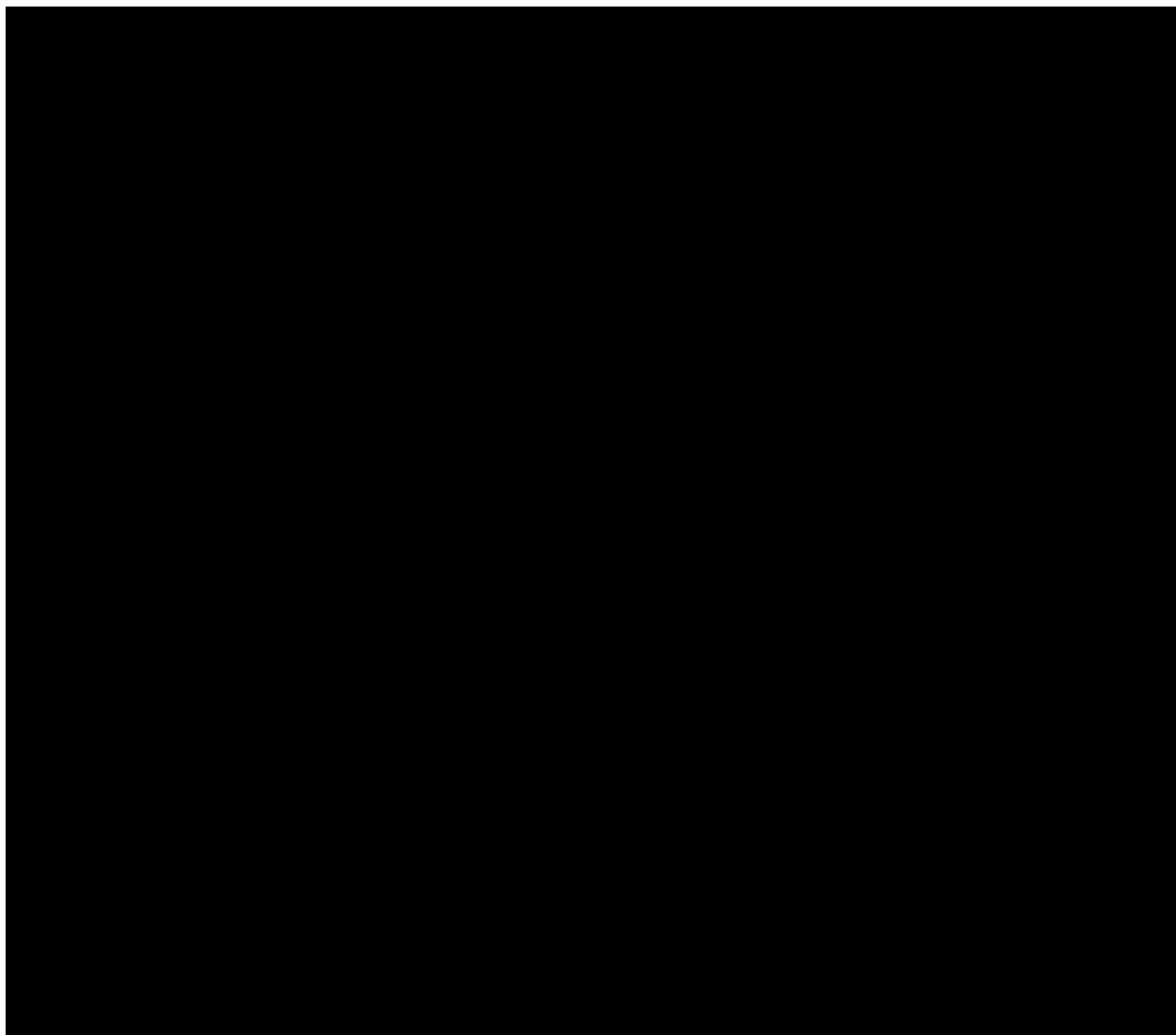
Additionally, the benefit-risk assessment for mitapivat in clinical studies is in the current edition of the IB.

### 3. OBJECTIVES AND ENDPOINTS

The study objectives and endpoints are provided in [Table 3](#).

**Table 3: Objectives and Endpoints**

<b>Primary Objective</b> <ul style="list-style-type: none"> <li>To compare the effect of mitapivat versus placebo on anemia in subjects with <math>\alpha</math>- or <math>\beta</math>-non-transfusion-dependent thalassemia</li> </ul>	<b>Primary Endpoint</b> <ul style="list-style-type: none"> <li>Hemoglobin (Hb) response, defined as a <math>\geq 1.0</math> g/dL increase in average Hb concentration from Week 12 through Week 24 compared with baseline</li> </ul>
<b>Key Secondary Objectives</b> <ul style="list-style-type: none"> <li>To compare the effect of mitapivat versus placebo on fatigue</li> <li>To compare the effect of mitapivat versus placebo on additional measures of anemia</li> </ul>	<b>Key Secondary Endpoints</b> <ul style="list-style-type: none"> <li>Change from baseline in average Functional Assessment of Chronic Illness Therapy-Fatigue subscale score from Week 12 through Week 24</li> <li>Change from baseline in average Hb concentration from Week 12 through Week 24</li> </ul>
<b>Secondary Objectives</b> <ul style="list-style-type: none"> <li>To evaluate the effect of mitapivat versus placebo on anemia and markers of hemolysis and erythropoiesis</li> <li>To evaluate the effect of mitapivat versus placebo on additional measures of fatigue</li> <li>To evaluate the effect of mitapivat versus placebo on physical activity</li> <li>To evaluate the effect of mitapivat versus placebo on iron metabolism</li> <li>To evaluate the safety of mitapivat</li> <li>To evaluate the pharmacokinetic and pharmacodynamic effects of mitapivat</li> </ul>	<b>Secondary Endpoints</b> <ul style="list-style-type: none"> <li>Hb 1.5+ response, defined as a <math>\geq 1.5</math> g/dL increase in average Hb concentration from Week 12 through Week 24 compared with baseline</li> <li>Change from baseline in indirect bilirubin, lactate dehydrogenase, and haptoglobin at Week 24</li> <li>Change from baseline in reticulocytes and erythropoietin at Week 24</li> <li>Improvement in the Patient Global Impression of Severity (PGIS)-Fatigue by at least 1 category at Weeks 12, 16, 20, and 24 compared with baseline, or “no change” if no or mild fatigue at baseline</li> <li>Improvement in the Patient Global Impression of Change (PGIC)-Fatigue at Weeks 12, 16, 20, and 24, or “no change” if no or mild fatigue at baseline</li> <li>Change from baseline in the 6-minute walk test distance at Week 24</li> <li>Change from baseline in markers of iron metabolism, including serum ferritin and transferrin saturation, at Week 24</li> <li>Type, severity, and relationship of adverse events and serious adverse events</li> <li>Plasma or blood concentrations and pharmacokinetic parameters of mitapivat and pharmacodynamic parameters, including adenosine triphosphate and 2,3-diphosphoglycerate</li> </ul>



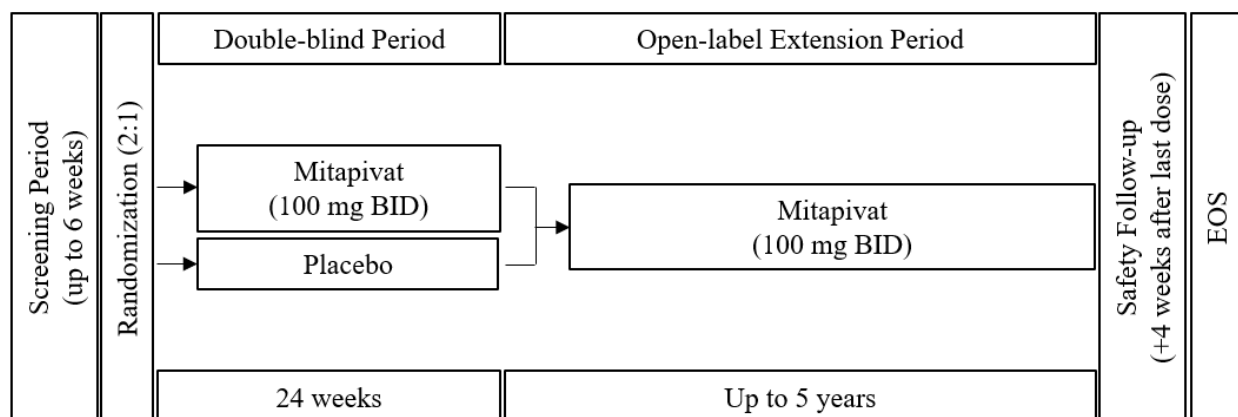
## 4. STUDY DESIGN

### 4.1. Overall 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  $\alpha$ - or  $\beta$ -NTDT followed by an Open-label Extension Period. Approximately 171 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](#). Study assessments will be performed at the time points in the Schedules of Assessments ([Section 1.1](#)).

**Figure 1: Study Design**



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

#### 4.1.1. Screening Period

Subject eligibility will be determined during the Screening Period (see [Section 5](#)). Baseline disease characteristics, including transfusion history from the 24-week period before randomization, will be recorded ([Section 8.7](#)). The Investigator will confirm the subject's eligibility before randomization. Screening extensions will not be granted. If a subject requires rescreening, screening assessments that do not need to be repeated are provided in [Section 8.2](#).

#### 4.1.2. Double-blind Period

Eligible subjects will be randomly assigned in a 2:1 ratio to receive study drug (mitapivat or placebo) for BID oral administration. Randomization will be stratified by baseline Hb concentration and thalassemia genotype (see [Section 6.3.1](#)). Study subjects, Investigators, clinical study center personnel, and the Sponsor will be blinded to the subject's treatment assignment (see [Section 6.3.2](#)). Subjects will receive either mitapivat or matched placebo for 24 weeks. Study visits will occur approximately every 4 weeks for efficacy and safety assessments. If allowed by local institutional guidelines, subjects will have the option for at-home study visits as designated in the Schedules of Assessments. Subjects who discontinue study drug before completing the Double-blind Period are not permitted to receive mitapivat in the Open-label Extension Period.

#### **4.1.3. Open-label Extension Period**

Subjects will be provided the opportunity to receive mitapivat for an additional 5 years in the Open-label Extension Period. Treatment assignment from the Double-blind Period will remain blinded until the study is unblinded for the analysis of the primary endpoint (see Section 6.3.2). During the Open-label Extension Period, subjects will continue to undergo safety and clinical activity assessments; however, the frequency of study visits will be reduced. If allowed by local institutional guidelines, subjects will have the option for at-home study visits as designated in the Schedules of Assessments.

#### **4.1.4. Duration of Treatment and Safety Follow-up**

The duration of participation of a subject in the study is up to 5.8 years. The duration of treatment is up to 5.6 years. Subjects will have a Safety Follow-up visit approximately 4 weeks after their last dose of study drug (including the recommended dose taper).

### **4.2. Study Design Rationale**

Study AG348-C-017 is a Phase 3, double-blind, randomized, placebo-controlled, multicenter study designed to demonstrate the clinical efficacy and safety of mitapivat in subjects with  $\alpha$ - or  $\beta$ -NTDT followed by an Open-label Extension Period.

The double-blind study design minimizes assessment bias. The proposed use of placebo as a comparator will allow an objective evaluation of the efficacy and safety of mitapivat. The choice of placebo control is supported by the lack of disease-specific, approved treatments for patients with  $\alpha$ - or  $\beta$ -NTDT. The double-blind study design minimizes assessment bias.

The primary endpoint will allow for a consistent measurement of the intended effect of mitapivat on Hb concentrations. The key secondary endpoint of Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-Fatigue) provides a direct patient-reported evaluation of how the subject feels and functions and constitutes a clinically meaningful endpoint.

Eligible subjects will be randomized assigned in a 2:1 ratio to receive mitapivat or matched placebo, providing an increased chance to receive active study drug. Placebo administration will be limited to a 24-week Double-blind Period, and subjects, including those who were randomized to placebo, will have the opportunity to receive mitapivat during the Open-label Extension Period. This allows for a longer-term assessment of safety while providing access to active drug. During the Double-blind Period, an Independent Data Monitoring Committee (IDMC) will be responsible for ongoing monitoring of the safety of subjects.

Randomization will be stratified by baseline Hb concentration and thalassemia genotype to ensure a balanced allocation of subjects to mitapivat or placebo.

Overall, the study design will allow for an evaluation of the efficacy and safety of mitapivat in subjects with  $\alpha$ - or  $\beta$ -NTDT while subjects continue receiving appropriate supportive care to manage symptoms and prevent secondary complications.

### 4.3. Dose Justification

Based on pharmacokinetic, pharmacodynamic, efficacy, and safety data from subjects with NTDT in Study AG348-C-010, a dose of 100 mg BID mitapivat has been selected for further evaluation in subjects with thalassemia. Data from this study indicate that a dose of 100 mg BID was associated with more subjects who achieved an Hb response. Further, safety data demonstrated that at 100 mg BID mitapivat, AEs were manageable and were consistent with those in other studies across the clinical development program (see Section 2.1.2.3.2).

Preliminary pharmacokinetic, pharmacodynamic, and exposure-response analyses were conducted for 14 subjects with NTDT (data cutoff date: 03 March 2020). Per protocol, subjects received an initial dose of 50 mg BID mitapivat and had their dose increased to 100 mg BID at Week 6. Exposure to mitapivat (as assessed by AUC from time 0 to the last measurable concentration [ $AUC_{last}$ ]) was approximately 30% higher at 100 mg BID compared with the exposure estimated from a single 50 mg dose on Day 1. The pharmacokinetics of mitapivat after a single 50 mg dose was similar to that observed at steady state after multiple 50 mg BID doses. This observation is consistent with previous studies where autoinduction was observed at 120 mg BID or higher in healthy subjects and at 300 mg BID in subjects with pyruvate kinase deficiency; autoinduction was not observed at the next lower doses of 60 mg BID in healthy subjects or 50 mg BID in subjects with pyruvate kinase deficiency. The mean ATP percent change from baseline was 82.7% (N=9) at Week 6 when subjects were still receiving 50 mg BID, increasing to 86.7% (N=12) and 92.3% (N=5) at Weeks 12 and 24, respectively, at which point subjects were receiving 100 mg BID.

A sequential population pharmacokinetic-pharmacodynamic modelling approach was used to characterize the exposure-efficacy relationship of mitapivat using Hb as the efficacy endpoint. The time course of Hb response was described by an indirect-response model, [REDACTED]

Although mitapivat was administered sequentially in Study AG348-C-010, and duration of exposure varied by dose (ie, several subjects received 100 mg BID for longer than 50 mg BID), there were no apparent differences in the spectrum of AEs reported while subjects were receiving either 50 mg BID or 100 mg BID (see Section 2.1.2.3.2).

Based on data from nonclinical and clinical studies, insomnia is an identified risk associated with mitapivat administration. An increased incidence of insomnia was observed in subjects with pyruvate kinase deficiency who received 300 mg BID compared with 50 mg BID (Study AG348-C-003). The 6-fold difference in dose resulted in an approximately 3.2-fold difference in total exposure at steady state. A logistic regression analysis was performed to examine the relationship between AEs of insomnia and mitapivat exposure. [REDACTED]

Recently, data from the randomized, placebo-controlled study (AG348-C-006) in which subjects received a maximum dose of 50 mg BID, AEs of insomnia as defined by Preferred Term (initial insomnia, insomnia, middle insomnia, and terminal insomnia) were observed with similar frequency between the mitapivat and placebo groups (17.5% vs 17.9%).

