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

NCT Number: NCT04770779

Document Date: Protocol Amendment 2.0: 28-Sep-2022

CLINICAL STUDY PROTOCOL

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

Study AG348-C-018

Study Sponsor:	Agios Pharmaceuticals, Inc. 88 Sidney Street Cambridge, MA 02139-4169 USA Phone: 617-649-8600 Facsimile: 617-649-8618
Medical Director:	[redacted] Medical Director Clinical Development, Agios Pharmaceuticals, Inc.
IND Number:	[redacted]
NCT Number:	NCT04770779
EudraCT Number:	2021-000212-34
Approval Date:	Original Protocol (27 January 2021) Amendment 1 (21 July 2021) Amendment 2 (28 September 2022)

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.

[redacted]

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 Transfusion-Dependent Alpha- or Beta-Thalassemia (ENERGIZE-T)
Approval Date:	28 September 2022

Sponsor Signatory:

[redacted], MD
Medical Director

Name and Title (Printed) | [redacted] Sign/Date (dd/mmm/yyyy)

[redacted] Ph.D.
Sr. Director, Biostatistics

Name and Title (Printed) | [redacted] 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 Transfusion-Dependent Alpha- or Beta-Thalassemia (ENERGIZE-T)
Approval Date:	28 September 2022

Investigator Signatory:

Name and Title (Printed)	Signature	Date (dd Month yyyy)

PROTOCOL AMENDMENT SUMMARY OF CHANGES

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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, and PYRUKYND)

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

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

Phase of Development: 3

Objectives and Endpoints:

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

• To evaluate the pharmacokinetic and pharmacodynamic effects of mitapivat	• Plasma or blood concentrations and pharmacokinetic parameters of mitapivat and pharmacodynamic parameters, including adenosine triphosphate (ATP) and 2,3-diphosphoglycerate (2,3-DPG)

Study Design: Study AG348-C-018 is a Phase 3, double-blind, randomized, placebo-controlled, multicenter study evaluating the efficacy and safety of mitapivat versus placebo in adult subjects with α- or β-TDT followed by an Open-label Extension Period. Subject eligibility will be determined during the (up to 8 weeks) Screening Period.

The primary objective of the study is to compare the effect of mitapivat versus placebo on transfusion burden. Other secondary objectives include the evaluation of markers of iron overload, 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.

Subjects will continue to receive appropriate supportive care to manage symptoms and prevent secondary complications as clinically indicated and according to applicable guidelines. Dose modifications are permitted for excessive hemoglobin response and study drug–related AEs.

During the 48-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 240 subjects are planned to be randomized in this study.

Eligibility Criteria:

Inclusion Criteria:

1. ≥18 years of age at the time of providing informed consent.
2. Documented diagnosis of thalassemia (β-thalassemia with or without α-globin gene mutations, HbE/β-thalassemia, or α-thalassemia/HbH disease) based on DNA analysis from the subject's medical record. If this information is not available from the subject's medical record, DNA analysis can be performed by a local laboratory during the Screening Period. If a local laboratory is unable to perform the test, results from the comprehensive α- and β-globin genotyping performed by the study central laboratory can be used.
3. Transfusion dependent, defined as 6 to 20 RBC units transfused and a ≤6-week transfusion-free period during the 24-week period before randomization.
4. If taking hydroxyurea, the hydroxyurea dose must be stable for ≥16 weeks before randomization.
5. Women of childbearing potential (WOCBP) must be abstinent of sexual activities that may induce 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.

6. 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 ≥36 weeks before randomization.
5. Currently receiving treatment with hematopoietic stimulating agents; the last dose must have been administered ≥36 weeks before randomization.
6. History of malignancy (active or treated) ≤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 ≤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 ≥450 milliseconds (males) or ≥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 × upper limit of normal (ULN); unless due to hemolysis and hepatic iron deposition) and alanine aminotransferase >2.5 × ULN (unless due to hepatic iron deposition)
9. Estimated glomerular filtration rate <45 mL/min/1.73 m^2 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 ≥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) ≤6 months before providing informed consent and/or a major surgical procedure planned during the study.
15. Current enrollment or past participation (≤12 weeks before administration of the first dose of study drug or a time frame equivalent to 5 half-lives of the investigational treatment, 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 ≥5 days or a time frame equivalent to 5 half-lives (whichever is longer), or strong CYP3A4 inducers that have not been stopped for ≥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 ≥12 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 6.3 years. The duration of treatment is up to 6.0 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 geographical region 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:

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

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

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

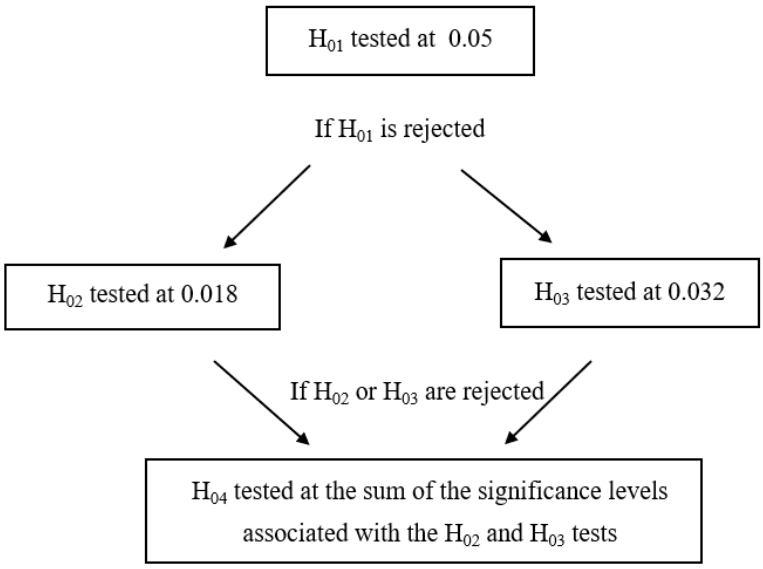
$H_{02}: p_{t2} - p_{c2} = 0$ vs $H_{12}: p_{t2} - p_{c2} \neq 0$

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

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

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

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



Sample Size Determination: Approximately 240 subjects will be randomized in a 2:1 ratio to receive mitapivat or placebo. With the planned sample size, the study will have 95% power to detect an increase in TRR rate from 12.5% in the placebo arm to 33.7% in the mitapivat arm at a 2-sided α-level of 0.05.

Populations for Analyses:

Population	Description
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 treatment. Subjects will be classified according to the treatment received. If a subject randomized to placebo receives at least 1 dose of mitapivat in the Double-blind Period, then the subject will be classified to the mitapivat arm.
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 TRR is defined as ≥50% reduction in transfused RBC units with a reduction of ≥2 units of transfused RBCs in any consecutive 12-week period through Week 48 compared with baseline. The proportion of subjects who achieve a TRR will be summarized for each treatment arm. The difference in TRR 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 TRR rate, along with the 95% CI and the 2-sided p-value will be provided. Subjects who are withdrawn from the study before Week 12 (Day 85) will be considered nonresponders.

Key Secondary Endpoints:

The key secondary endpoints are TRR2, TRR3, and TRR4. The same analysis method presented for the primary endpoint will be used for the analysis of TRR2, TRR3 and TRR4. Subjects who are withdrawn from the study before Week 24 will be considered nonresponders in TRR2. Subjects who are withdrawn from the study before Week 48 will be considered nonresponders in TRR3 and TRR4.

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 the 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), 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

	Screening Period		On-Treatment Period[1]																			
			Double-blind Period													Open-label Extension Period						
Study Visit Week:	--	--	1	4[3]	8[3]	12	16[3]	20	24	28[3]	32	36	40[3]	44[3]	48	52	56[3]	60[3]	72	84[3]	96	108[3]
Study Visit Day:	-56 to -8	-7 to -1	1	29	57	85	113	141	169	197	225	253	281	309	337	365	393	421	505	589	673	757
Study Visit Window (Days):	--	--	--	±12	±12	±12	±12	±12	±12	±12	±12	±12	±12	±12	±12	±12	±12	±12	±14	±14	±14	±14
Procedures:																						
Informed consent	X																					
Demographics	X																					
Eligibility	X		X																			
Documentation of thalassemia diagnosis[4]	X																					
Comprehensive α- and β-globin genotyping[5]	X																					
[redacted]																						
Medical, procedural, surgical, and disease history	X																					
Transfusion history[6]	X																					
Prior medications[7]	X																					

Table 1: Schedule of Assessments

	Screening Period		On-Treatment Period[1]																		
			Double-blind Period													Open-label Extension Period					
Study Visit Week:	--	--	**1**	**4**[3]	**8**[3]	**12**	**16**[3]	**20**	**24**	**28**[3]	**32**	**36**	**40**[3]	**44**[3]	**48**	**52**	**56**[3]	**60**[3]	**72**	**84**[3]	**96**	**108**[3]
Study Visit Day:	**-56 to -8**	**-7 to -1**	**1**	**29**	**57**	**85**	**113**	**141**	**169**	**197**	**225**	**253**	**281**	**309**	**337**	**365**	**393**	**421**	**505**	**589**	**673**	**757**
Study Visit Window (Days):	--	--	--	**±12**	**±12**	**±12**	**±12**	**±12**	**±12**	**±12**	**±12**	**±12**	**±12**	**±12**	**±12**	**±12**	**±12**	**±12**	**±14**	**±14**	**±14**	**±14**
Hepatitis and HIV screening[8]	X																					
Pregnancy testing (WOCBP only)[9]	X		X																			
Randomization[10]			X																			
BMD by DXA scan[11]	X														X						X	
[redacted]																						
Physical examination			X												X							
Vital signs, weight, and height[13]	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
12-lead ECG[14]	X																					
Hematology[15]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Clinical chemistry[16]	X		X	X		X			X			X			X	X		X	X	X	X	X
Liver tests [redacted][17]	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Lipid panel[18]	X														X						X	

Table 1: Schedule of Assessments

	Screening Period		On-Treatment Period[1]																		
			Double-blind Period													Open-label Extension Period					
Study Visit Week:	--	--	**1**	**4**[3]	**8**[3]	**12**	**16**[3]	**20**	**24**	**28**[3]	**32**	**36**	**40**[3]	**44**[3]	**48**	**52**	**56**[3]	**60**[3]	**72**	**84**[3]	**96**	**108**[3]
Study Visit Day:	**-56 to -8**	**-7 to -1**	**1**	**29**	**57**	**85**	**113**	**141**	**169**	**197**	**225**	**253**	**281**	**309**	**337**	**365**	**393**	**421**	**505**	**589**	**673**	**757**
Study Visit Window (Days):	--	--	--	**±12**	**±12**	**±12**	**±12**	**±12**	**±12**	**±12**	**±12**	**±12**	**±12**	**±12**	**±12**	**±12**	**±12**	**±12**	**±14**	**±14**	**±14**	**±14**
Coagulation tests[19]		X	X																			
Sex hormones[20]			X			X			X						X			X	X		X	
[redacted]																						
Iron panel[21]		X	X						X						X				X		X	
[redacted]																						
Blood sample for pharmacodynamic analysis		X[23]	X[23]			X[24]			X[24]			X[25]										
Blood sample for pharmacokinetic analysis						X[24]			X[24]			X[25]										
[redacted]																						

Table 1: Schedule of Assessments

	Screening Period		On-Treatment Period[1]																		
			Double-blind Period													Open-label Extension Period					
Study Visit Week:	--	--	1	4[3]	8[3]	12	16[3]	20	24	28[3]	32	36	40[3]	44[3]	48	52	56[3]	60[3]	72	84[3]	96	108[3]
Study Visit Day:	-56 to -8	-7 to -1	1	29	57	85	113	141	169	197	225	253	281	309	337	365	393	421	505	589	673	757
Study Visit Window (Days):	--	--	--	±12	±12	±12	±12	±12	±12	±12	±12	±12	±12	±12	±12	±12	±12	±12	±14	±14	±14	±14
[redacted]																						
Study drug administration[28]			X (continuous)																			
AEs and SAEs[29]	X (continuous)																					
Concomitant medications, procedures, and surgeries[30]			X (continuous)																			
On-treatment transfusion record[31]			X (continuous)																			

Abbreviations: 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_2 = carbon dioxide; DXA = dual-energy x-ray absorptiometry; ECG = electrocardiogram; [redacted]; EOS = End of Study; [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; [redacted] INR = international normalized ratio; LCMS = liquid chromatography-mass spectrometry; [redacted]; LDL-C = low-density lipoprotein cholesterol; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; [redacted] RBC = red blood cell; RDW-CV = red cell distribution width coefficient of variation; SAE = serious adverse event;

T_0 = transfusion zero; Thal = thalassemia; [redacted] WBC = white blood cell; WOCBP = women of childbearing potential.

Notes: [redacted] **All blood samples must be collected before study drug administration and before an RBC transfusion, including T_0, if an RBC transfusion is scheduled on the same day (see Section 8.7).**

[1] 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.

[2] The first dose of study drug must be administered within 7 days after T_0. Transfusion 0 is defined as the last RBC transfusion that was administered before randomization.

[3] 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. 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).

[4] Documentation of thalassemia diagnosis by 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 should be performed during the Screening Period (see Section 8.2).

[5] A blood sample will be collected for comprehensive α- and β-globin genotyping. Results of this analysis are not required for eligibility.

[6] Transfusion history will be recorded for the 24-week period before randomization. Transfusion history includes T0. A pretransfusion Hb concentration threshold will be determined for each subject based on transfusion history (see Section 8.7). Definition of an RBC unit and information to be recorded for each RBC transfusion is also in this section.

[7] Prior medications are defined as those administered any time ≤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.

[8] 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.

[9] 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.

[10] Randomization can occur up to 48 hours before administration of the first dose of study drug on Day 1. Randomization must not occur until T0 has been completed.

[11] DXA scan of the hip and spine.

[redacted]

[13] 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.

[14] 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.

[15] 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).

[16] Clinical chemistry includes albumin, BUN or urea, total calcium, CO_2 or bicarbonate, chloride, creatinine, [redacted] magnesium, phosphorus, potassium, sodium, total protein, and uric acid.

[17] Liver tests include ALP, ALT/SGPT, AST/SGOT, and bilirubin (direct/conjugated, indirect/unconjugated, and total). [redacted]

[18] Lipid panel includes nonfasting HDL-C, LDL-C, total cholesterol, and triglycerides.