In summary, 100 mg BID mitapivat has been shown to have a manageable safety profile for chronic dosing. [REDACTED]

██████████. Based on these findings, 100 mg BID mitapivat was selected for administration in subjects with NTDT in this study.

Dose modifications are presented in Section 6.7.

#### 4.4. End of Study Definition

The end of the study 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.



## 5. STUDY POPULATION

Protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, are not permitted.

### 5.1. Inclusion Criteria

Subjects are eligible to be included in the study only if all the following criteria apply:

1.  $\geq 18$  years of age at the time of providing informed consent.
2. Documented diagnosis of thalassemia ( $\beta$ -thalassemia with or without  $\alpha$ -globin gene mutations, HbE/ $\beta$ -thalassemia, or  $\alpha$ -thalassemia/HbH disease) based on Hb electrophoresis, Hb high-performance liquid chromatography, and/or DNA analysis from the subject's medical record. If this information is not available from the subject's medical record, the test(s) can be performed by a local laboratory during the Screening Period. If a local laboratory is unable to perform the test(s), results from the comprehensive  $\alpha$ - and  $\beta$ -globin genotyping performed by the study central laboratory can be used (see Section 8.2 and Section 8.9).
3. Hb concentration  $\leq 10.0$  g/dL (100.0 g/L), based on an average of at least 2 Hb concentration measurements (separated by  $\geq 7$  days) collected during the Screening Period.
4. Non-transfusion dependent, defined as:  $\leq 5$  RBC units during the 24-week period before randomization **and** no RBC transfusions  $\leq 8$  weeks before providing informed consent **and** no RBC transfusions during the Screening Period.
5. If taking hydroxyurea, the hydroxyurea dose must be stable for  $\geq 16$  weeks before randomization.
6. Women of childbearing potential (WOCBP) must be abstinent of sexual activities that may result in pregnancy as part of their usual lifestyle or agree to use 2 forms of contraception, 1 of which must be considered highly effective, from the time of providing informed consent, throughout the study, and for 28 days after the last dose of study drug. The second form of contraception can be an acceptable barrier method (see [Appendix 2](#) for the definition of WOCBP and acceptable contraception methods).
7. Written informed consent before any study-related procedures are conducted and willing to comply with all study procedures for the duration of the study.

### 5.2. Exclusion Criteria

Subjects are excluded from the study if any of the following criteria apply:

1. Pregnant, breastfeeding, or parturient.
2. Documented history of homozygous or heterozygous HbS or HbC.
3. Prior exposure to gene therapy or prior bone marrow or stem cell transplantation.
4. Currently receiving treatment with luspatercept; the last dose must have been administered  $\geq 18$  weeks before randomization.

5. Currently receiving treatment with hematopoietic stimulating agents; the last dose must have been administered  $\geq 18$  weeks before randomization.
6. History of malignancy (active or treated)  $\leq 5$  years before providing informed consent, except for nonmelanomatous skin cancer in situ, cervical carcinoma in situ, or breast carcinoma in situ.
7. History of active and/or uncontrolled cardiac or pulmonary disease  $\leq 6$  months before providing informed consent, including but not limited to:
  - a. New York Heart Association Class III or IV heart failure or clinically significant dysrhythmia
  - b. Myocardial infarction or unstable angina pectoris; hemorrhagic, embolic, or thrombotic stroke; deep venous thrombosis; or pulmonary or arterial embolism
  - c. Heart rate–corrected QT interval using Fridericia’s method  $\geq 450$  milliseconds (males) or  $\geq 470$  milliseconds (females), except for right or left bundle branch block
  - d. Severe pulmonary fibrosis as defined by severe hypoxia, evidence of right-sided heart failure, and radiographic pulmonary fibrosis  $> 50\%$
  - e. Severe pulmonary hypertension as defined by severe symptoms associated with hypoxia, right-sided heart failure, and oxygen indicated
8. Hepatobiliary disorders, including but not limited to:
  - a. Liver disease with histopathological evidence of cirrhosis or severe fibrosis
  - b. Clinically symptomatic cholelithiasis or cholecystitis (prior cholecystectomy is not exclusionary)
  - c. History of drug-induced cholestatic hepatitis
  - d. Aspartate aminotransferase (AST)  $> 2.5 \times$  upper limit of normal (ULN); unless due to hemolysis and hepatic iron deposition) and alanine aminotransferase (ALT)  $> 2.5 \times$  ULN (unless due to hepatic iron deposition)
9. Estimated glomerular filtration rate  $< 45$  mL/min/1.73 m<sup>2</sup> by Chronic Kidney Disease Epidemiology Collaboration creatinine equation ([Levey et al, 2009](#)).
10. Nonfasting triglycerides  $> 440$  mg/dL (5 mmol/L).
11. Active infection requiring systemic antimicrobial therapy at the time of providing informed consent. If antimicrobial therapy is required during the Screening Period, screening procedures should not be performed while antimicrobial therapy is being administered, and the last dose of antimicrobial therapy must be administered  $\geq 7$  days before randomization.
12. Positive test for hepatitis C virus (HCV) antibody (Ab) with evidence of active HCV infection, or positive test for hepatitis B surface antigen (HBsAg).
13. Positive test for HIV-1 Ab or HIV-2 Ab.
14. History of major surgery (including splenectomy)  $\leq 16$  weeks before providing informed consent and/or a major surgical procedure planned during the study.
15. Current enrollment or past participation ( $\leq 12$  weeks before administration of the first dose of study drug or a time frame equivalent to 5 half-lives of the investigational study

drug, whichever is longer) in any other clinical study involving an investigational treatment or device.

16. Receiving strong CYP3A4/5 inhibitors that have not been stopped for  $\geq 5$  days or a time frame equivalent to 5 half-lives (whichever is longer), or strong CYP3A4 inducers that have not been stopped for  $\geq 4$  weeks or a time frame equivalent to 5 half-lives (whichever is longer), before randomization.
17. Receiving anabolic steroids that have not been stopped for at least 4 weeks before randomization. Testosterone replacement therapy to treat hypogonadism is allowed; the testosterone dose and preparation must be stable for  $\geq 10$  weeks before randomization.
18. Known allergy, or other contraindication, to mitapivat or its excipients (microcrystalline cellulose, croscarmellose sodium, sodium stearyl fumarate, mannitol, and magnesium stearate, Opadry® II Blue [hypromellose, titanium dioxide, lactose monohydrate, triacetin, and FD&C Blue #2]).
19. Any medical, hematological, psychological, or behavioral condition(s) or prior or current therapy that, in the opinion of the Investigator, may confer an unacceptable risk to participating in the study and/or could confound the interpretation of the study data. Also excluded are:
  - Subjects who are institutionalized by regulatory or court order
  - Subjects with any condition(s) that could create undue influence (including but not limited to incarceration, involuntary psychiatric confinement, and financial or familial affiliation with the Investigator or Sponsor)

### **5.3. Lifestyle Considerations**

Not applicable

### **5.4. Screen Failures**

Screen failures are defined as subjects who provide informed consent to participate in the clinical study but who do not meet the eligibility criteria for participation in this study and who are not subsequently randomized. Information to be collected for screen failures includes the reason for screen failure, demography, eligibility criteria, SAEs, and deaths.

Individuals who do not meet the criteria for participation in this study (screen failure) may be rescreened. See Section 8.2 for criteria for which rescreening is permitted.

## 6. STUDY DRUG

### 6.1. Study Drug Administered

Study drug is defined as mitapivat or matched placebo. Study drug is for investigational use only and to be used only within the context of this study protocol.

Subjects will receive 100 mg BID mitapivat or matched-placebo for oral administration. Tablets are to be swallowed whole with water and may be taken with or without food. Tablets are not to be crushed, chewed, or dissolved in water. If a dose of mitapivat is missed by  $\leq 4$  hours, the dose should be taken as soon as possible. If a dose of mitapivat is missed by  $>4$  hours, the dose should be skipped and taken at the time of the next scheduled dose. The regular dosing schedule should resume the following day.

Subjects who discontinue study drug should undergo the recommended dose taper and be monitored as clinically indicated for signs and symptoms of acute hemolysis and worsening anemia. If immediate or abrupt study drug discontinuation is required for an AE or medical emergency, subjects should be monitored as clinically indicated for signs of acute hemolysis or worsening anemia. Study drug dose modifications are provided in Section 6.7.

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.

Study drug will be supplied according to the specifications in Table 5.

**Table 5: Study Drug**

Treatment Arm:	Active	Matched Placebo
Study drug name (INN)	Mitapivat (AG-348)	NA
Dose formulation	Tablet	Tablet
Tablet unit strength(s)	100 mg	NA
Appearance	Oblong blue tablet	Oblong blue tablet
Route of administration	Oral	Oral
Sourcing	Provided by the Sponsor	Provided by the Sponsor
Packaging and labeling	Provided in containers with child resistant closures and labeled as required per local regulations.	Provided in containers with child resistant closures and labeled as required per local regulations.

Abbreviations: INN = international nonproprietary name; NA = not applicable.

Additional information will be provided in the Pharmacy Manual.

## **6.2. Preparation, Handling, Storage, and Accountability**

Only subjects enrolled in the study may receive study drug, and only authorized clinical study center personnel may supply or administer study drug.

All study drug must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the Investigator and authorized clinical study center personnel.

Accountability for the study drug at the clinical study center is the responsibility of the Investigator. The Investigator will ensure that the study drug is used only in accordance with this protocol. Where allowed, the Investigator may choose to assign drug accountability responsibilities to a pharmacist or other appropriate individual.

The Investigator (or designee) will maintain accurate drug accountability records indicating the study drug delivery date to the clinical study center, inventory at the center, use by each subject, and return to the Sponsor (or designee), or disposal of study drug, if approved by the Sponsor. These records will adequately document that the subjects were provided the doses as specified in the protocol and should reconcile all study drug received from the Sponsor. Accountability records will include dates, quantities, batch/serial numbers, expiration dates (if applicable), and subject numbers. The Sponsor (or designee) will review study drug accountability records at the study center on an ongoing basis during monitoring visits.

Study drug must not be used for any purpose other than the present clinical study. Study drug that has been dispensed to a subject and returned unused must not be redispensed to a different subject.

All used, unused, or expired study drug should be retained at the study center until inventoried and verified by study center personnel and/or the study monitor. All used, unused, or expired study drug will be returned to the Sponsor (or designee) or if authorized, disposed of at the study center per the study center standard operating procedures (SOPs) and documented. All material containing mitapivat or matched placebo will be treated and disposed of as hazardous waste in accordance with governing regulations.

Additional information will be provided in the Pharmacy Manual.

## **6.3. Randomization and Blinding**

### **6.3.1. Randomization**

In this study, eligible subjects will be randomly assigned in a 2:1 ratio to receive study drug (mitapivat or placebo). Randomization will be stratified by baseline Hb concentration ( $\leq 9.0$  g/dL or 9.1-10.0 g/dL) and by thalassemia genotype (see Section 8.2). Randomization assignment will be generated and implemented by an interactive voice/web response system (IXRS) provider. Mitapivat and matched placebo will be packaged and labeled identically to maintain the study blind. Randomization can occur up to 48 hours before administration of the first dose of study drug on Day 1.

### **6.3.2. Blinding**

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 Open-label Extension Period. At the last study visit of the Double-blind Period, subjects who continue in the Open-label Extension 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.

### **6.3.3. Unblinding**

The IXRS will be programmed with blind-breaking instructions. In the event of a medical emergency or confirmed pregnancy in a female subject, if knowledge of the investigational product assignment is critical to the subject's management, the Investigator has the sole responsibility for determining if unblinding of a subject's treatment assignment is warranted. Subject safety must always be the first consideration in making such a determination. If the Investigator decides that unblinding is warranted, the Investigator should make every effort to contact the Medical Monitor before unblinding a subject's treatment assignment unless this could delay emergency treatment of the subject. If a subject's treatment assignment is unblinded, the Medical Monitor must be notified within 24 hours after breaking the blind. The Medical Monitor must remain blinded to the subject's treatment assignment. The date and reason that the blind was broken must be recorded in the IXRS.

## **6.4. Study Drug Compliance**

Subjects will receive instructions for home administration of study drug. Subjects must return study drug bottles (including empty bottles) and unused tablets at each visit to the clinical study center. Compliance with the study drug dosing regimen will be assessed. Deviation(s) from the assigned dose or dose regimen should be recorded in the electronic case report form (eCRF).

## **6.5. Overdose**

Overdose is the accidental or intentional use of the study drug in an amount higher than the dose being studied. No information is available on the treatment of an overdose of mitapivat. In the event of an overdose, observation and general supportive measures should be instituted, and the Medical Monitor should be contacted. Any study drug overdose or incorrect administration of study drug should be recorded on the eCRF.

See the current edition of the IB for details. See Section [9.4.1](#) for reporting of AEs associated with an overdose.

## **6.6. Prior and Concomitant Therapy**

### **6.6.1. Prior Therapy**

All medications administered and procedures and surgeries conducted  $\leq 4$  weeks before providing informed consent until administration of the first dose of study drug are to be recorded on the eCRF. This includes over-the-counter or prescription medicines, vitamins, and herbal supplements.

Use of iron chelation therapy for up to 1 year before administration of the first dose of study drug will be recorded on the eCRF.

Transfusion history will also be recorded (see Section 8.7).

### **6.6.2. Permitted Concomitant Therapy**

Transfusions and other supportive care therapies are permitted as clinically indicated, unless otherwise specified in Section 6.6.3. Concomitant administration of iron chelation therapy will be recorded on the eCRF. Red blood cell transfusions are discussed in Section 8.7.

Initiation of new Herbal/dietary supplements (HDS) is permitted only after discussion with the Investigator.

### **6.6.3. Prohibited Concomitant Therapy**

Concomitant use of investigational treatments or devices is not permitted during study participation.

Concomitant administration of strong CYP3A4/5 inhibitors is prohibited during the study. These therapies must be discontinued  $\geq 5$  days or a time frame equivalent to 5 half-lives (whichever is longer) before randomization.

Products known to inhibit CYP3A4 (eg, grapefruit or grapefruit juice) are also prohibited during the study.

Concomitant administration of strong CYP3A4 inducers is prohibited during the study. These therapies must be discontinued  $\geq 4$  weeks or a time frame equivalent to 5 half-lives (whichever is longer) before randomization.

Concomitant administration of luspatercept is prohibited during the study; the last dose must have been administered  $\geq 18$  weeks before randomization.

Concomitant administration of erythropoietin is prohibited during the study; the last dose must have been administered  $\geq 18$  weeks before randomization.

Concomitant administration of anabolic steroids, including testosterone preparations, is prohibited during the study; the last dose must have been administered at least 4 weeks before randomization. However, continuation of testosterone replacement therapy to treat hypogonadism is allowed if the dose and preparation has been stable for at least 10 weeks before randomization. It is desirable to maintain a stable dose during the Double-blind Period.

Subjects requiring a prohibited concomitant therapy will be discontinued from study drug and withdrawn from the study.



See the current edition of the IB for examples of prohibited concomitant therapies.

#### **6.6.3.1. Concomitant Therapy to be Used With Caution or Replaced**

Medications under the following categories should be replaced with alternative treatments before administration of the first dose of study drug:

- Corticosteroids
- Sensitive CYP3A4 substrates
- Moderate CYP3A4 inhibitors
- Moderate inducers of CYP3A4
- Gastric acid-reducing agents, namely proton-pump inhibitors and histamine 2-receptor antagonists. (Concomitant use of antacids such as magnesium hydroxide and aluminum hydroxide is permitted.)