[19] Coagulation tests include fibrinogen, aPTT, and INR.

[20] 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.

[21] Iron panel includes iron, serum ferritin, transferrin, transferrin saturation, and total iron binding capacity.

[redacted]

[23] A blood sample for pharmacodynamic analysis will be collected at T0 (up to 3 days before transfusion) and on Day 1 (≤60 min predose).

[24] Blood samples for pharmacokinetic and pharmacodynamic analysis will be collected at Week 12 (≤60 min predose) and Week 24 (≤60 min predose). If an RBC transfusion takes place during this visit, the blood samples for pharmacokinetic and pharmacodynamic analysis should be collected before the RBC transfusion.

[25] Blood samples for pharmacokinetic and pharmacodynamic analysis will be collected at Week 36 (≤60 min predose, and 0.5 hr [±5 min], 1 hr [±5 min], 3 hr [±15 min], 5 hr [±15 min], and 7 hr [±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.

[redacted]

[redacted]

[28] 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 48 visit. The first dose of open-label mitapivat will be administered on the evening of the Week 48 visit.

[29] Adverse events and SAEs will be collected throughout the study.

[30] 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).

[31] During the study, the subject's RBC transfusion schedule will be modified according to the guidance in Section 8.7. Information to be recorded for each transfusion is also in this section.

Table 2: Schedule of Assessments (Continued)

	Open-label Extension Period[1]																	Safety Follow-up[2]
Study Visit Week:	**120**	**132**	**144**	**156[3]**	**168**	**180[3]**	**192**	**204[3]**	**216**	**228[3]**	**240**	**252[3]**	**264**	**276[3]**	**288**	**300[3]**	**312[4]**	**28 Days After Last Dose/ EOS**
Study Visit Day:	**841**	**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**	**2,101**	**2,185**	
Study Visit Window (Days):	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**	**+4**
Procedures:																		
Pregnancy testing (WOCBP only)[5]																		X
BMD by DXA scan[6]			X				X				X				X			
[redacted]																		
Physical examination																	X	X
Vital signs and weight[8]	X	X	X		X		X		X		X		X		X		X	X
Hematology[9]	X	X	X		X		X		X		X		X		X		X	X
Clinical chemistry[10]	X	X	X		X		X		X		X		X		X		X	X
Liver tests [redacted][11]	X	X	X		X		X		X		X		X		X		X	X
Lipid panel[12]			X				X				X				X		X	X
Sex hormones[13]	X		X		X		X		X		X		X		X		X	X
[redacted]																		
Iron panel[14]	X		X		X		X		X		X		X		X		X	
[redacted]																		

Table 2: Schedule of Assessments (Continued)

	Open-label Extension Period[1]																	Safety Follow-up[2]
Study Visit Week:	**120**	**132**	**144**	**156[3]**	**168**	**180[3]**	**192**	**204[3]**	**216**	**228[3]**	**240**	**252[3]**	**264**	**276[3]**	**288**	**300[3]**	**312[4]**	**28 Days After Last Dose/ EOS**
Study Visit Day:	**841**	**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**	**2,101**	**2,185**	
Study Visit Window (Days):	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**	**±14**	**+4**
Procedures:																		
[redacted]																		
Study drug administration[17]	X (continuous)																X	
AEs and SAEs[18]	X (continuous)																	X
Concomitant medications, procedures, and surgeries[19]	X (continuous)																	X
On-treatment transfusion record[20]	X (continuous)																	

Abbreviations: AE = adverse event; ALP = alkaline phosphatase; ALT = alanine aminotransferase; AST = aspartate aminotransferase; BMD = bone mineral density; BUN = blood urea nitrogen; CO_2 = carbon dioxide; DXA = dual-energy x-ray absorptiometry; [redacted] EOS = End of Study; Hb = hemoglobin; [redacted] HCT = hematocrit; HDL-C = high-density lipoprotein cholesterol; [redacted]; LDL-C = low-density lipoprotein cholesterol; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; MCV = mean corpuscular volume; [redacted] RBC = red blood cell; RDW-CV = red cell distribution width coefficient of variation; SAE = serious adverse event; [redacted] WBC = white blood cell; WOCBP = women of childbearing potential.

Notes: [redacted] **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).**

[1] 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.

[2] 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).

[3] 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. These visits (Investigator Assessment only) will be conducted by the Investigator (or designee) (see Section 8).
[4] After completing the study visit at Week 312, subjects should begin the dose taper.
[5] 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.
[6] DXA scan of the hip and spine.
[redacted]
[8] 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.
[9] 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).
[10] Clinical chemistry includes albumin, BUN or urea, total calcium, CO_2 or bicarbonate, chloride, creatinine, [redacted] magnesium, phosphorus, potassium, sodium, total protein, and uric acid.
[11] Liver tests include ALP, ALT/SGPT, AST/SGOT, and bilirubin (direct/conjugated, indirect/unconjugated, and total).[redacted]
[12] Lipid panel includes nonfasting HDL-C, LDL-C, total cholesterol, and triglycerides.
[13] 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.
[14] Iron panel includes iron, serum ferritin, transferrin, transferrin saturation, and total iron binding capacity.
[redacted]
[17] 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.
[18] Adverse events and SAEs will be collected throughout the study.
[19] 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).
[20] During the study, the subject's RBC transfusion schedule will be modified according to the guidance in Section 8.7. Definition of an RBC unit and information to be recorded for each transfusion is also in this section.

2. INTRODUCTION

2.1. Background

2.1.1. Thalassemia

The thalassemias are a group of disorders in which the normal ratio of α- to β-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 α- and β-thalassemias, which result from the defective synthesis of the α- or β-globin chains of adult Hb (HbA). The second group includes structural Hb variants, such as sickle cell hemoglobin (HbS), HbC, and HbE (Musallam et al, 2013; Taher et al, 2017). Alpha-globin aggregates (found in β-thalassemia) readily precipitate, which disrupts the red blood cell (RBC) membrane and results in oxidative stress. Beta-globin tetramers (found in α-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 α- and β-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 β-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 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 is 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-018) is focused on a population of subjects with TDT, the following sections focus more specifically on disease characteristics and available treatments for patients with TDT.

2.1.1.1. Transfusion-Dependent Thalassemia

Patients with TDT include those with β-thalassemia major or severe forms of β-thalassemia intermedia, HbE/β-thalassemia, or α-thalassemia/HbH disease. Patients with β-thalassemia major present in early childhood and require life-long regular RBC transfusions for survival. Patients with severe forms of β-thalassemia intermedia, HbE/β-thalassemia, or α-thalassemia/HbH disease can range from requiring chronic transfusions in early childhood to developing transfusion dependence later in adulthood (Cappellini et al, 2014).

Over time, anemia with or without transfusions can lead to widespread organ damage and dysregulated compensatory mechanisms. Regular transfusions are associated with iron overload; however, in the absence of RBC transfusions, iron overload can also be caused by chronic hemolysis, ineffective erythropoiesis, and hypoxia, ultimately leading to increased intestinal iron absorption. Clinical consequences of thalassemia include splenomegaly, extramedullary expansion of hematopoiesis, bone deformities, osteoporosis, and potentially, fractures (Angastiniotis et al, 2013; Taher et al, 2011; Taher, Musallam, et al, 2015). The death rate of adults with TDT remains higher than that of the general United States (US) population (Fung et al, 2007), and high morbidity and mortality persist (Borgna-Pignatti et al, 2004; Modell et al, 2000). Access to transfusions and specialized supportive care varies by region and is challenging particularly in resource-constrained countries, creating a gap in access for some patients (Betts et al, 2020; Cappellini et al, 2018; Cappellini et al, 2014; Vichinsky, 2012; Weatherall, 2011).

2.1.1.2. Transfusion-Dependent Thalassemia Treatment

Besides RBC transfusions, most patients with TDT receive supportive care, which may include iron chelation therapy and splenectomy (Cappellini et al, 2014). Chronic RBC transfusions are associated with iron overload, transfusion reactions, infections, and alloimmunization. Patients with TDT often require treatment with iron chelators to avoid long-term complications from tissue iron deposition, which may specifically cause hepatic, cardiac, and endocrine toxicity. Iron overload–related cardiac events are the leading cause of mortality in patients with TDT. These therapies have limitations in efficacy and safety, and access and adherence to treatment are not uniform (Cappellini 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 and infections after splenectomy (Cappellini et al, 2000). Despite recent improvements in the clinical management of patients with TDT, including increased access to blood supply, reduction of transfusion-associated infections, iron chelation therapy, and advanced imaging to evaluate hepatic and cardiac iron concentrations, the complication rate remains high (Borgna-Pignatti et al, 2004; Modell et al, 2000).

Recently, targeted therapies have become available for patients with TDT, but they either have substantial restrictions associated with their administration or address only a subpopulation of patients with β-thalassemia. Hematopoietic stem cell transplantation (HSCT) and gene therapy are potentially curative treatments (Angelucci et al, 2014; Schuessler-Lenz et al, 2020). Zynteglo® (autologous CD34+ cells encoding βA-T87Q-globin gene) was approved in the European Union in 2019 for patients 12 years and older with β-thalassemia (non-(β^0/β^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® (luspatercept), an activin receptor fusion protein, was approved in the US in 2019 and the European Union in

2020 to treat anemia in adults with β-thalassemia who require regular RBC transfusions (Reblozyl (luspatercept-aamt) USPI, 2019).

Mitapivat offers 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 × 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 ≥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 have been 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 α- or β-NTDT (EudraCT 2018-002217-35; ClinicalTrials.gov NCT03692052). Subjects with a baseline Hb concentration ≤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 α-thalassemia and 15 with β-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 α- and β-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 α-thalassemia and 11 with β-thalassemia) met the primary endpoint of Hb response, defined as an increase in Hb concentration of ≥1.0 g/dL from baseline at ≥1 assessment between Week 4 and Week 12. The time to the first Hb response (ie, ≥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 (±standard deviation [SD]) increase from baseline in Hb concentration over a continuous 12-week interval (Week 4 to Week 12) was 1.15±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 ≥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 ≥1.0 g/dL from baseline at ≥1 assessment from Week 16 to Week 24.

In the 19 subjects who completed 24 weeks of mitapivat treatment, the mean (±SD) increase from baseline in Hb concentration over a continuous 12-week interval (Week 12 to Week 24) was 1.3±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 α-thalassemia and β-thalassemia. Fourteen of 20 subjects (70.0%; 4 with α-thalassemia, 10 with β-thalassemia) had a ≥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±27.536 μmol/L, 269.35±113.055 U/L, and 870.53±2,659.950 IU/L, respectively. Over a continuous 12-week interval (Week 12 to Week 24), the mean (±SD) change from baseline in indirect bilirubin, LDH, and erythropoietin was -17.72±18.861 μmol/L, -40.68±123.699 U/L, and -627.00±2404.460 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 α- and β-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 ≥20% or ≥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. While subjects with TDT were not evaluated in this study, the study

included 4 subjects with a baseline Hb concentration below 7 g/dL, a threshold that renders patients eligible for chronic transfusions based on current treatment guidelines (Cappellini et al, 2014), with 3 of them achieving a ≥1.0 g/dL increase in average Hb concentration from Week 12 through Week 24 compared with baseline. The clinical benefit observed in Study AG348-C-010 is considered relevant to subjects with transfusion-dependence as well, because the pathophysiology of the disease is the same regardless of transfusion status (see Section 2.1.1). The goal for thalassemia treatment in general, independent of transfusion status, is to decrease the ineffective erythropoiesis and hemolysis and subsequently improve chronic anemia to prevent downstream clinical sequelae (Taher et al, 2018). PKR activation by mitapivat (see Section 2.1.2) in AG348-C-010 resulted in improvement in hemolytic and erythropoietic markers (see Section 2.1.2.3.2). Therefore, the data from the Phase 2 study are supportive of the development of mitapivat in patients with TDT.

A similar approach was taken in the development of mitapivat for patients with PK deficiency. The knowledge gained from Study AG348-C-003 in a non-transfusion dependent PK deficiency population allowed for the development of 2 Phase 3 studies, one in patients who do not require regular transfusions (AG348-C-006) and the other in patients who require regular transfusions (AG348-C-007). Preliminary review of safety data from Study AG348-C-007 has not demonstrated a change in the safety profile from that observed in Studies AG348-C-006 or AG348-C-003.

Study AG348-C-018 is a Phase 3, double-blind, randomized, placebo-controlled, multicenter study designed to demonstrate the clinical efficacy and safety of mitapivat in subjects with α- or β-TDT. The primary objective of the study is to compare the effect of mitapivat versus placebo on transfusion burden. Secondary objectives include the evaluation of markers of iron overload as well as safety and pharmacokinetic and pharmacodynamic parameters.

Although recently approved therapies offer new options for patients with β-TDT, many patients do not respond, luspatercept is not approved for patients with α-thalassemia, and thus is ample reason to test mitapivat based on data from the Phase 2 study. Luspatercept, indicated for β-TDT, requires subcutaneous administration every 3 weeks by a health-care provider and includes a warning for hypertension and thromboembolic events (Reblozyl (luspatercept-aamt) USPI, 2019). In addition to luspatercept, betibeglogene autotemcel (EMA, 2019) and HSCT are potential treatment options for a limited group of patients with β-TDT, but these options require access to specialized treatment centers and are also associated with substantial clinical risks.

Therefore, a serious unmet medical need still exists for patients with transfusions-dependent α- or β-thalassemia. Mitapivat, has the potential to improve the transfusion burden in patients with TDT with the added benefit 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. While some patients with thalassemia needing transfusions may have more limited access to these in certain regions of the world because of pandemic restrictions or disruptions, the Investigator can exclude patients via the last exclusion criterion (#19), if this situation is relevant to the patient and if this creates a medical condition that does not allow for safe participation. 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.