If alternative treatments are not possible, subjects receiving these medications concomitantly with study drug should be carefully monitored. Investigators must monitor subjects for lack of efficacy of the prescribed medication or for side effects arising from the medication and make appropriate modifications to the dose of the prescribed medication or find alternatives to the prescribed medication.

In vitro data suggest that mitapivat has the potential to induce uridine 5'-diphospho-glucuronosyltransferase (UGT) enzymes and therefore has the potential to decrease systemic exposure of UGT enzyme substrates. Iron chelators that are substrates of UGT enzymes may be administered concomitantly with mitapivat. Subjects receiving these medications concomitantly should, however, be closely monitored.

See the current edition of the IB for examples of concomitant therapies to be used with caution.

#### **6.6.3.2. Rescue Medicine**

Not applicable

### **6.7. Dose Modifications**

Dose modifications for excessive Hb response, study drug-related AEs and [REDACTED] are detailed in Section 6.7.1.1, Section 6.7.1.2, and Section 6.7.1.3.

Dose modification decisions should be made by the Investigator based on a clinical evaluation of the subject. The Investigator should notify the Medical Monitor of any dose modifications that are planned or instituted.

Subjects who discontinue study drug should undergo the recommended dose taper (Table 4) and be monitored as clinically indicated for signs and symptoms of acute hemolysis and worsening anemia. If immediate or abrupt study drug discontinuation is required for an AE or medical emergency, subjects should be monitored as clinically indicated for signs of acute hemolysis or worsening anemia.



### 6.7.1. Management of Adverse Events and Clinical Laboratory Test Result Changes

#### 6.7.1.1. Dose Modifications for Excessive Hb Response

Study drug dose modification guidance is provided for excessive Hb response, in the absence of RBC transfusions, to avoid abrupt discontinuation of mitapivat. Excessive Hb response is defined as an increase in Hb concentration that is greater than the ULN (by sex), in the absence of RBC transfusions. Dose modification guidelines for excessive Hb response are provided in [Table 6](#).

**Table 6: Dose Modifications for Excessive Hemoglobin Response**

Clinical Laboratory Test Result	Study Drug Modification
Hb concentration >ULN (by sex), in the absence of RBC transfusions for ≥4 weeks	<p>Reduce to 100 mg QD.</p> <ul style="list-style-type: none"> <li>If Hb concentration decreases to ≤ULN (by sex) within 28 days of the dose reduction to 100 mg QD, increase to 100 mg BID.</li> <li>If Hb concentration does not decrease ≤ULN (by sex) within 28 days of the dose reduction to 100 mg QD, contact the Medical Monitor.</li> </ul>

Abbreviations: BID = twice daily; Hb = hemoglobin; QD = once daily; RBC = red blood cell; ULN = upper limit of normal.

#### 6.7.1.2. Dose Modifications for Study Drug–Related Adverse Events

Dose modifications for study drug–related AEs, except for events of excessive Hb response [REDACTED], are provided in [Table 7](#).

**Table 7: Dose Modifications for Study Drug–Related Adverse Events Except for Events of Excessive Hemoglobin Response [REDACTED]**

AE Severity <sup>1</sup>	Study Drug Modification	
Grade 1 (mild)	None required	
Grade 2 (moderate)	None required	
Grade 3 (severe)	First occurrence	<p>Reduce to 100 mg QD.</p> <ul style="list-style-type: none"> <li>If the event resolves to Grade ≤2 within 28 days of the dose reduction, increase to 100 mg BID.</li> <li>If the event does not resolve to Grade ≤2 within 28 days of the dose reduction, discontinue study drug.</li> </ul>
	Second occurrence	Reduce to 100 mg QD for 7 days, then discontinue study drug.
Grade 4 (life-threatening)	Reduce to 100 mg QD for 7 days, then discontinue study drug.	

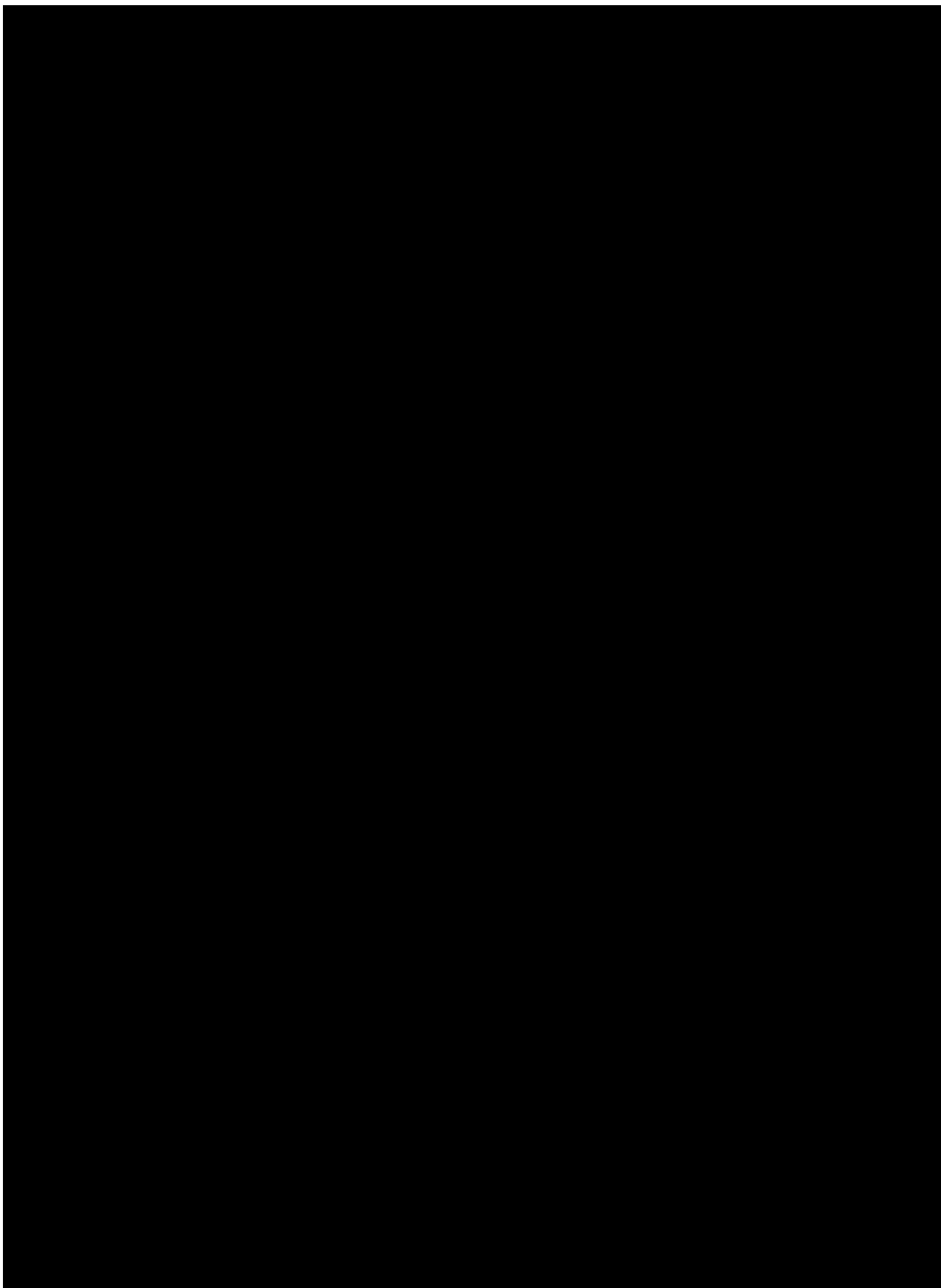
Abbreviations: BID = twice daily; QD = once daily.

<sup>1</sup> Adverse event severity assessed as mild, moderate, or severe, or according to National Cancer Institute Common Terminology Criteria for Adverse Events.

[REDACTED]

[REDACTED]

[REDACTED]





## **7. STUDY DRUG DISCONTINUATION AND SUBJECT WITHDRAWAL FROM STUDY**

### **7.1. Study Drug Discontinuation**

In some instances, it may be necessary for a subject to discontinue study drug. Subjects may be discontinued from study drug for the following reasons:

- Completed
- Adverse event
- Death
- Lost to follow-up
- Physician decision
- Pregnancy
- Protocol deviation
- Study terminated by sponsor
- Withdrawal by subject
- Other

The primary reason for study drug discontinuation will be recorded in the eCRF. All efforts will be made to complete and report protocol-defined study observations as thoroughly as possible. Subjects who are discontinuing study drug should undergo the recommended dose taper and be monitored as clinically indicated for signs and symptoms of acute hemolysis and worsening anemia. Assessments to be completed at the time of study drug discontinuation are specified in the Schedules of Assessments ([Table 2](#)).

### **7.2. Subject Withdrawal From the Study**

A subject may withdraw consent from participation in the study at any time at the subject's request or may be withdrawn from the study at any time at the discretion of the Investigator for safety, behavioral, compliance, or administrative reasons. Subjects may be withdrawn from the study for the following reasons:

- Death
- Lost to follow-up
- Withdrawal by subject
- Study terminated by sponsor
- Other

The primary reason for withdrawal from the study will be recorded in the eCRF. If a subject withdraws consent from study participation, no further evaluations should be performed, and no attempts should be made to collect additional data. The subject will be discontinued from study drug and withdrawn from the study at that time.

Subjects who withdraw consent for further participation in the study will not be replaced.

If the subject withdraws consent for disclosure of future information, the Sponsor may retain and continue to use any data collected before withdrawal of consent.

If a subject withdraws consent for further study participation, the subject may request destruction of any samples taken and not tested, and the Investigator must document this in the clinical study center records.

### **7.3. Lost to Follow-up**

A subject will be considered lost to follow-up if the subject repeatedly fails to attend study visits (at the clinical study center or at home) and is unable to be contacted by the clinical study center.

If a subject fails to attend a study visit, the clinical study center must attempt to contact the subject to reschedule the missed visit as soon as possible, counsel the subject on the importance of maintaining the assigned visit schedule, and ascertain whether the subject wishes to and/or should continue in the study. Before a subject is considered lost to follow-up, the Investigator (or designee) must make every effort to regain contact with the subject. These contact attempts should be documented in the subject's medical record. Should the subject continue to be unreachable, the subject will be deemed lost to follow-up and withdrawn from the study.

## 8. STUDY ASSESSMENTS AND PROCEDURES

Study procedures and their timing are outlined in the Schedules of Assessments in Section 1.1. Protocol waivers or exemptions are not allowed.

Details on the processes for collection and handling of blood samples will be provided in the Laboratory Manual.

If allowed by local institutional guidelines, and at the discretion of the Investigator and the subject, the subject can complete some visits and study-related assessments at home. The designated time points and assessments for these at-home study visits, instead of in-person visits to the clinical study center, are in the Schedules of Assessments.

An at-home visit consists of 1 or 2 of the following components:

1. **Investigator Assessment:** A visit conducted by the Investigator (or designee) either via video conference or telephone call (telemedicine) or in person at home for assessments that can be completed via this mode (eg, AEs and concomitant medications).
2. **Home Health:** A visit at home with a nurse or home health-care provider for assessments that can be completed via this mode (eg, vital signs and blood collection for clinical laboratory assessments). Patient-reported outcome measures may also be administered during at-home study visits, as applicable.

During the Double-blind and Open-label Extension Periods through Week 108, the at-home visits will include both Investigator Assessment and Home Health components. Starting at Week 132 during the Open-label Extension Period, the visits will consist of the Investigator Assessment component only.

### 8.1. Informed Consent

Informed consent will be obtained before any study procedures are performed.

### 8.2. Screening and Eligibility

All screening evaluations must be completed and documented by the Investigator in the eCRF within the specified screening window (see Schedules of Assessments; Table 1). The Investigator will confirm the subject's eligibility before randomization. Screening extensions will not be granted.

A blood sample will be collected for comprehensive  $\alpha$ - and  $\beta$ -globin genotyping during the Screening Period for analysis by the study central laboratory. Results from the comprehensive  $\alpha$ - and  $\beta$ -globin genotyping are not required for eligibility if test results confirming the diagnosis of  $\alpha$ - or  $\beta$ -thalassemia are available in the subject's medical record or can be obtained by a local laboratory during the Screening Period.

Randomization will be stratified by thalassemia genotype as either  $\alpha$ -thalassemia/HbH disease or  $\beta$ -thalassemia, which includes  $\beta$ -thalassemia with or without  $\alpha$ -globin gene mutations and HbE/ $\beta$ -thalassemia. If it is unclear how the subject should be classified for stratification, the Investigator should contact the Sponsor to facilitate a review of the testing results before randomization. If a subject's screening window expires before the availability of genetic testing

results from local or central laboratory analyses, or before the determination of their stratification class, the subject may be rescreened.

If a subject is ineligible due to a transient condition (eg, prohibited concomitant medication, curable medical condition), the subject can be rescreened after the criterion that the subject failed to meet has resolved. If a subject was ineligible according to a previous version of the protocol, the subject may be rescreened if the subject could be eligible according to the current version of the protocol.

Rescreened subjects will be assigned a different subject number than for the previous screening. Rescreened subjects need to re-sign the informed consent form (ICF). Each subject may be rescreened a maximum of 2 times (ie, a subject may be screened a total of 3 times).

If a subject requires rescreening, the following screening assessments do not need to be repeated: demographics, documentation of thalassemia diagnosis, and comprehensive  $\alpha$ - and  $\beta$ -globin genotyping. If the [REDACTED] and dual-energy x-ray absorptiometry (DXA) scan are within 6 months of randomization, these screening assessments do not need to be repeated.

### **8.3. Demographic Data**

Demographic data will be collected during the Screening Period and include sex, year of birth, race, and ethnicity, according to local regulatory requirements.

### **8.4. Medical, Procedural, Surgical, and Disease History**

A complete medical, procedural, surgical, and disease history will be obtained during the Screening Period. The medical history is to include all relevant medical history and all current medical conditions.

### **8.5. Efficacy Assessments**

#### **8.5.1. Clinical Laboratory Efficacy Assessments**

Study-required clinical laboratory efficacy assessment samples will be analyzed by a central laboratory. If samples for local laboratory analysis are collected, the study-required samples for central laboratory analysis must also be collected at the same time.

Clinical laboratory efficacy assessment parameters are listed in [Table 9](#).

**Table 9: Clinical Laboratory Efficacy Assessments**

Assessment	Parameters
Anemia	Hb [REDACTED]
Hemolysis	Indirect bilirubin, haptoglobin, LDH [REDACTED]
Erythropoiesis	Reticulocytes, [REDACTED] erythropoietin, [REDACTED]
Iron metabolism	Iron, serum ferritin, transferrin, [REDACTED] TSAT, and TIBC <sup>1</sup>

Abbreviations: Hb = hemoglobin; [REDACTED] LDH = lactate dehydrogenase; TIBC = total iron binding capacity; TSAT = transferrin saturation.

**Note:** Study-required clinical laboratory efficacy assessment samples will be analyzed by a central laboratory.

<sup>1</sup> TSAT and TIBC will be calculated.

Clinical laboratory safety assessment parameters are listed in Section 8.6.3.

## 8.6. Safety Assessments

### 8.6.1. Vital Signs and Physical Examinations

Vital signs should be measured after 5 minutes of recumbency and before blood sample collection. Vital signs include systolic and diastolic blood pressure, pulse rate, and body temperature. Weight will also be recorded.

Height will be measured and recorded during the Screening Period.

### 8.6.2. Electrocardiograms

A single, 12-lead electrocardiogram (ECG) will be recorded and read at the clinical study center after 5 minutes of recumbency and before blood sample collection.

### 8.6.3. Clinical Laboratory Safety Assessments

Study-required clinical laboratory assessment samples will be analyzed by a central laboratory. Local laboratory analysis is allowed, at the discretion of the Investigator, if faster turnaround time is required to support clinical decision-making. If samples for local laboratory analysis are collected, the study-required samples for central laboratory analysis must also be collected at the same time. Subjects will be monitored for potential aromatase inhibition by assessments of sex hormone levels.



Clinical laboratory safety assessment parameters are listed in [Table 10](#).

**Table 10: Clinical Laboratory Safety Assessments**

Assessment	Parameters
Hematology	<ul style="list-style-type: none"> <li>Complete blood count (Hb, HCT, MCHC, MCH, MCV, RBC count, nucleated RBCs, RDW-CV, % reticulocyte count, absolute reticulocyte count, and platelet count) with WBC count and differential (neutrophils, basophils, eosinophils, lymphocytes, and monocytes)</li> </ul>
Clinical chemistry	<ul style="list-style-type: none"> <li>Albumin, BUN or urea, total calcium, CO<sub>2</sub> or bicarbonate, chloride, creatinine, [REDACTED] magnesium, phosphorus, potassium, sodium, total protein, and uric acid</li> </ul>
Liver tests	<ul style="list-style-type: none"> <li>ALP, ALT/SGPT, AST/SGOT, and bilirubin (direct/conjugated, indirect/unconjugated, and total)</li> <li>LDH</li> </ul>
Coagulation tests	<ul style="list-style-type: none"> <li>Fibrinogen, aPTT, and INR</li> </ul>
Lipid panel	<ul style="list-style-type: none"> <li>Nonfasting HDL-C, LDL-C, total cholesterol, and triglycerides</li> </ul>
Sex hormones	<ul style="list-style-type: none"> <li>Nonfasting estradiol, estrone, and testosterone (total and free)</li> </ul>
[REDACTED]	
Screening virology	<ul style="list-style-type: none"> <li>HBsAg, HCV Ab (positive results for HCV Ab testing require confirmation by a test for HCV-RNA), HIV-1 Ab, and HIV-2 Ab</li> </ul>
Screening FSH and serum hCG	<ul style="list-style-type: none"> <li>FSH (for women in the absence of 12 months of amenorrhea to confirm non-reproductive potential) and a serum (hCG) pregnancy (for WOCBP)</li> </ul>

Abbreviations: AB = antibody; ALP = alkaline phosphatase; ALT = alanine aminotransferase; aPTT = activated partial thromboplastin time; AST = aspartate aminotransferase; BUN = blood urea nitrogen; CO<sub>2</sub> = carbon dioxide; [REDACTED]; FSH = follicle stimulating hormone; Hb = hemoglobin; HBsAg = hepatitis B surface antigen; hCG = human chorionic gonadotropin; HCT = hematocrit; HCV = hepatitis C virus; HDL-C = high-density lipoprotein cholesterol; INR = international normalized ratio; LCMS = liquid chromatography–mass spectrometry; LDH = lactate dehydrogenase; LDL-C = low density lipoprotein cholesterol; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; RBC = red blood cell; RDW-CV = red cell distribution width coefficient of variation; WBC = white blood cell; WOCBP = women of childbearing potential.

**Note: Study-required clinical laboratory assessment samples will be analyzed by a central laboratory.**

[REDACTED]

Clinical laboratory efficacy assessment parameters are listed in [Section 8.5](#).

#### 8.6.4. Dual-Energy X-ray Absorptiometry Scans

Subjects will be monitored for potential aromatase inhibition by assessments of bone mineral density. Bone mineral density will be assessed by DXA scans of the hip and spine performed according to the instructions provided by the central-read vendor. The DXA scans will be read and interpreted by the central vendor.

### **8.6.5. Adverse Events and Serious Adverse Events**

The definitions and reporting requirements for AEs and SAEs are provided in Section 9.

### **8.7. Red Blood Cell Transfusions**

All HRQOL assessments and the 6-minute walk test (6MWT) (see Section 8.11) must be conducted before all other study visit assessments, including blood sampling. All blood samples must be collected before administration of the study drug and before the RBC transfusion, if an RBC transfusion is scheduled on the same day.

Transfusion history will be recorded for the 24-week period before randomization. On-treatment transfusions will also be recorded. The transfusion history and on-treatment transfusion record includes transfusion dates, number of RBC units transfused, and pretransfusion Hb concentration. An RBC unit refers to packed red blood cell (pRBC) preparations. Due to hematocrit concentration differences in blood products used per local standards of care, 2 units of whole blood are equivalent to 1 unit of pRBCs and constitute an RBC unit. If available, the volume and the hematocrit of each transfused RBC unit should also be recorded. All RBC transfusions, including those received outside of the clinical study center, must be recorded.

Subjects may receive RBC transfusions at the discretion of the Investigator as clinically indicated.

### **8.8. Pharmacokinetics and Pharmacodynamics**

Blood samples will be collected for measurement of concentrations of mitapivat and other pharmacokinetic parameters as appropriate, and for pharmacodynamic measurement of 2,3-DPG and ATP.

On days when there are pharmacokinetic or pharmacodynamic assessments, the morning dose of study drug will be administered at the clinical study center.

The actual date and time (24-hour clock time) of sample collection will be recorded in the source documents or eCRF. An explanation should be provided in the source documents for any missed or mishandled pharmacokinetic samples, and for any samples collected outside the time windows. The actual date and time (24-hour clock time) of study drug administration for the morning dose and the last dose taken the previous evening will also be recorded in the source documents or eCRF.

Pharmacokinetic samples will be analyzed using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. Plasma pharmacokinetic parameters will be computed from measured plasma concentrations and using actual sample collection times.

Pharmacodynamic samples will be analyzed for 2,3-DPG and ATP using an LC-MS/MS method.

[REDACTED]

## 8.9. Genetics

Blood samples collected for comprehensive  $\alpha$ - and  $\beta$ -globin genotyping [REDACTED] will be analyzed by the study central laboratory. Samples will be maintained in a secure storage facility with adequate measures to protect subject confidentiality. Samples will be retained for a maximum of 10 years (or according to local regulations).

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

## 8.11. Health-Related Quality of Life and Performance Outcome Assessments

All HRQOL assessments and the 6MWT must be conducted before all other study visit assessments. The 6MWT should be conducted before the remaining assessments at the study visit, including blood samples.

### 8.11.1. Functional Assessment of Chronic Illness Therapy-Fatigue Subscale

The FACIT-Fatigue subscale was originally developed as an additional subscale to the Functional Assessment of Cancer Therapy-General (FACT-G) to assess cancer-related fatigue (Acaster et al, 2015). The full FACIT-Fatigue instrument consists of the 27-item FACT-G, a generic measure of HRQOL consisting of physical, functional, emotional, and social and family well-being aspects, and a 13-item self-reported fatigue subscale, which assesses the severity and impact of fatigue (including the impact on daily activities and functioning). The subscale has a 7-day recall period and is scored on a 5-point Likert scale: 0 (not at all) to 4 (very much) (Acaster et al, 2015). The total score ranges from 0 to 52, with a higher score indicating better HRQOL.

### 8.11.2. Patient Global Impressions of Severity and Change

The Patient Global Impression of Severity (PGIS) and Patient Global Impression of Change (PGIC) questions are patient-reported prospective and retrospective ratings, respectively, of disease-related concepts or overall status. The PGIS is a validated, single-item measure used to assess the HRQOL impact or severity of a specific health condition (US FDA, 2018; Viktrup et al, 2012). The PGIC is a validated, single-item measure used to assess the change over time in a concept or overall status (Guy, 1976; US FDA, 2018). The PGIS questions will be administered

to assess severity (on a 4-point scale ranging from “None” to “Severe”) of the following 3 concepts as perceived by the subject over the recall periods noted below:

- Fatigue (7-day recall)

■ [REDACTED]

■ [REDACTED]

Similarly, the PGIC questions will be used to assess subject-perceived change compared with baseline in the same concepts on a 5-point scale ranging from “Much better” to “Much worse.”

■ [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

■ [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

■ [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

#### **8.11.7. Six-Minute Walk Test**

The 6MWT is a well-established PerfO measure that is widely used to evaluate physical activity in terms of distance walked in patients with a variety of conditions. The test measures the distance an individual can walk on a hard, flat surface in 6 minutes and only requires a hallway that is  $\geq 100$  feet. The test is self-paced, and the individual can rest during the test as needed ([ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories, 2002](#)). In healthy adults, the normal range is from 400 to 700 meters ([Enright, 2003](#)). A lower score, based on less distance covered in 6 minutes, indicates worse level of physical activity.

## **9. ADVERSE EVENTS: DEFINITIONS AND PROCEDURES FOR RECORDING AND REPORTING**

### **9.1. Definitions of Adverse Events**

#### **9.1.1. Definition of an Adverse Event**

An AE is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product that does not necessarily have a causal relationship with this treatment. An AE can therefore be any of the following:

- Any unfavorable and unintended sign (eg, an abnormal laboratory finding), symptom, or disease temporally associated with the use of a study drug, whether or not considered related to the study drug.
- Any new disease or exacerbation of an existing disease (ie, a worsening in the character, frequency, or severity of a known condition)
- Recurrence of an intermittent medical condition (eg, headache) not present at baseline
- Any deterioration in a laboratory value or other clinical test (eg, ECG, x-ray) that is associated with clinical signs and symptoms, leads to a modification or discontinuation of study drug or concomitant therapy, requires medical or surgical intervention, or is considered by the Investigator to be clinically significant
- AEs that are related to a protocol-mandated intervention, including those that occur before assignment of study drug (eg, invasive screening procedures such as biopsies)

#### **9.1.2. Definition of a Serious Adverse Event**

An SAE is any AE or suspected adverse reaction that:

- Results in death
- Is immediately life-threatening
  - Life-threatening means that the subject was at immediate risk of death from the reaction as it occurred (ie, it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form).
- Requires inpatient hospitalization or prolongation of existing hospitalization
  - Planned hospital admissions or surgical procedures for an illness or disease which existed before the subject was enrolled in the study or before study drug was administered are not to be considered AEs unless the condition deteriorated in an unexpected manner during the study (eg, surgery was performed earlier or later than planned).
- Results in persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- Results in congenital anomaly/birth defect

- Is considered an important medical event
  - An important medical event is an event that may not result in death, be life-threatening, or require hospitalization but may be considered an SAE when, based upon appropriate medical judgment, it may jeopardize the subject or may require medical or surgical intervention to prevent one of the outcomes listed in the definitions for SAEs. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias, or the development of drug dependency or drug abuse.

The terms “severe” and “serious” are not synonymous. Severity refers to the intensity of an AE (mild, moderate, or severe, or according to NCI CTCAE); the event itself may be of relatively minor medical significance (eg, severe headache without any further findings). Severity and seriousness need to be independently assessed for each AE recorded on the eCRF.

### **9.1.3. Abnormal Clinical Laboratory Events**

A clinical laboratory test result should be reported as an AE if it meets any of the following criteria:

- Associated with clinical signs and symptoms
- Results in a dose modification or study drug discontinuation
- Requires medical or surgical intervention or a change in concomitant therapy
- Clinically significant in the Investigator’s judgment

The Investigator is responsible for reviewing all clinical laboratory findings. Medical and scientific judgment should be exercised in deciding whether an isolated laboratory abnormality should be classified as an AE.

If a clinically significant laboratory abnormality is a sign or symptom of a disease or syndrome, only the diagnosis should be recorded on the AE eCRF.

If a clinically significant laboratory abnormality is not a sign or symptom of a disease or syndrome, the abnormality itself should be recorded on the AE eCRF, along with a descriptor indicating if the test result is above or below the normal range.

## **9.2. Procedures for Recording Adverse Events and Serious Adverse Events**

### **9.2.1. Recording Adverse Events**

Adverse events and SAEs that occur from the time of providing informed consent until 28 days after the administration of the last dose of study drug will be recorded on the appropriate pages of the eCRF.

Adverse events spontaneously reported by the subject and/or in response to open nonleading questions (eg, “How are you feeling?”) from study personnel or revealed by observation,

physical examination, or other diagnostic procedures will be collected at every visit and recorded in the subject's medical record and in the appropriate pages of the eCRF.

Adverse events will be followed until 28 days after administration of the last dose of study drug or until study termination. Subjects will be assessed at the Safety Follow-up visit to determine if any new AEs have occurred. Adverse events that are ongoing at the time of study drug discontinuation should be followed through the Safety Follow-up visit. After this period, Investigators should report only SAEs that are considered related to study drug. Subjects who are withdrawn from the study due to a study drug-related SAE [REDACTED] will continue to be followed until the SAE resolves or is declared chronic by the Investigator, or until the subject is lost to follow-up.

Any clinically significant deterioration in clinical laboratory test results (see Section 9.1.3) or other clinical finding that is considered an AE must be recorded in the eCRF. When possible, signs and symptoms indicating a common underlying pathology should be noted as one comprehensive event.

Information to be reported in the description of each AE includes:

- A medical diagnosis of the event (if a medical diagnosis cannot be determined, a description of each sign or symptom characterizing the event should be recorded)
- Event onset date
- Event resolution date
- Whether the event is serious (Section 9.1.2)
- Severity of the event (Section 9.2.2)
- Relationship of the event to study drug (Section 9.2.3)
- Action taken with study drug
- Event outcome

### 9.2.2. Severity of Adverse Events

The severity of all AEs, including clinically significant treatment emergent- clinical laboratory abnormalities, will be graded by the Investigator according to Version 4.03 of the NCI CTCAE on a 5-point severity scale (Grade 1 through Grade 5). Adverse events not specified by the NCI CTCAE will be graded according to Table 11.

**Table 11: Adverse Event Severity**

Severity	Definition	NCI CTCAE
Mild	The event is noticeable to the subject but does not interfere with routine activity.	Grade 1
Moderate	The event interferes with routine activity but responds to symptomatic therapy or rest.	Grade 2
Severe	The event significantly limits the subject's ability to perform routine activities despite symptomatic therapy.	Grade 3



Life-threatening	The subject was at risk of death at the time of the event.	Grade 4
Fatal	An event that results in the death	Grade 5

Abbreviations: NCI CTCAE = National Cancer Institute Common Terminology Criteria for Adverse Events.

An AE of severe intensity may not be considered serious. Severity is a measure of intensity, whereas seriousness is defined as described in Section 9.1.2.

### 9.2.3. Relationship to Study Drug

Investigators must determine whether an AE is considered to be related to the study drug, indicating “yes” or “no” according to the causal attribution guidance in Table 12.

**Table 12: Causal Attribution Guidance**

Is the AE suspected to be caused by the study drug based on facts, evidence, science-based rationales, and clinical judgment?	
Yes	There is a plausible temporal relationship between the onset of the AE and administration of the study drug, and the AE cannot be readily explained by the subject’s clinical state, intercurrent illness, or concomitant therapies; and/or the AE follows a known pattern of response to the study drug; and/or the AE abates or resolves upon discontinuation of the study drug or dose reduction and, if applicable, reappears upon rechallenge.
No	Adverse events will be considered related unless evidence exists that the AE has an etiology other than the study drug (eg, preexisting medical condition, underlying disease, intercurrent illness, or concomitant therapy); and/or the AE has no plausible temporal relationship to study drug administration (eg, cancer diagnosed 2 days after first dose of study drug).