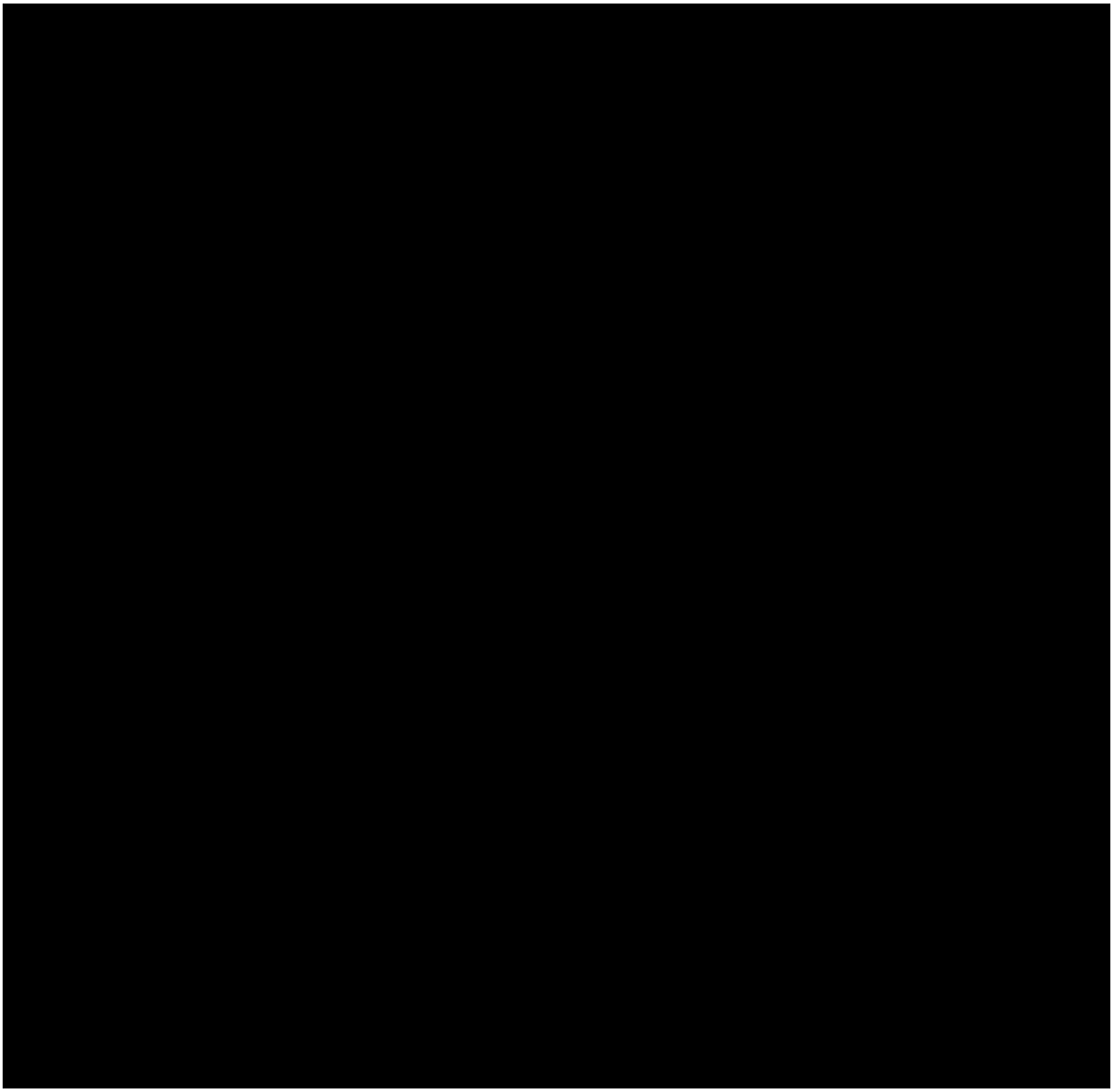
Additionally, the benefit-risk assessment for mitapivat in clinical studies is in the current edition of the IB.

3. OBJECTIVES AND ENDPOINTS

The study objectives and endpoints are provided in Table 3.

Table 3: Objectives and Endpoints

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



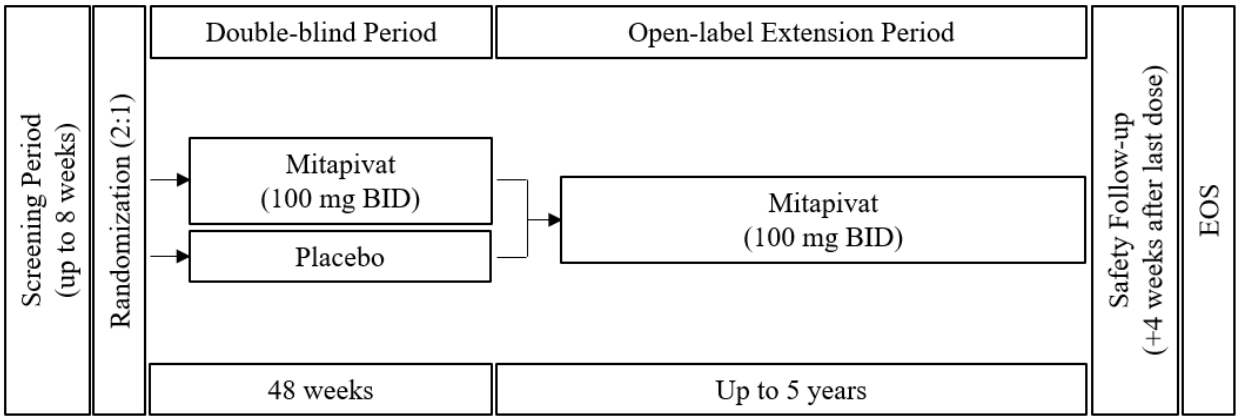
4. STUDY DESIGN

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 α- or β-TDT followed by an Open-label Extension Period. Approximately 240 subjects are planned to be randomized in this study. This multicenter study will be conducted internationally.

An overview of the study design is presented in Figure 1. Study assessments will be performed at the time points in the Schedules of Assessments (see Section 1.2).

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 (see 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 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 thalassemia genotype and geographical region (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 48 weeks. During the study, subjects will receive RBC transfusions according to the guidance in Section 8.7. Study visits will occur approximately every 4 weeks for efficacy and safety assessments and will be aligned, if possible, to occur with the subject's routine transfusion schedule. 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. Study visits will continue to be aligned, if possible, with the subject's routine transfusion schedules and modified according to the guidance in Section 8.7. 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 6.3 years. The duration of treatment is up to 6.0 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-018 is a Phase 3, double-blind, randomized, placebo-controlled, multicenter study designed to demonstrate the clinical efficacy and safety of mitapivat in subjects with α- or β-TDT 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 widely available and disease-specific, approved treatments for patients with α-TDT and patients with β-TDT.

The primary endpoint, in conjunction with the key secondary endpoints, will allow for a consistent and reliable measurement of the intended effect of mitapivat on transfusion burden.

Eligible subjects will be randomized 2:1 to receive mitapivat or matched placebo, providing an increased chance to receive active study drug. Placebo administration will be limited to the 48-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 study, an Independent Data Monitoring Committee (IDMC) will be responsible for ongoing monitoring of the safety of subjects.

Randomization will be stratified by thalassemia genotype to ensure that the comparison reflects a balanced allocation of subjects to mitapivat or placebo within each genotype, and geographical region to allow for a characterization of the effect across areas with regional care variations.