Abbreviation: AE = adverse event.

When determining a causal attribution to study drug, the following guidance should be considered:

- Temporal relationship of event onset to the initiation of study drug
- Course of the event, considering especially the effects of dose reduction, study drug discontinuation, or study drug rechallenge, as applicable
- Known association of the event with the study drug or with similar treatments
- Known association of the event with the disease under study
- Presence of risk factors for the subject or use of concomitant therapies known to increase the occurrence of the event
- Presence of non–treatment-related factors that are known to be associated with the occurrence of the event

## 9.3. Procedures for Reporting Adverse Events

### 9.3.1. Reporting of Serious Adverse Events

All SAEs, regardless of relationship to study drug, that occur from the time of providing informed consent until 28 days after administration of the last dose of study drug must be reported to the Sponsor within 24 hours from the time when the Investigator becomes aware of

the SAE. This 24-hour notification applies to the initial SAE information and all follow-up SAE information.

Serious adverse events that occur more than 28 days after administration of the last dose of study drug that are considered by the Investigator to be related to study drug must be reported to the Sponsor any time the Investigator becomes aware of such an event.

The SAE eCRF must be completed and will include the following information: subject number, a narrative description of the event, and an assessment by the Investigator of the severity of the event and its relationship to study drug. Additional follow-up information on the SAE may be requested by the Sponsor or Medical Monitor.

If the electronic data capture (EDC) system is unavailable for more than 24 hours, SAEs must be reported using the EDC Downtime SAE Report Form. The SAE Report Form and completion instructions, a Safety Cover Letter, as well as a list of country-specific fax numbers are provided by the sponsor. Completed SAE reports may be submitted to Agios via email at [Safety\\_Operations@agios.com](mailto:Safety_Operations@agios.com).

If there are suspected unexpected serious adverse reactions associated with the use of mitapivat, the Sponsor will notify the appropriate regulatory agency(ies) and all participating Investigators on an expedited basis. The local Institutional Review Board (IRB)/Independent Ethics Committee (IEC) will be promptly notified based on local regulations where required by the IRB/IEC of all serious, unexpected adverse drug reactions involving risk to human subjects.

Any on-treatment deaths, regardless of relationship to study drug, must be recorded on the appropriate eCRF and reported to the Sponsor within 24 hours. Deaths that occur during the protocol-specified SAE reporting period that are attributed by the Investigator solely to disease under study should not be reported as SAEs and should only be recorded on the End of Study eCRF page.

## **9.4. Other Safety-Related Issues**

### **9.4.1. Overdose or Dose Administration Error**

Overdose is the accidental or intentional use of the study drug in an amount higher than the dose being studied. An overdose or incorrect administration of study drug is not an AE unless it results in untoward medical effects. All AEs associated with an overdose or incorrect administration of study drug should be recorded on the AE eCRF. If the associated AE fulfills the criteria for an SAE, the event must be reported as specified in Section 9.3.1.

### **9.4.2. Pregnancy**

Pregnancy is not reported as an AE unless there is a suspicion that an investigational product may have interfered with the effectiveness of a contraceptive medication or a complication relating to the pregnancy occurs that may qualify as an SAE (eg, spontaneous abortion). The Investigator must report any pregnancy that occurs during the treatment period and within 28 days after administration of the last dose of study drug, even in the absence of an AE. Pregnancy must be reported to the Sponsor within 24 hours of learning of its occurrence using a Pregnancy Report Form. Abnormal pregnancy outcomes (eg, maternal serious complications,

spontaneous or therapeutic abortion, ectopic pregnancy, stillbirth, neonatal death, congenital anomaly, or birth defect) are considered SAEs and must be reported as specified in Section 9.3.1.

#### 9.4.2.1. Pregnancy in Female Subjects

Female subjects who become pregnant while participating in the study will be immediately discontinued from study drug. Subjects should be encouraged to undergo the assessments at the Safety Follow-up visit. All AEs that occur during this time, including those related to the pregnancy, will be reported.

Investigators should counsel the subject regarding the risks of continuing the pregnancy and the possible effects on the fetus. The Investigator must follow-up and document the course and outcome of all pregnancies. Monitoring of the subject should continue until conclusion of the pregnancy. After completion of the pregnancy, attempts should be made to collect and report details of the outcome of the pregnancy and development of the child.

#### 9.4.2.2. Pregnancy Follow-up

All outcomes of a pregnancy from a female subject must be reported by the Investigator to the Sponsor or Medical Monitor in the pregnancy outcome section of the paper Pregnancy Reporting Form immediately after becoming aware of the outcome of the pregnancy. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for the procedure.

The Sponsor will attempt to collect follow-up information where possible and allowable by local practice and regulations (ie, with the consent of the subject) regarding the course and outcome of the pregnancy, including any postnatal sequelae in the infant and the development of the child in order to perform an independent medical assessment of the reported pregnancy.

After study termination, the Investigator may report any outcomes or AEs directly to the sponsor on a paper SAE form.

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

## 10. STATISTICAL CONSIDERATIONS

### 10.1. Statistical Hypotheses

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 with an Hb response in the mitapivat arm and placebo arm, respectively. Hb response is defined as an increase in average Hb concentration from Week 12 through Week 24 that is  $\geq 1.0$  g/dL from baseline.

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

$$H_{02}: \mu_{t1} - \mu_{c1} = 0 \text{ vs } H_{12}: \mu_{t1} - \mu_{c1} \neq 0;$$

$$H_{03}: \mu_{t2} - \mu_{c2} = 0 \text{ vs } H_{13}: \mu_{t2} - \mu_{c2} \neq 0;$$

where  $\mu_{t1}$  and  $\mu_{c1}$  are the mean change from baseline in average FACIT-Fatigue subscale score from Week 12 through Week 24 in the mitapivat arm and placebo arm respectively; and  $\mu_{t2}$  and  $\mu_{c2}$  are the mean change from baseline in average Hb concentration from Week 12 through Week 24 in the mitapivat arm and placebo arm, respectively.

Overall type I error will be maintained at or below 2-sided significance level of 0.05 by a fixed-sequence testing procedure ([Westfall and Krishen, 2001](#)).  $H_{01}$  will be tested first; if  $H_{01}$  is rejected, then  $H_{02}$  will be tested; if  $H_{02}$  is rejected, then  $H_{03}$  will be tested. All tests will be conducted at the 2-sided 0.05 significance level.

The study will have met its primary objective if the difference in the Hb response rate between the mitapivat arm and the placebo arm is statistically significant at the corresponding  $\alpha$ -level per the statistical testing strategy at the time of the analysis of the primary endpoint when all subjects have completed the Double-blind Period (or discontinued earlier).

### 10.2. Sample Size Determination

Assuming an Hb response rate of 10% in the placebo arm, 171 subjects (114 subjects randomized to mitapivat and 57 subjects randomized to placebo) are needed to provide a 95% power to detect an increase in Hb response rate from 10% in the placebo arm to 35% in the mitapivat arm based on a 2-sided significance level of 0.05.

Given the large variability of Hb concentration in patients with anemia, the SD of change from baseline can be as high as 1.3 g/dL ([Kalantar-Zadeh and Aronoff, 2009](#)). Assuming no changes in Hb concentration in subjects receiving placebo, approximately 10% of subjects could achieve an Hb response simply because of the variability in Hb concentration measurements. Therefore, a 10% Hb response rate is being assumed for the placebo arm.

The assumption of a 35% Hb response rate was informed by the results from Study AG348-C-010. As of 20 August 2020, 70% (14/20 overall, 10/15 in those with  $\beta$ -thalassemia, and 4/5 in those with  $\alpha$ -thalassemia) subjects achieved a  $\geq 1.0$  g/dL increase in average Hb concentration from Week 12 through Week 24 compared with baseline. Considering a more heterogeneous population in this large, double-blind, Phase 3 study and a commonly noted

decrease in efficacy between Phase 2 and Phase 3 studies, a 35% response rate for mitapivat arm is deemed to be a reasonable assumption.

The sample size of 171 subjects will also provide 80% power at a 2-sided significance level of 0.05 to detect a 5.5 difference in the change from baseline in average FACIT-Fatigue subscale score from Week 12 through Week 24 between the mitapivat arm and the placebo arm. These calculations assume:

- a change from baseline in average FACIT-Fatigue subscale score from Week 12 through Week 24 in the mitapivat arm of 4.5 and in the placebo arm of -1
- a common SD of 12

Assumptions of the FACIT-Fatigue score change from baseline are from several sources. FACIT-Fatigue results from placebo-controlled studies in NTDT have not yet been published. The available literature and feedback from the thalassemia experts suggest an increase of 3 points in FACIT-Fatigue score for every 1 g/dL increase in Hb. Results of the open-label luspatercept NTDT study with similar eligibility criteria included mean FACIT-Fatigue score change from baseline to Week 24 of approximately +2.8 (N=17) and mean Hb change from baseline to Week 24 of approximately +1.2 g/dL (N=25) ([Piga et al, 2016](#)). In a recent randomized, open-label, controlled trial in paroxysmal nocturnal hemoglobinuria, the observed FACIT-Fatigue score change from baseline to Week 16 was +9.2 in the pegcetacoplan treatment arm, and Hb change over the same period was +2.4 g/dL (N=41 patients randomized) ([Hillmen et al, 2020](#)). Based on the Hb data observed in the Phase 2 Study AG348-C-010 and these results from trials, an increase in the FACIT-Fatigue score of approximately +4.5 in the mitapivat arm over Weeks 12 to 24 and a small decrease in the score of -1 in the placebo group are expected, with an estimated SD of 12 for the difference of changes.

### 10.3. Populations for Analyses

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 ICF.
Full Analysis Set (FAS)	All subjects who are randomized. Subjects will be classified according to the randomized treatment arm according to the 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 do not receive at least 1 dose of the randomized treatment will be excluded from the PPS. Other criteria leading to exclusion of subjects from the PPS will be prespecified in the statistical analysis plan.
Safety Analysis Set	All subjects who receive at least 1 dose of the study drug. 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.
Pharmacodynamic Analysis Set	A subset of the Safety Analysis Set including all subjects with at least 1 blood 2,3-DPG or ATP concentration measurement above LLQ.
Pharmacokinetic Analysis Set	A subset of the Safety Analysis Set including all subjects with at least 1 plasma mitapivat concentration measurement above LLQ.

Abbreviations: 2,3-DPG = 2,3-diphosphoglycerate; ATP = adenosine triphosphate; ITT = intent-to-treat; LLQ = lower limit of quantitation.

### 10.4. Statistical Analyses

The statistical analysis plan (SAP) will be finalized before database lock and unblinding and will include a more detailed description of the statistical analyses described in this section.

#### 10.4.1. General Considerations

##### 10.4.1.1. Baseline Definitions

##### Efficacy Evaluations

For efficacy parameters (Hb concentration-based endpoints and markers of hemolysis and erythropoiesis), baseline is defined as the average of all assessments within 42 days before randomization for subjects randomized and not dosed or within 44 (42+2) days before the start of study treatment for subjects randomized and dosed. Assessments collected within 8 weeks after an RBC transfusion will be excluded from the baseline derivation for these efficacy parameters and for endpoints associated with measures of fatigue.

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.

The first assessments related to physical activity are planned to occur on Day 1 before administration of the first dose of study drug. Therefore, the last assessment before

randomization for subjects randomized and not dosed or before the start of study treatment for subjects randomized and dosed will be used as the baseline.

### **Baseline Characteristics**

For summaries of baseline characteristics based on the FAS, baseline will be defined as follows:

- For subjects randomized and not dosed: the last assessment before randomization
- For subjects randomized and dosed: the last assessment before the start of study treatment

### **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.

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

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

#### **10.4.1.2. On-Treatment Period**

The on-treatment period begins on the date of the start of study drug and ends 28 days after the end of study drug administration (including the recommended dose taper).

#### **10.4.1.3. Pooling of Data Across Clinical Study Centers**

To provide overall estimates of treatment effects, data will be pooled across clinical study centers (“sites”). The “site” factor will not be considered in statistical models or subgroup analyses given the high number of participating centers in contrast to the anticipated small number of subjects randomized at each study center.

#### **10.4.1.4. Continuous and Categorical Variables**

Continuous variables will be summarized using descriptive statistics (ie, number of nonmissing values, mean, SD, median, quartiles, minimum, and maximum).

Categorical variables will be summarized by frequency distributions (ie, 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.

#### **10.4.1.5. 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. Descriptive statistics (mean, SD, median, quartiles, minimum, and maximum) by nominal visit or time point for safety endpoints (eg, clinical laboratory measurements and vital signs) will include only data from scheduled visits. Summaries of outliers (eg, worst value, worst change from baseline, worst severity grade) during the on-treatment period for safety endpoints (eg, AEs and clinical laboratory assessments) will include data from both scheduled and unscheduled visits.

### **10.4.2. Primary Endpoint**

#### **10.4.2.1. Hemoglobin Response**

The primary endpoint of Hb response is defined as a  $\geq 1.0$  g/dL increase in average Hb concentration from Week 12 through Week 24 compared with baseline.

The proportion of subjects who achieved an Hb response (response rate) will be summarized for each treatment arm. The difference in response rate between the mitapivat arm and the placebo arm will be estimated by a Mantel-Haenszel stratum weighted method adjusting for randomization stratification factors. The adjusted difference in response rate, along with the 95% CI and the 2-sided p-value, will be provided.

Hemoglobin concentrations assessed within 8 weeks after an RBC transfusion will be excluded from the analysis of the primary endpoint. Subjects who do not have at least 2 on-treatment Hb concentration assessments from Week 12 to Week 24 will be considered nonresponders.

#### **10.4.2.2. Sensitivity Analyses**

The following sensitivity analyses will be performed:

- Hb response will be summarized using the methodology described for the primary analysis but based on the PPS.
- Hb response will be summarized using the methodology described for the primary analysis but including only those subjects who completed 24 weeks of study treatment and who did not initiate any concomitant medications that could affect the Hb concentrations from randomization through the completion of 24 weeks of study treatment.



Additional sensitivity analyses may be performed and will be prespecified in the SAP.

#### **10.4.2.3. Subgroup Analyses**

Hemoglobin response rate will be summarized for subgroups defined by each randomization stratification factor. Within each subgroup, Hb response rate will be summarized by treatment arm, and the difference in Hb response rate between the mitapivat arm and the placebo arm will be estimated (difference in Hb response rate and 95% CI) using an unstratified method.

Hemoglobin response may be summarized for other subgroups prespecified in the SAP.

#### **10.4.3. Secondary Efficacy Endpoints**

##### **10.4.3.1. Key Secondary Efficacy Endpoints**

###### **10.4.3.1.1. FACIT-Fatigue**

The FACIT-Fatigue subscale will be scored according to published scoring guidelines (see Section 8.11.1). The change from baseline in average FACIT-Fatigue subscale score from Week 12 through Week 24 will be compared between the mitapivat arm and the placebo arm using an analysis of covariance (ANCOVA) model. The model will include the score change from baseline as the dependent variable, treatment arm as the independent variable, and covariates for randomization stratification factors and baseline FACIT-Fatigue subscale score. Missing data will not be imputed for the primary analysis of FACIT-Fatigue. FACIT-Fatigue subscale scores within 8 weeks after an RBC transfusion will be excluded from the analysis.

Descriptive statistics will be used to summarize the individual items and change from baseline in the average subscale scores at each scheduled assessment time point by treatment arm.

Additionally, anchor-based analyses may be used to establish the threshold for clinically meaningful change in the FACIT-Fatigue using PGIS and PGIC ratings as a basis for the anchors.

#### **Sensitivity Analyses**

The following sensitivity analyses will be performed:

- To assess the durability of effect of the study treatment and longitudinal trending, the FACIT-Fatigue score change from baseline at each month after Week 12 will be summarized using an ANCOVA model as described for the primary analysis of FACIT-Fatigue.
- Pattern-mixture model with control-based pattern imputation for missing data.

Missing FACIT-Fatigue data will be imputed using multiple imputation methodology (Rubin, 1987) by replacing each missing value with a set of plausible values that represent the uncertainty about the correct value to impute. A Markov Chain Monte Carlo method (Schafer, 1997) will first be used to generate data sets following monotone missing patterns, and a regression-based pattern-mixture method will then be used to impute the remaining missing values based on the distribution data from the subjects in the placebo arm. This conservative approach is based on the assumption that after discontinuation from study treatment, subjects in the mitapivat arm will exhibit the same future evolution of the disease as subjects in the placebo

arm; this approach will tend to move the estimate of the treatment effect toward a smaller value compared with missing-at-random-based analysis methods, such as mixed models with repeated measures or standard multiple imputation models. The same ANCOVA model as described for the primary analysis of FACIT-Fatigue will be used on each of the multiply-imputed datasets and the statistics will be combined according to Rubin's rules ([Rubin, 1987](#)).

#### **10.4.3.1.2. Change From Baseline in Average Hemoglobin Concentration**

The change from baseline in average Hb concentration from Week 12 through Week 24 will be compared between the mitapivat arm and the placebo arm using an ANCOVA model. The model will include the score change from baseline as the dependent variable, treatment arm as the independent variable, and covariates for randomization stratification factors and baseline Hb concentration. The least squares mean of the change from baseline in average Hb concentration (average from Week 12 to Week 24) will be presented for each treatment arm.

Hemoglobin concentrations assessed within 8 weeks after an RBC transfusion will be excluded from the analysis.

#### **10.4.3.2. Other Secondary Efficacy Endpoints**

##### **10.4.3.2.1. Hemoglobin (1.5+) Response**

The secondary endpoint of Hb 1.5+ response is defined as a  $\geq 1.5$  g/dL increase in average Hb concentration from Week 12 through Week 24 compared with baseline. The proportion of subjects who achieve an Hb 1.5+ response will be summarized for each treatment arm. The difference in Hb 1.5+ response rate between the mitapivat arm and the placebo arm will be estimated by a Mantel-Haenszel stratum weighted method adjusting for randomization stratification factors. The adjusted difference in response rate, along with the 95% CI, will be provided.

Hemoglobin concentrations assessed within 8 weeks after an RBC transfusion will be excluded from the analysis.

##### **10.4.3.2.2. Mean Hemoglobin Concentration Increase for Hemoglobin Responders ( $\geq 1.0$ g/dL)**

For subjects who achieve an Hb response ( $\geq 1.0$  g/dL increase in average Hb concentration from Week 12 through Week 24 compared with baseline), descriptive statistics for the mean Hb concentration increase will be provided for each treatment arm.

##### **10.4.3.2.3. Biomarker Endpoints**

The change from baseline in markers of hemolysis and erythropoiesis at Week 24 will be summarized using the same statistical methodology described for the Hb 1.5+ response endpoint (see Section [10.4.3.2.1](#)).

##### **10.4.3.2.4. Other Health-Related Quality of Life and Performance Outcome Endpoints**

For the PGIS-Fatigue and PGIC-Fatigue, the number and proportion of subjects in each response level will be summarized by treatment arm and study visit. The change from baseline in PGIS-Fatigue levels and PGIC-Fatigue ratings at Week 12, 16, 20, and 24 between the mitapivat

arm and the placebo arm will be summarized. Specifically, the proportion of subjects with no, or mild, fatigue on the PGIS-Fatigue at baseline who remain stable or who improve from mild fatigue to no fatigue at the specified week compared with baseline will be summarized. The proportion of subjects with moderate or severe fatigue on the PGIS-Fatigue at baseline who report improvement by  $\geq 1$  level at the specified week compared with baseline will also be summarized.

For the PGIC-Fatigue, the proportion of subjects with no, or mild, fatigue on the PGIS-Fatigue at baseline who report “no change” or any level of improvement on the PGIC-Fatigue at the specified week, and the proportion of subjects with moderate or severe fatigue on the PGIS-Fatigue at baseline who report any level of improvement on the PGIC-Fatigue at the specified week, will be summarized. Analyses for PGIS-Fatigue and PGIC-Fatigue will be conducted separately at Weeks 12, 16, 20, and 24.

Descriptive statistics will also be used to summarize the 6MWT distance and change from baseline at each scheduled assessment time point by treatment arm. The change from baseline in 6MWT distance at Week 24 between the mitapivat arm and the placebo arm will be summarized. Anchor-based analyses may also be used to establish the threshold of a clinically meaningful change for the 6MWT using PGIS and PGIC ratings as a basis for the anchors.

#### **10.4.3.2.5. Pharmacokinetic and Pharmacodynamic Endpoints**

Descriptive statistics of pharmacokinetic (arithmetic and geometric means, SD, percent coefficient of variation [CV%], CV% geometric mean, minimum, median, and maximum) and pharmacodynamic parameters, including ATP and 2,3-DPG, will be summarized as appropriate.

#### **10.4.4. Safety Endpoints**

Summaries of safety data from the Double-blind Period will be presented by treatment arm based on the Safety Analysis Set. Summaries of safety data from the Open-label Extension Period will be presented by treatment arm and overall based on the Safety Analysis Set.

##### **10.4.4.1. Adverse Events**

Treatment-emergent adverse events are defined as AEs with a first onset date during the on-treatment period or worsening from baseline. All summaries will be based on treatment-emergent adverse events, unless otherwise specified. Treatment-emergent adverse events will be summarized according to the latest version of Medical Dictionary for Regulatory Activities terminology by System Organ Class and/or Preferred Term, severity (based on the NCI CTCAE Version 4.03 grading), seriousness, and relationship to study drug, unless otherwise specified.

##### **10.4.4.2. Clinical Laboratory Assessments**

The actual values and the changes from baseline will be summarized by study visit for clinical laboratory tests.

###### **10.4.4.2.1. Parameters With CTCAE Grades Available**

Clinical laboratory test results will be graded according to the CTCAE as applicable. Grading will be derived based on the numerical thresholds defined by the CTCAE. Nonnumerical

qualifiers will not be taken into consideration in the derivation of CTCAE grading. Clinical laboratory test results classified according to the CTCAE will be described using the worst grade.

The frequency of subjects with clinical laboratory toxicities during the on-treatment period will be tabulated as follows.

- Summaries of clinical laboratory parameters by CTCAE grade will include the number and percentage of subjects with Grade 1, 2, 3, 4; Grades 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.
- Shift tables 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, Grades 3-4) during the on-treatment period will also be summarized.

The denominator used to calculate percentages for each clinical laboratory test is the number of subjects evaluable for CTCAE grading for that parameter (ie, subjects for whom a Grade of 0, 1, 2, 3, or 4 can be derived).

#### **10.4.4.2.2. Parameters With CTCAE Grades Not Available**

Clinical laboratory test results that are not specified by the CTCAE will be presented according to the following categories: < the lower limit of normal (LLN), within normal limits, and >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.

#### **10.4.4.3. Dual-Energy X-ray Absorptiometry Scans**

Results from bone mineral density on DXA scan will be summarized.

#### **10.4.4.4. Vital Signs and Physical Examinations**

The actual values and the changes from baseline will be summarized by study visit for vital signs assessments. Clinically significant changes in physical examinations will also be presented.

[REDACTED]

### **10.5. Interim Analyses**

An interim analysis is not planned.

## **10.6. Independent Data Monitoring Committee**

An unblinded IDMC will be responsible for ongoing monitoring of the safety of subjects according to the IDMC Charter. The recommendations for study conduct (eg, continue as planned, continue with modifications, terminate) made by the IDMC will be provided to the Sponsor (or designee) for the final decision. The Sponsor (or designee) will forward such decisions, which may include summaries of aggregate analyses of endpoint events and of safety data that are not endpoints, to regulatory authorities, as appropriate.

The IDMC will perform the first review of safety data after approximately 30 subjects have been randomized in the study. Subsequently, the IDMC will convene to monitor safety in the study approximately every 3 months until the study is unblinded for the analysis of the primary endpoint. Recommendations for study conduct (continue as planned, continue with modifications, terminate) will be conveyed to Agios by the IDMC chair.

## **11. REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS**

### **11.1. Regulatory and Ethical Considerations**

This study will be conducted in accordance with the protocol and with the following:

- Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences International Ethical Guidelines
- Applicable International Council for Harmonisation (ICH) GCP Guidelines
- Applicable laws and regulations

The protocol, ICF, IB, and other relevant documents (eg, advertisements) must be reviewed and approved by an IRB/IEC before the study is initiated.

Any amendments to the protocol will require IRB/IEC approval before implementation of changes, except for changes necessary to eliminate an immediate hazard to study subjects in which case approval will be obtained as soon as possible following implementation.

The Investigator will be responsible for the following:

- Providing written summaries of the study status to the IRB/IEC in accordance with the requirements, policies, and procedures established by the IRB/IEC (at least annually and at study closeout)
- Notifying the IRB/IEC of SAEs or other significant safety findings as required
- Overseeing the conduct of the clinical study at the study center and adhering to all applicable regulations

#### **11.1.1. Temporary Modifications Allowed During Public Health Emergencies**

In the event of a declared public health emergency or natural disaster that affects a geographic area (eg, state, province, country, region, continent) and impedes adherence to protocol-specified procedures, certain modifications (see Section 11.1.1.1) are temporarily allowable to ensure subject safety, maintain compliance with GCP, and minimize risks to study integrity; the protocol must be followed to the fullest extent possible.

These modifications are only allowable 1) when consistent with applicable regulations and guidance and 2) for the duration of the declared public health emergency or natural disaster. During this period, the need for all implemented modifications will be reassessed and the Sponsor will no longer allow these modifications once the situation resolves.

Examples of declared public health emergencies and natural disasters are:

- The public health emergency related to COVID-19 declared by the US Secretary of Health and Human Services in 2020
- The Australian Bushfires Disaster declared by the Australian Attorney General in 2020

Documented approval from the Sponsor is required before these modifications can be implemented.

#### **11.1.1.1. Allowable Temporary Modifications**

The following temporary modifications are allowed in the event of a declared public health emergency or natural disaster and must be reported as protocol deviations; refer to the Schedules of Assessments (Section 1.1) for the timing of assessments.

- Alternative distribution of study drug
  - Study drug may be shipped to a local health-care provider or pharmacy or, if necessary, directly to a subject. The quantity to be shipped must be reviewed and approved in advance by the Sponsor (or designee), in agreement with the Investigator.
  - Secure, trackable delivery methods (delivery service companies [eg, DHL], couriers, and hand delivery) must be used.
  - Sponsor (or designee) approval is required before each shipment. Shipment will be permitted only if, at minimum, a telemedicine visit has been conducted that incorporates appropriate safety assessments.
- Returning unused study drug and empty study drug packaging
  - Return of unused study drug and empty study drug packaging may be delayed until the subject's next visit to the study site. In certain circumstances, the nature of the return process may vary (eg, personal protective equipment may be required).
- Telemedicine visits for assessments other than PROs
  - Telemedicine visits, preferably via video conference, are permissible for all assessments that can be completed via this mode (eg, medical history, concomitant medications, review of AEs).
- Telemedicine for collection of PROs
  - Sponsor agreement is needed before implementing alternative solutions for PROs that were intended to be collected at the site.
- Use of laboratories and health-care providers not specified in the clinical study documentation (must be reported as a protocol deviation)
  - For assessments that cannot be completed via telemedicine, the use of health-care providers and laboratories that are not specified in the clinical trial documentation (eg, an imaging facility, clinic, or local practice that is more readily accessible by the subject) is permissible for all assessments that can be completed via this mode (eg, blood collection for laboratory assessments, ECG, physical examinations, imaging).

- Use of a laboratory or health-care provider not specified in the clinical study documentation requires coordination between the subject, the Investigator, and the subject's local health-care provider.
- The Investigator must document their review of the results provided by laboratories and health-care providers not specified in the clinical study documentation.
- Home health study support
  - For assessments that cannot be completed via telemedicine, home health-care provider visits are permissible for all assessments that can be completed via this mode (eg, physical examination, collection of laboratory samples).
  - The Investigator must document their review of the results of home health-care provider visits.
- Virtual informed consent/reconsent in lieu of in-person informed consent/reconsent
  - Reconsent (ie, consenting to an amended version of the protocol) may be completed virtually and documented in the relevant subject medical records. In these instances, reconsent may be completed virtually where allowed by the applicable regulations and documented in the relevant subject medical records.
  - The other allowable modifications described in this section may require consent from the subject because their implementation requires a variation from the specifications in the protocol to which the subject has consented (eg, consent for a home visit, consent to provide name and address to a third-party delivery service, consent to a new mode of completing study procedures and receiving study drug). In these instances, consent may be completed virtually where allowed by the applicable regulations and documented in the relevant subject medical records.

## 11.2. Informed Consent Process

The Investigator (or designee) will explain the nature of the study to subjects (or their legally authorized representative) and answer all questions regarding the study. Subjects will be informed that their participation is voluntary.

Subjects (or their legally authorized representative) will be required to sign a statement of informed consent that meets the requirements of Title 21 Code of Federal Regulations Part 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act of 1996 requirements, where applicable, and the IRB/IEC. The authorized person obtaining the informed consent must also sign the ICF. A copy of the ICF will be provided to subjects (or their legally authorized representative).

The medical record must include a statement that written informed consent was obtained before the subject was enrolled in the study and the date the written informed consent was obtained.

During participation in the study, subjects (or their legally authorized representative) must provide written informed consent to the most current and IRB/IEC-approved version of the study ICF.





### 11.3. Data Protection


Subjects will be assigned a unique identifier by the Sponsor (or designee). Any subject records or datasets transferred to the Sponsor will be labeled with only this unique identifier; neither subject names nor other information that readily enables subject identification will be transferred. The key needed to connect the unique identifier with the subject's name will be held by the Principal Investigator and approved research staff at the clinical study center.

The subject will be informed that personal study-related data will be used by the Sponsor in accordance with applicable data protection laws. Where required by applicable data protection laws, the subject will be required to provide consent for their data to be used as described in the informed consent.

The subject must be informed that his/her medical records and personal data collected in the study may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the Sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities. The subject will also be informed that his/her information will be disclosed to certain other parties working for or with the Sponsor in conducting the study (eg, CROs, central laboratories, and research collaborators).

Should the Sponsor become aware of a serious data breach, the Sponsor will evaluate the breach to determine whether notification to regulatory authorities or study subjects is appropriate in accordance with applicable law.

### 11.4. Dissemination of Clinical Study Data

All information regarding study drug supplied by the Sponsor (or designee) to the Investigator is privileged and  information. The Investigator agrees to use this information only to conduct the study and not to use it for any other purpose without explicit consent from the Sponsor.

It is understood that there is an obligation on the part of the Investigator to provide the Sponsor with the complete data obtained during the study. Such information will be used in the clinical development of the study drug and may be disclosed to regulatory authorities, other Investigators, corporate partners, or consultants, as required.

## 11.5. Quality Compliance

All subject data relating to the study will be recorded on a printed CRF or eCRF unless transmitted to the Sponsor (or designee) electronically (eg, clinical laboratory data). The Investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.