Additionally, during the study, subjects will continue to receive appropriate supportive care to manage symptoms and prevent secondary complications as clinically indicated and according to applicable guidelines. Study visit windows were developed to facilitate alignment with the subjects' routine transfusion schedules for flexibility and convenience.

Overall, the study design will allow for an evaluation of the efficacy and safety of mitapivat in subjects with α- or β-TDT 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 a Hb responses. 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, and the effect of mitapivat on Hb formation rate was described by a maximum effect 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 and mitapivat exposure. [redacted]

[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%).

[redacted]

In summary, 100 mg BID mitapivat has been shown to have a manageable safety profile for chronic dosing. [redacted] Based on these findings, a dose of 100 mg BID of mitapivat has been selected for subjects with NTDT (Study AG348-C-017). Because the underlying molecular and pathophysiological basis for TDT and NTDT are similar, the same dose (100 mg BID of mitapivat) has been selected for this current 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. ≥18 years of age at the time of providing informed consent.
2. Documented diagnosis of thalassemia (β-thalassemia with or without α-globin gene mutations, HbE/β-thalassemia, or α-thalassemia/HbH disease) based on DNA analysis from the subject's medical record. If this information is not available from the subject's medical record, DNA analysis can be performed by a local laboratory during the Screening Period. If a local laboratory is unable to perform the test, results from the comprehensive α- and β-globin genotyping performed by the study central laboratory can be used (see Section 8.2 and Section 8.9).
3. Transfusion dependent, defined as 6 to 20 RBC units transfused and a ≤6-week transfusion-free period during the 24-week period before randomization.
4. If taking hydroxyurea, the hydroxyurea dose must be stable for ≥16 weeks before randomization.
5. Women of childbearing potential (WOCBP) must be abstinent of sexual activities that may induce 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).
6. 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 ≥36 weeks before randomization.
5. Currently receiving treatment with hematopoietic stimulating agents; the last dose must have been administered ≥36 weeks before randomization.
6. History of malignancy (active or treated) ≤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 ≤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 ≥450 milliseconds (males) or ≥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 × upper limit of normal (ULN); unless due to hemolysis and hepatic iron deposition) and alanine aminotransferase (ALT) >2.5 × ULN (unless due to hepatic iron deposition)
9. Estimated glomerular filtration rate <45 mL/min/1.73 m^2 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 ≥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) ≤6 months before providing informed consent and/or a major surgical procedure planned during the study.
15. Current enrollment or past participation (≤12 weeks before administration of the first dose of study drug or a time frame equivalent to 5 half-lives of the investigational treatment, 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 ≥5 days or a time frame equivalent to 5 half-lives (whichever is longer), or strong CYP3A4 inducers that have not been stopped for ≥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 ≥12 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 ≤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 geographical region (North America and Europe; Asia-Pacific; and Rest of World) 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 ≤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.

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 ≥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 prohibited during the study.

Concomitant administration of strong CYP3A4 inducers is prohibited during the study. These therapies must be discontinued ≥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 ≥36 weeks before randomization.

Concomitant administration of erythropoietin is prohibited during the study; the last dose must have been administered ≥36 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 12 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 are permitted for excessive Hb response and study drug–related AEs. 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 Result Test 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.

During the study, Investigators will monitor changes in Hb concentration levels with study drug administration and modify the subject's RBC transfusion schedule according to guidelines in Section 8.7. If a subject has not required an RBC transfusion for ≥4 weeks and their Hb

concentration is greater than the ULN (by sex), dose modification guidelines for excessive Hb response in Table 6 should be followed.

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	Reduce to 100 mg QD. • If Hb concentration decreases to ≤ULN (by sex) within 28 days of the dose reduction to 100 mg QD, increase to 100 mg BID. • If Hb concentration does not decrease to <ULN (by sex) within 28 days of the dose reduction to 100 mg QD, contact the Medical Monitor.

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 are provided in Table 7.

Table 7: Dose Modifications for Study Drug–Related Adverse Events

AE Severity[1]	Study Drug Modification	
Grade 1 (mild)	None required	
Grade 2 (moderate)	None required	
Grade 3 (severe)	First occurrence	Reduce to 100 mg QD. • If the event resolves to Grade ≤2 within 28 days of the dose reduction, increase to 100 mg BID. • If the event does not resolve to Grade ≤2 within 28 days of the dose reduction, discontinue study drug.
	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.

[1] Adverse event severity assessed as mild, moderate, or severe, or according to National Cancer Institute Common Terminology Criteria for Adverse Events.

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 participation in the study, 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 1.2. 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 (PRO) 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 156 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 α- and β-globin genotyping during the Screening Period for analysis by the study central laboratory. Results from the comprehensive α-and β-globin genotyping are not required for eligibility if conclusive DNA analysis results 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 subjects who do not have a β^0 mutation at both alleles of the β-globin gene (non-[β^0/β^0]), including subjects with HbE/β-thalassemia and α-thalassemia/HbH disease; or subjects who have a β^0 mutation at both alleles of the β-globin gene (β^0/β^0). 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 α- and β-globin genotyping. If the magnetic resonance imaging (MRI) 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 provided in Table 8.

Table 8: Clinical Laboratory Efficacy Assessments

Assessment	Parameters
Anemia	Hb [redacted]
[redacted]	[redacted]
[redacted]	[redacted] soluble transferrin receptor
Iron metabolism	Iron, serum ferritin, transferrin, hepcidin, TSAT, and TIBC[1]

Abbreviations: Hb = hemoglobin; [redacted]; [redacted]; TIBC = total iron binding capacity; TSAT = transferrin saturation.
Note: Study-required clinical laboratory efficacy assessment samples will be analyzed by a central laboratory.
[1] TSAT and TIBC will be calculated.

Clinical laboratory safety assessment parameters are provided in Section 8.6.3.

[redacted]

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 provided in Table 9.

Table 9: Clinical Laboratory Safety Assessments

Assessment	Parameters
Hematology	• 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)
Clinical chemistry	• Albumin, BUN or urea, total calcium, CO_2 or bicarbonate, chloride, creatinine, [redacted] magnesium, phosphorus, potassium, sodium, total protein, and uric acid
Liver tests	• Liver tests include ALP, ALT/SGPT, AST/SGOT, and bilirubin (direct/conjugated, indirect/unconjugated, and total). [redacted]
Coagulation tests	• Fibrinogen, aPTT, and INR
Lipid panel	• Nonfasting HDL-C, LDL-C, total cholesterol, and triglycerides
Sex hormones	• Nonfasting estradiol, estrone, and testosterone (total and free)
Spot urine sample	• Albumin to creatinine ratio
Screening virology	• HBsAg, HCV Ab (positive results for HCV Ab testing require confirmation by a test for HCV-RNA), HIV-1 Ab, and HIV-2 Ab
Screening FSH and serum hCG	• FSH (for women in the absence of 12 months of amenorrhea to confirm non–reproductive potential) and a serum (hCG) pregnancy (for WOCBP)

Abbreviations: Ab = antibody; ALP = alkaline phosphatase; ALT = alanine aminotransferase; aPTT = activated partial thromboplastin time; AST = aspartate aminotransferase; BUN = blood urea nitrogen; CO2 = 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; [redacted]; 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 provided in Section 8.5.1.

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

8.7. Red Blood Cell Transfusions and Transfusion Modifications

The first dose of study drug must be administered within 7 days after Transfusion 0. Transfusion 0 is defined as the last RBC transfusion that was administered before randomization. Randomization must not occur until T0 has been completed.

[redacted] All blood samples must be collected before administration of the study drug and before an RBC transfusion, including Transfusion 0, if an RBC transfusion is scheduled on the same day.

Transfusion history will be recorded for the 24-week period before randomization. Transfusion history includes T0. 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.

A pretransfusion Hb threshold will be determined for each subject based on transfusion history. The pretransfusion Hb threshold is defined as the mean of all documented pretransfusion Hb concentration values recorded for the RBC transfusions administered during the 24-week transfusion history.

During the study, Investigators will monitor changes in Hb concentration and modify the subject's RBC transfusion schedule according to the guidelines in Table 10. Study drug dose modification guidance for excessive Hb response is provided in Section 6.7.1.1.

Subjects may receive RBC transfusions at the discretion of the Investigator for acute symptoms related to anemia or other comorbidities requiring immediate treatment (eg, infection or cardiopulmonary compromise).

Table 10: Red Blood Cell Transfusion Modification Guidance

Pretransfusion Hb Concentration[1]	RBC Transfusion Modification
Hb concentration ≥1 g/dL above the pretransfusion Hb threshold and ≤ULN (by sex)	Delay RBC transfusion or decrease the number of RBC units to be transfused.
>ULN (by sex)	Delay RBC transfusion. • Recheck Hb concentration within 28 days. • If Hb concentration remains >ULN (by sex) for ≥4 weeks, in the absence of RBC transfusions, modify the study drug dose as described in Section 6.7.1.1.

Abbreviations: Hb = hemoglobin; RBC = red blood cell; ULN = upper limit of normal.
[1] The pretransfusion Hb concentration is the Hb concentration obtained before a planned transfusion event to guide its transfusion prescription, irrespective of whether a transfusion is subsequently delivered or deferred.

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.

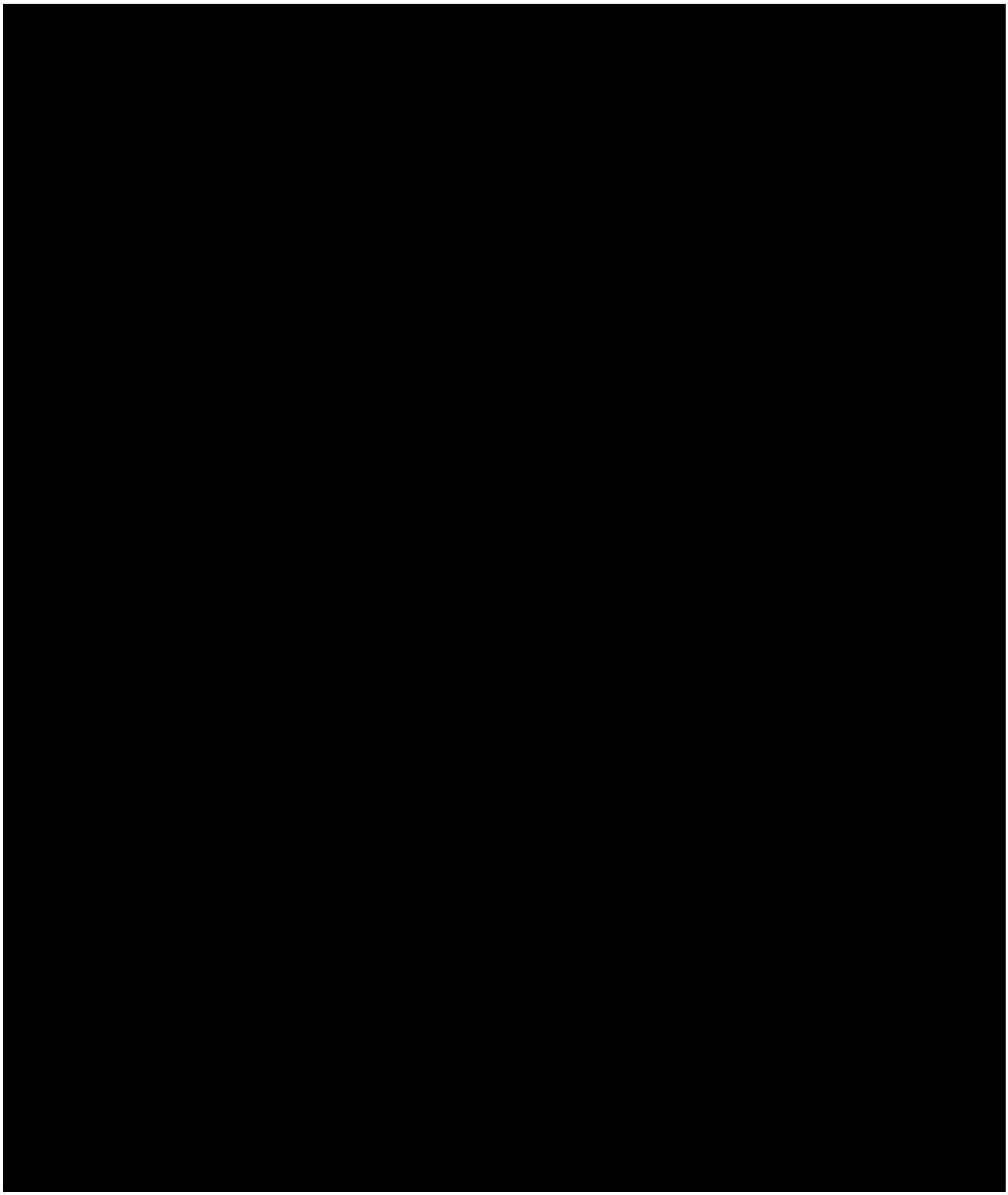
Pharmacokinetic samples will be analyzed using a validated liquid chromatography-tandem mass spectrometry (LC-MS/MS) method. Plasma pharmacokinetic parameters will be computed from measured plasma concentrations and using actual sample collection times.

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

Samples may be retained for a maximum of 5 years (or according to local regulations) after the end of the study at a facility selected by the Sponsor for further analysis of pharmacokinetic or pharmacodynamic responses to mitapivat for subjects who consent to this optional exploratory research in the ICF (see Section 11.2).

8.9. Genetics

Blood samples collected for comprehensive α- and β-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).



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 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 assessments (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.

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. Pregnancies 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 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.

10. STATISTICAL CONSIDERATIONS

10.1. Statistical Hypotheses

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

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

where p_{t1} and p_{c1} are the proportion of subjects achieving a transfusion reduction response (TRR), defined as ≥50% reduction in transfused RBC units with a reduction of ≥2 units of transfused RBCs in any consecutive 12-week period through Week 48 compared with baseline, in the mitapivat arm and placebo arm, respectively.