The Investigator must maintain accurate documentation (source data) supporting the information entered in the CRF.

The Investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.

Monitoring details describing strategy (eg, risk management and mitigation strategies and analytical risk-based monitoring), methods, responsibilities, and requirements, including handling of noncompliance issues and monitoring techniques (eg, central, remote, or on-site monitoring), are in the Monitoring Plan.

The Sponsor (or designee) is responsible for the data management of this study including quality checking of the data.

The Sponsor assumes accountability for actions delegated to other individuals (eg, CROs).

Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized clinical study center personnel are accurate, complete, and verifiable from source documents; that the safety and rights of subjects are being protected; and that the study is being conducted in accordance with the currently approved protocol, applicable SOPs, work instructions, policies and any other study agreements, GCP (including but not limited to ICH, European Medicines Agency, and US FDA), and all applicable regulatory requirements.

Essential documents pertaining to the conduct of this study, including signed ICFs, must be retained by the Investigator until at least 2 years after the last approval of a marketing application for the investigational product or until at least 2 years have elapsed since the formal discontinuation of the clinical development of the investigational product. These documents should be retained for a longer period if required by applicable regulatory requirements, by an agreement with the Sponsor, or by local regulations or institutional policies. No records may be destroyed during the retention period without the written approval of the Sponsor. No records may be transferred to another location or party without written notification to the Sponsor.

Record destruction is to be documented, comply with applicable laws and regulations, and ensure subject confidentiality.

## 11.6. Source Documents

Source documents will be completed for each study subject. It is the Investigator's responsibility to ensure the accuracy, completeness, and timeliness of the data reported in the subject's source document/eCRF (or on the paper CRF, if applicable). The source document should indicate the subject's participation in the study and should document the dates and details of study procedures, AEs, and subject status.

The Investigator, or designated representative, should complete the source document as soon as possible after information is collected for a subject's treatment, assessments, and study

procedures. Any outstanding entries must be completed after the final study visit. An explanation should be provided for all missing data.

### **11.7. Study Termination and Clinical Study Center Closure**

Clinical study centers will be closed upon study completion. A study center is considered closed when all required documents and study supplies have been collected and a study center closure visit has been performed.

The Sponsor reserves the right to terminate the study or close the clinical study center at any time for any reason at the sole discretion of the Sponsor.

The Investigator may initiate study center closure at any time, provided there is reasonable cause and enough notice is provided to the Sponsor in advance of the intended termination.

Reasons for study termination or the early closure of a clinical study center by the Sponsor or Investigator may include but are not limited to the following:

- Failure of the Investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the Sponsor's procedures, or GCP guidelines
- Inadequate recruitment of subjects by the Investigator
- Discontinuation of further study drug development

### **11.8. Publication Policy**

The results of this study may be published or presented at scientific meetings. If this is foreseen, the Investigator agrees to submit all manuscripts or abstracts to the Sponsor before submission. This allows the Sponsor to protect proprietary information and to provide comments.

The Sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the Sponsor will generally support publication of multicenter studies only in their entirety and not as individual clinical study center data. In this case, a coordinating Investigator will be designated by mutual agreement.

Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements.

## 12. REFERENCES

Acaster S, Dickerhoof R, DeBusk K, Bernard K, Strauss W, Allen LF. Qualitative and quantitative validation of the FACIT-fatigue scale in iron deficiency anemia. *Health Qual Life Outcomes*. 2015;13:60. doi:10.1186/s12955-015-0257-x

Amid A, Leroux R, Merelles-Pulcini M, et al. Factors impacting quality of life in thalassemia patients; Results from the IntercontinenThal collaborative study. *Blood*. 2016;128(22):3633-3633. doi:10.1182/blood.V128.22.3633.3633

Angelucci E, Matthes-Martin S, Baronciani D, et al. Hematopoietic stem cell transplantation in thalassemia major and sickle cell disease: indications and management recommendations from an international expert panel. *Haematologica*. 2014;99(5):811-820. doi:10.3324/haematol.2013.099747

ATS Committee on Proficiency Standards for Clinical Pulmonary Function Laboratories. ATS statement: guidelines for the six-minute walk test. *Am J Respir Crit Care Med*. 2002;166(1):111-117. doi:10.1164/ajrccm.166.1.at1102

Borgna-Pignatti C, Rugolotto S, De Stefano P, et al. Survival and complications in patients with thalassemia major treated with transfusion and deferoxamine. *Haematologica*. 2004;89(10):1187-1193.

Cappellini M, Viprakasit V, Taher A, et al. The Believe Trial: results of a phase 3, randomized, double-blind, placebo-controlled study of luspatercept in adult beta-thalassemia patients who require regular red blood cell (RBC) transfusions. *Blood*. 2018;132(suppl 1):163. doi:10.1182/blood-2018-163

Cappellini MD, Cohen A, Perter J, Taher A, Viprakasit V. *Guidelines for the Management of Transfusion Dependent Thalassemia (TDT)*. 3rd ed: Thalassemia International Federation; 2014.

Cappellini MD, Porter JB, Viprakasit V, Taher AT. A paradigm shift on beta-thalassaemia treatment: How will we manage this old disease with new therapies? *Blood Rev*. 2018;32(4):300-311. doi:10.1016/j.blre.2018.02.001

Cappellini MD, Robbiolo L, Bottasso BM, Coppola R, Fiorelli G, Mannucci AP. Venous thromboembolism and hypercoagulability in splenectomized patients with thalassaemia intermedia. *Br J Haematol*. 2000;111(2):467-473. doi:10.1046/j.1365-2141.2000.02376.x

Chakraborty I, Mishra R, Gachhui R, Kar M. Distortion of beta-globin chain of hemoglobin alters the pathway of erythrocytic glucose metabolism through band 3 protein. *Arch Med Res*. 2012;43(2):112-116. doi:10.1016/j.arcmed.2012.02.003

Enright PL. The six-minute walk test. *Respir Care*. 2003;48(8):783-785.

Galanello R. Recent advances in the molecular understanding of non-transfusion-dependent thalassemia. *Blood Rev*. 2012;26(suppl 1):S7-S11. doi:10.1016/S0268-960X(12)70004-8

Galanello R, Cao A. Gene test review. Alpha-thalassemia. *Genet Med*. 2011;13(2):83-88. doi:10.1097/GIM.0b013e3181fcb468

Grace R, Layton D, Galactéros F, et al. Long-term safety and efficacy of mitapivat (AG-348), a pyruvate kinase activator, in patients with pyruvate kinase deficiency: The DRIVE PK study. *Blood*. 2019;134(suppl 1):3512-3512. doi:10.1182/blood-2019-123406

Grace RF, Rose C, Layton DM, et al. Safety and efficacy of mitapivat in pyruvate kinase deficiency. *N Engl J Med*. 2019;381(10):933-944. doi:10.1056/NEJMoa1902678

Guy W. *ECDEU Assessment Manual for Psychopharmacology*. U.S. Dept. of Health, Education, and Welfare, Public Health Service, Alcohol, Drug Abuse, and Mental Health Administration, National Institute of Mental Health, Psychopharmacology Research Branch, Division of Extramural Research Programs; 1976.

[REDACTED]

Hillmen P, Szer J, Weitz I, et al. Results of the PEGASUS phase III randomized trial demonstrating superiority of the C3 inhibitor, pegcetacoplan, compared to eculizumab in patients with paroxysmal nocturnal hemoglobinuria. *HemaSphere*. 2020;4(1)(suppl):52. doi:10.1097/HS9.0000000000000404

Kalantar-Zadeh K, Aronoff GR. Hemoglobin variability in anemia of chronic kidney disease. *J Am Soc Nephrol*. 2009;20(3):479-487. doi:10.1681/ASN.2007070728

Karimi M, Cohan N, De Sanctis V, Mallat NS, Taher A. Guidelines for diagnosis and management of Beta-thalassemia intermedia. *Pediatr Hematol Oncol*. 2014;31(7):583-596. doi:10.3109/08880018.2014.937884

Kung C, Hixon J, Kosinski PA, et al. AG-348 enhances pyruvate kinase activity in red blood cells from patients with pyruvate kinase deficiency. *Blood*. 2017;130(11):1347-1356. doi:10.1182/blood-2016-11-753525

Levey AS, Stevens LA, Schmid CH, et al. A new equation to estimate glomerular filtration rate. *Ann Intern Med*. 2009;150(9):604-612. doi:10.7326/0003-4819-150-9-200905050-00006

Mihailescu AM, Musallam KM, Cappellini MD, Taher AT. Less 'reds' more 'blues': hemoglobin level and depression in non-transfusion-dependent thalassemia. *Ann Hematol*. 2020;99(4):903-904. doi:10.1007/s00277-020-03958-y

Modell B, Khan M, Darlison M. Survival in beta-thalassaemia major in the UK: data from the UK Thalassaemia Register. *Lancet*. 2000;355(9220):2051-2052. doi:10.1016/s0140-6736(00)02357-6

Musallam KM, Angastiniotis M, Eleftheriou A, Porter JB. Cross-talk between available guidelines for the management of patients with beta-thalassemia major. *Acta Haematol*. 2013;130(2):64-73. doi:10.1159/000345734

Musallam KM, Cappellini MD, Viprakasit V, Kattamis A, Rivella S, Taher AT. Revisiting the non-transfusion-dependent (NTDT) vs. transfusion-dependent (TDT) thalassemia classification 10 years later. *Am J Hematol*. 2020. doi:10.1002/ajh.26056

Musallam KM, Rivella S, Vichinsky E, Rachmilewitz EA. Non-transfusion-dependent thalassemias. *Haematologica*. 2013;98(6):833-844. doi:10.3324/haematol.2012.066845

[REDACTED]  
[REDACTED]  
[REDACTED]

Piga A, Tartaglione I, Gamberini R, et al. Luspatercept Increases Hemoglobin, Decreases Transfusion Burden, and Improves Patient-Reported Outcomes in Adults with Beta-Thalassemia. Acceleron Pharma. 05 December 2016. <https://acceleronpharma.com/wp-content/uploads/2017/03/20161205-Luspatercept-Increases-Hemoglobin.pdf>

Pyrukynd (mitapivat). USPI. Agios Pharmaceuticals; 2022.

Reblozyl (luspatercept-aamt). USPI. Celgene; 2019.

Rubin D. Introduction. In: *Multiple Imputation for Nonresponse in Surveys*. John Wiley and Sons, Inc.; 1987:1-26:chap 1.

Schafer J. *Analysis of Incomplete Multivariate Data*. 1st ed: Chapman and Hall; 1997.

Schuessler-Lenz M, Enzmann H, Vamvakas S. Regulators' advice can make a difference: European Medicines Agency approval of Zynteglo for beta thalassemia. *Clin Pharmacol Ther*. 2020;107(3):492-494. doi:10.1002/cpt.1639

Shaeffer JR. ATP-dependent proteolysis of hemoglobin alpha chains in beta-thalassemic hemolysates is ubiquitin-dependent. *J Biol Chem*. 1988;263(27):13663-13669.

Taher A, Musallam K, Cappellini M. *Guidelines for the Management of Non-Transfusion Dependent Thalassaemia (NTDT)*. 2nd ed: Thalassaemia International Federation; 2017.

Taher AT, Musallam KM, Cappellini MD, Weatherall DJ. Optimal management of beta thalassaemia intermedia. *Br J Haematol*. 2011;152(5):512-523. doi:10.1111/j.1365-2141.2010.08486.x

Taher AT, Musallam KM, Saliba AN, Graziadei G, Cappellini MD. Hemoglobin level and morbidity in non-transfusion-dependent thalassemia. *Blood Cells Mol Dis*. 2015;55(2):108-109. doi:10.1016/j.bcmd.2015.05.011

Taher AT, Radwan A, Viprakasit V. When to consider transfusion therapy for patients with non-transfusion-dependent thalassaemia. *Vox Sang*. 2015;108(1):1-10. doi:10.1111/vox.12201

Taher AT, Weatherall DJ, Cappellini MD. Thalassaemia. *Lancet*. 2018;391(10116):155-167. doi:10.1016/s0140-6736(17)31822-6

Thein SL. The molecular basis of beta-thalassemia. *Cold Spring Harb Perspect Med*. 2013;3(5):a011700. doi:10.1101/cshperspect.a011700

Ting YL, Naccarato S, Qualtieri A, Chidichimo G, Brancati C. In vivo metabolic studies of glucose, ATP and 2,3-DPG in beta-thalassaemia intermedia, heterozygous beta-thalassaemic and normal erythrocytes: <sup>13</sup>C and <sup>31</sup>P MRS studies. *Br J Haematol*. 1994;88(3):547-554.

US FDA. *Methods to identify what is important to patients and select, develop or modify fit-for-purpose clinical outcomes assessments*. United States Food and Drug Administration; 2018. Discussion document for patient-focused drug development public workshop on guidance 3.

[REDACTED]

Vichinsky E. Advances in the treatment of alpha-thalassemia. *Blood Rev*. 2012;26 Suppl 1:S31-34. doi:10.1016/S0268-960X(12)70010-3

Vichinsky E. Non-transfusion-dependent thalassemia and thalassemia intermedia: epidemiology, complications, and management. *Curr Med Res Opin*. 2016;32(1):191-204. doi:10.1185/03007995.2015.1110128

Viktrup L, Hayes RP, Wang P, Shen W. Construct validation of patient global impression of severity (PGI-S) and improvement (PGI-I) questionnaires in the treatment of men with lower urinary tract symptoms secondary to benign prostatic hyperplasia. *BMC Urol*. 2012;12:30. doi:10.1186/1471-2490-12-30

Weatherall DJ. The challenge of haemoglobinopathies in resource-poor countries. *Br J Haematol*. 2011;154(6):736-744. doi:10.1111/j.1365-2141.2011.08742.x

Westfall PH, Krishen A. Optimally weighted, fixed sequence, and gatekeeping multiple testing procedures. *J Stat Plan Inference*. 2001;99(1):25-40. doi:10.1016/S0378-3758(01)00077-5

Zynteglo. Summary of Product Characteristics. bluebird bio (Netherlands) B.V.; 2019.