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

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

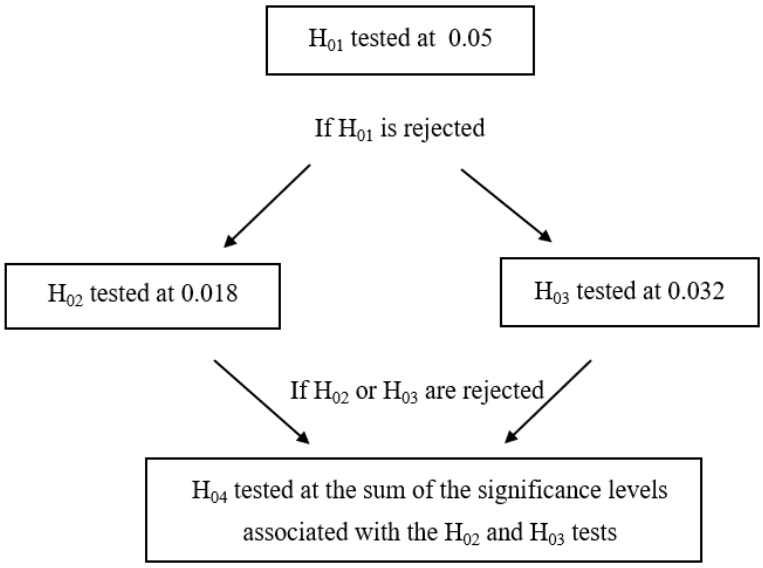
$H_{03}: p_{t3} - p_{c3} = 0$ vs $H_{13}: p_{t3} - p_{c3} \neq 0$

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

- where p_{t2} and p_{c2} are the proportion of subjects achieving a ≥50% reduction in transfused RBC units in any consecutive 24-week period through Week 48 compared with baseline (referred to as TRR2) in the mitapivat arm and placebo arm, respectively.
- p_{t3} and p_{c3} are the proportion of subjects achieving a ≥33% reduction in transfused RBC units from Week 13 through Week 48 compared with baseline (referred to as TRR3) in the mitapivat arm and placebo arm, respectively.
- p_{t4} and p_{c4} are the proportion of subjects achieving a ≥50% reduction in transfused RBC units from Week 13 through Week 48 compared with baseline (referred to as TRR4) in the mitapivat arm and placebo arm, respectively.

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

Figure 2: Statistical Testing Strategy



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

The study will have met its primary objective if the primary endpoint is statistically significant at the time of the final analysis at the 2-sided α-level of 0.05.

10.2. Sample Size Determination

Approximately 240 subjects will be randomly assigned in a 2:1 ratio to receive study drug (mitapivat or placebo). With the planned sample size, the study will have 95% power to detect an increase in TRR rate from 12.5% in the placebo arm to 33.7% in the mitapivat arm at a 2-sided α-level of 0.05.

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

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 treatment. Subjects will be classified according to the treatment received. If a subject randomized to placebo receives at least 1 dose of mitapivat in the Double-blind Period, then the subject will be classified to the mitapivat arm.
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

Baseline transfusion burden is defined as the total number of RBC units transfused during the 24-week period before randomization for subjects randomized and not dosed or before the start of study treatment for subjects randomized and dosed. Baseline transfusion burden will be standardized to match the postbaseline assessment duration specified for each endpoint associated with reduction of transfusion burden from baseline.

For other efficacy endpoints (markers of iron overload and iron metabolism), baseline is defined as the last assessment before Transfusion 0. 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.

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.

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 measurements) will include data from both scheduled and unscheduled visits.

10.4.2. Primary Endpoint

10.4.2.1. Transfusion Reduction Response

The primary endpoint of TRR (as defined in Section 10.1) will be tested using the Mantel-Haenszel stratum weighted method adjusting for randomization stratification factors. Subjects who are withdrawn from the study before Week 12 (Day 85) will be considered nonresponders. The proportion of subjects who achieved a TRR will be summarized for each treatment arm. The adjusted difference in TRR rate between the mitapivat arm and placebo arm, along with the 95% CI and the 2-sided p-value, will be provided.

10.4.2.2. Sensitivity Analyses

TRR will be summarized using the methodology described for the primary analysis but based on PPS.

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

10.4.2.3. Subgroup Analyses

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

Transfusion reduction 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

The key secondary endpoints (TRR2, TRR3, and TRR4, as defined in Section 10.1) will be tested using the Mantel-Haenszel stratum weighted method adjusting for randomization stratification factors. The response rate based on different definitions of response will be summarized for each treatment arm. The adjusted differences in response rate between the mitapivat arm and placebo arm, along with the 95% CI and the 2-sided p-value, will be provided.

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

10.4.3.2. Other Secondary Efficacy Endpoints

The change from baseline in transfused RBC units from Week 13 through Week 48 will be summarized. The frequency of subjects within the following percentage reduction categories will also be provided: <0, 0 to <20, 20 to <33, 33 to <50, and ≥50%.

The frequency of subjects achieving transfusion independence will be summarized for each treatment arm. Transfusion independence is defined as transfusion-free for ≥8 consecutive weeks

through Week 48. The adjusted difference in the proportion of subjects who achieve transfusion independence between the mitapivat arm and the placebo arm using a Mantel-Haenszel stratum weighted method adjusting for randomization stratification factors, along with the 95% CI, will be provided.

The change from baseline in iron, serum ferritin, transferrin saturation, and total iron binding capacity will be summarized by treatment arm and visit.

10.4.3.3. 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 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 Assessment (Section 1.2) 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.

The ICF will contain a separate section that addresses the use of remaining mandatory samples for optional exploratory research. The Investigator (or designee) will explain to each subject the objectives of the exploratory research. Subjects will be told that they are free to refuse to participate and may withdraw their consent to allow any remaining samples to be used for optional exploratory research at any time and for any reason during the storage period. A separate signature will be required to document a subject's agreement to allow any remaining specimens to be used for exploratory research. Subjects who decline to participate in this optional research will not provide this separate signature.

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, contract research organizations [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 confidential 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, the European Medicines Agency, and the 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.

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13. APPENDICES

APPENDIX 1. LIST OF ABBREVIATIONS

Abbreviation	Term
2,3-DPG	2,3-diphosphoglycerate
Ab	antibody
AE	adverse event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ATP	adenosine triphosphate
AUC	area under the plasma concentration × time curve
$AUC_{0-\infty}$	area under the plasma concentration × time curve from time 0 to infinity
AUC_{last}	area under the plasma concentration × time curve from time 0 to the last measurable concentration
AUC_{0-t}	area under the plasma concentration × time curve from time 0 to time t
BID	twice daily
C_{max}	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
[redacted]	
FDA	Food and Drug Administration
FSH	follicle stimulating hormone
GCP	Good Clinical Practice
Hb	hemoglobin
HbA	adult hemoglobin
HbS	sickle cell hemoglobin
HBsAg	hepatitis B surface antigen

Abbreviation	Term
HCV	hepatitis C virus
[redacted]	
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
[redacted]	
LLN	lower limit of normal
[redacted]	
NCI	National Cancer Institute
NTDT	non–transfusion-dependent thalassemia
PEP	phosphoenolpyruvate
[redacted]	
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
[redacted]	
QD	once daily
RBC	red blood cell
SAE	serious adverse event
SAP	statistical analysis plan
SD	standard deviation

Abbreviation	Term
SOP	standard operating procedure
$t_{1/2}$	half-life
TDT	transfusion-dependent thalassemia
t_{max}	time to maximum (peak) concentration
[redacted]	
TRR	transfusion reduction response
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.

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