## **13. APPENDICES**



**APPENDIX 1. LIST OF ABBREVIATIONS**

<b>Abbreviation</b>	<b>Term</b>
2,3-DPG	2,3-diphosphoglycerate
6MWT	6-minute walk test
Ab	antibody
AE	adverse event
ALT	alanine aminotransferase
ANCOVA	analysis of covariance
AST	aspartate aminotransferase
ATP	adenosine triphosphate
AUC	area under the plasma concentration $\times$ time curve
AUC <sub>0-<math>\infty</math></sub>	area under the plasma concentration $\times$ time curve from time 0 to infinity
AUC <sub>last</sub>	area under the plasma concentration $\times$ time curve from time 0 to the last measurable concentration
AUC <sub>0-t</sub>	area under the plasma concentration $\times$ time curve from time 0 to time t
BID	twice daily
C <sub>max</sub>	maximum (peak) concentration
COVID-19	coronavirus disease 2019
CRO	contract research organization
CTCAE	Common Terminology Criteria for Adverse Events
CV%	percent coefficient of variation
CYP	cytochrome P450
DXA	dual-energy x-ray absorptiometry
ECG	electrocardiogram
eCRF	electronic case report form
EDC	electronic data capture
FACIT-Fatigue	Functional Assessment of Chronic Illness Therapy-Fatigue
FDA	Food and Drug Administration
FSH	follicle stimulating hormone
GCP	Good Clinical Practice

<b>Abbreviation</b>	<b>Term</b>
Hb	hemoglobin
HbA	adult hemoglobin
HbS	sickle cell hemoglobin
HBsAg	hepatitis B surface antigen
HCV	hepatitis C virus
HRQOL	health-related quality of life
HRT	hormone replacement therapy
HSCT	hematopoietic stem cell transplantation
IB	Investigator's Brochure
ICF	Informed Consent Form
ICH	International Council for Harmonisation
IDMC	Independent Data Monitoring Committee
IEC	Independent Ethics Committee
IRB	Institutional Review Board
IXRS	Interactive Voice/Web Response System
LC-MS/MS	liquid chromatography-tandem mass spectrometry
LDH	lactate dehydrogenase
LLN	lower limit of normal
NCI	National Cancer Institute
NTDT	non-transfusion-dependent thalassemia
PEP	phosphoenolpyruvate
PerfO	performance outcome
PGIC	Patient Global Impression of Change
PGIS	Patient Global Impression of Severity
P-gp	P-glycoprotein
PKL	liver-specific form of pyruvate kinase
PKM	pyruvate kinase muscle isozyme
PKR	red blood cell-specific form of pyruvate kinase
pRBC	packed red blood cell
PRO	patient-reported outcome

Abbreviation	Term
QD	once daily
RBC	red blood cell
SAE	serious adverse event
SAP	statistical analysis plan
SD	standard deviation
SOP	standard operating procedure
$t_{1/2}$	half-life
TDT	transfusion-dependent thalassemia
$t_{\max}$	time to maximum (peak) concentration
UGT	uridine 5'-diphospho-glucuronosyltransferase
ULN	upper limit of normal
US	United States
WOCBP	women of childbearing potential

## **APPENDIX 2. DEFINITION OF WOMEN OF CHILDBEARING POTENTIAL AND CONTRACEPTION GUIDANCE**

### **Definition of Women of Childbearing Potential**

A woman is considered fertile after menarche and until becoming postmenopausal unless permanently sterile. If fertility is unclear (eg, amenorrhea in adolescents or athletes) and a menstrual cycle cannot be confirmed before administration of the first dose of study drug, additional evaluation should be considered.

A woman is not considered a WOCBP if any of the following apply:

1. Premenarchal
2. Premenopausal female with 1 of the following:
  - Documented hysterectomy
  - Documented bilateral salpingectomy
  - Documented bilateral oophorectomy

For individuals with permanent infertility due to an alternate medical cause other than the above (eg, mullerian agenesis, androgen insensitivity), Investigator discretion should be applied to determining study eligibility.

Documentation can come from the subject's medical records, medical examination, or medical history interview.

3. Postmenopausal female
  - A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.
    - A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a postmenopausal state in women not using hormonal contraception or hormone replacement therapy (HRT); however, in the absence of 12 months of amenorrhea, confirmation with >1 FSH measurement is required.
  - Females on HRT and whose menopausal status is in doubt will be required to use 1 of the nonestrogen hormonal highly effective contraception methods if they wish to continue their HRT during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

### **Acceptable Contraception Methods**

A highly effective form of contraception is defined as combined (estrogen and progestin containing) hormonal contraceptives (oral, intravaginal, or transdermal) known to be associated with inhibition of ovulation; progestin-only hormonal contraceptives (oral, injectable, or implantable) known to be associated with inhibition of ovulation; intrauterine device; intrauterine hormone releasing system; bilateral tube occlusion; or vasectomized partner. Hormonal contraception being used as a highly effective form of contraception must be accompanied by an acceptable barrier method.

An acceptable barrier method of contraception includes male or female condoms with or without spermicide, and cervical cap, diaphragm, or sponge with spermicide.

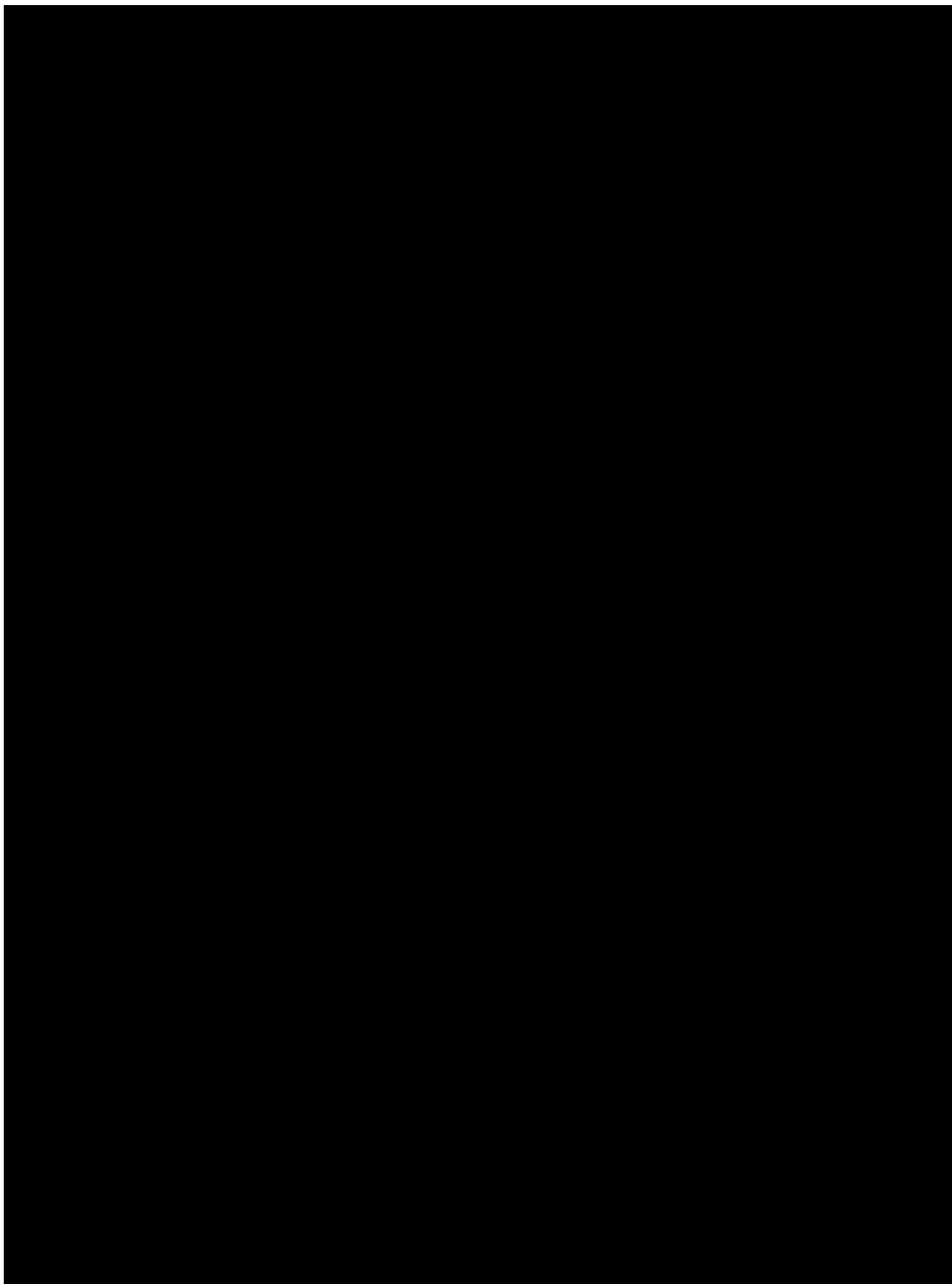
[REDACTED]

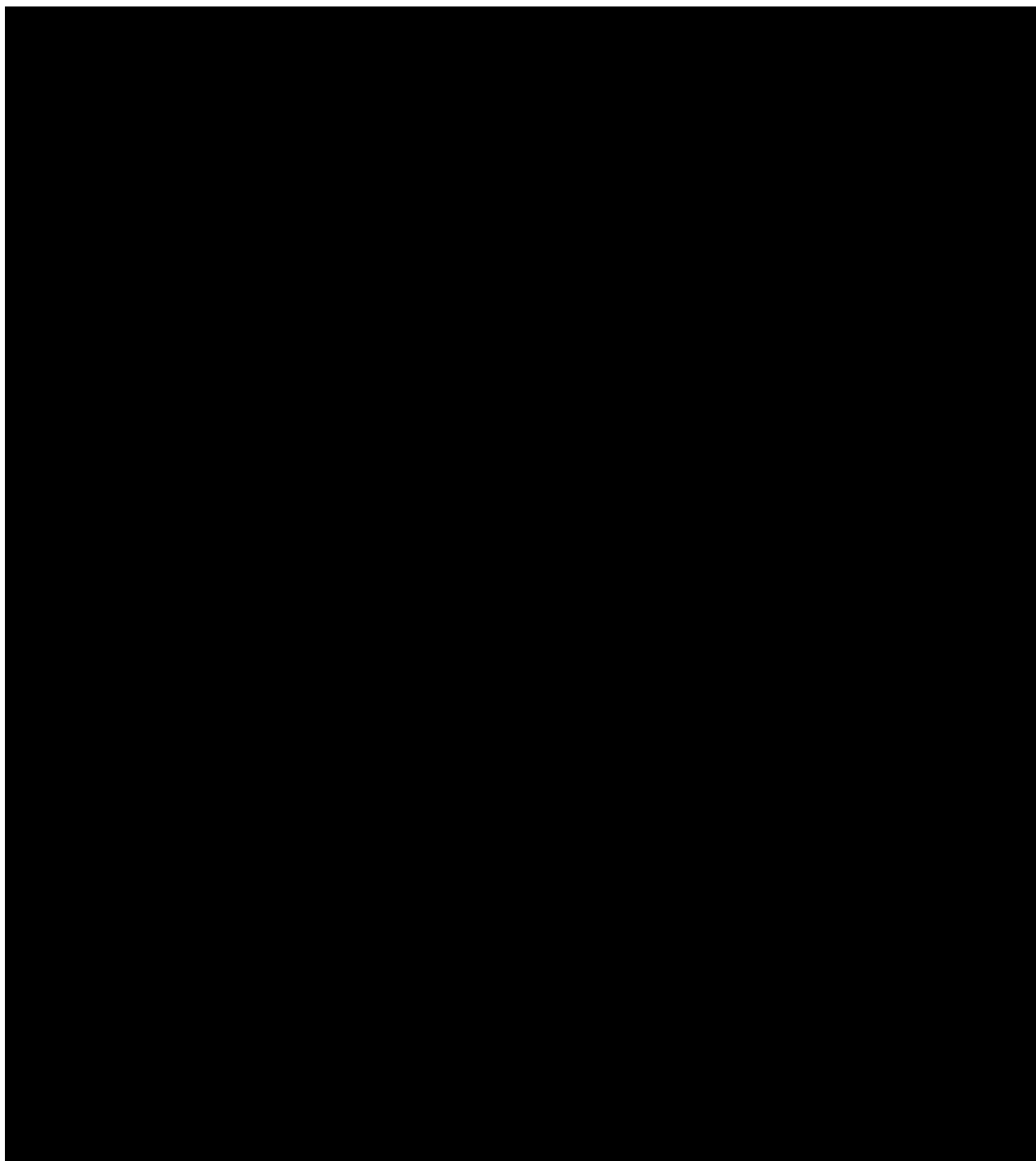
[REDACTED]

[REDACTED]

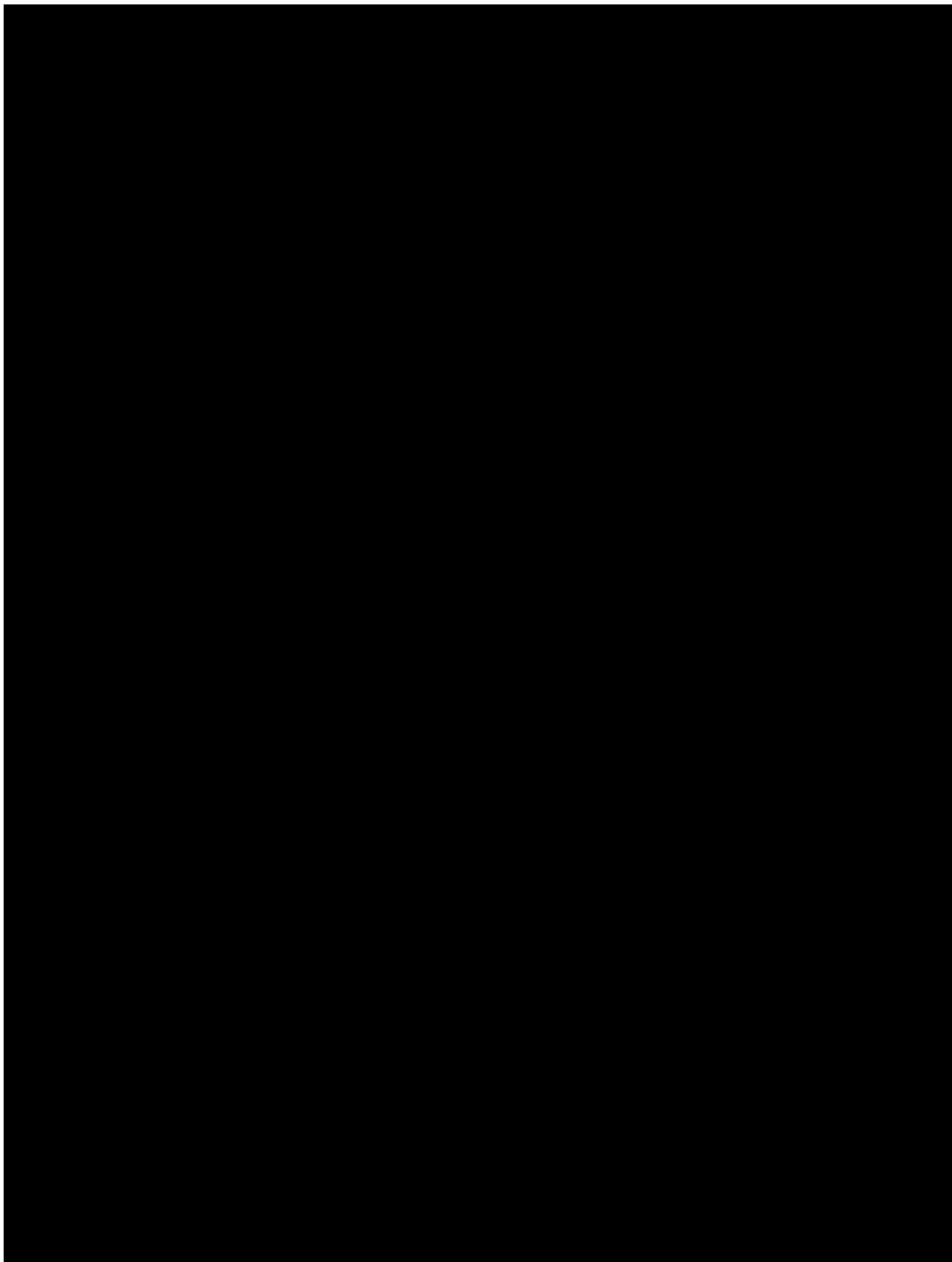
[REDACTED]

[REDACTED]









	<ul style="list-style-type: none"><li>• The exclusion criterion related to subjects who have received anabolic steroids was modified to allow the use of testosterone replacement therapy to treat hypogonadism. This change was incorporated as the testosterone replacement therapy to treat hypogonadism is part of the standard of care of male thalassemia subjects and it is not expected to affect the safety or efficacy of mitapivat in this study.</li></ul>
--	--