16 October 2025
EMA/352964/2025
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Pyrukynd

International non-proprietary name: Mitapivat

Procedure No. EMEA/H/C/005540/X/0010/G

Note

Assessment report as adopted by the CHMP with all information of a commercially confidential nature deleted.

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List of abbreviations

Abbreviation	Definition
$\%BR_{max}$	maximum percent change from baseline
$\%BR_{min}$	minimum percent change from baseline
2,3-DPG	2,3-diphosphoglycerate
Ab	Antibody
ADP	adenosine diphosphate
AE	adverse event
AESI	adverse event of special interest
Al	Aluminium
ALP	alkaline phosphatase
ALT	alanine aminotransferase
ANCOVA	analysis of covariance
aPTT	activated partial thromboplastin time
AST	aspartate aminotransferase
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
$AUC\_Net\_B_{0-7}$	net change in area under the concentration-time curve from baseline to Day 7
AUC_{0-last}	area under the concentration-time curve from 0 to T_{last} on dosing day
BID	twice daily
BMD	bone mineral density
CFU	Colony Forming Units
CI	confidence interval
C_{last}	last quantifiable concentration after a single dose or within the dosing interval (tau) for multiple doses
C_{max}	maximum concentration
CQA	Critical Quality Attribute
CRO	contract research organization
CSR	clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
CYP	cytochrome P450
DAD	Diode Array Detector
DXA	dual-energy x-ray absorptiometry
EC	European Commission

Abbreviation	Definition
ECG	Electrocardiogram
eCRF	electronic case report form
EDC	electronic data capture
eDISH	evaluation of drug-induced serious hepatotoxicity
EQ-5D-5L	EuroQoL Group 5-Level EQ-5D
EQ-VAS	EuroQoL-Visual Analogue Scale
EU	European Union
FAS	Full Analysis Set
FDA	Food and Drug Administration
FSH	follicle-stimulating hormone
GCP	Good Clinical Practice
Hb	Hemoglobin
HBsAg	hepatitis B surface antigen
hCG	human chorionic gonadotropin
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
HPLC	High performance liquid chromatography
HRQOL	health-related quality of life
HSC	hematopoietic stem cell
HSCT	hematopoietic stem cell transplantation
ICF	Informed Consent Form
ICH	International Council for Harmonisation
IDMC	Independent Data Monitoring Committee
IEC	Independent Ethics Committee
INR	international normalized ratio
IP	investigational product
IRB	Institutional Review Board
IXRS	Interactive Voice/Web Response System
LC	Liquid Chromatography
LDH	lactate dehydrogenase
LLN	lower limit of normal
LS	least squares
MedDRA	Medical Dictionary for Regulatory Activities
MRI	magnetic resonance imaging

Abbreviation	Definition
MS	Mass Spectrometry
OLE	Open-label Extension
PCTFE	Polychlorotrifluoroethylene
PD	pharmacodynamic(s)
PE	physical examination
PEP	Phosphoenolpyruvate
PGIC	Patient Global Impression of Change
PGIS	Patient Global Impression of Severity
Ph. Eur.	European Pharmacopoeia
PK	pharmacokinetic(s)
PKR	red blood cell–specific form of pyruvate kinase
PPS	Per-Protocol Set
PRO	patient-reported outcome
PROMIS®	Patient Reported Outcomes Measurement Information System®
PT	Preferred Term
PVC	Polyvinyl chloride
QD	once daily
QTL	quality tolerance limit
RBC	red blood cell
RH	Relative Humidity
R_{max}	maximum response
R_{min}	minimum response
SAE	serious adverse event
SAP	statistical analysis plan
SD	standard deviation
SE	standard error
SF-8	Short form-8 item physical function component
SmPC	Summary of Product Characteristics
SOC	System Organ Class
SOP	standard operating procedure
Study 010	AG348-C-010
Study 018	AG348-C-018
TEAE	treatment-emergent adverse event
T_{last}	Time of the last quantifiable concentration
t_{max}	time to maximum concentration

Abbreviation	Definition
TranQoL	Transfusion-Dependent Quality of Life Questionnaire
TRR	transfusion reduction response
TSE	Summary of Product Characteristics
ULN	upper limit of normal
US	United States
UV	Ultraviolet
WOCBP	Women of childbearing potential

1. Background information on the procedure

1.1. Submission of the dossier

Agios Netherlands B.V. submitted on 8 November 2024 a group of variations consisting of an extension of the marketing authorisation and the following variations:

Variations requested		**Type**	**Annex affected**
C.I.4	C.I.4 - Change(s) in the SPC, Labelling or PL due to new quality, preclinical, clinical or pharmacovigilance data	II	I

Extension application to introduce a new strength (100 mg film-coated tablet) associated with an extension of indication in adults for the treatment of anaemia associated with transfusion-dependent and non-transfusion-dependent alpha- or beta-thalassaemia (see section 5.1) and a new orphan indication for the "Pyrukynd is indicated in adults for the treatment of anaemia associated with transfusion-dependent and non-transfusion-dependent alpha- or beta-thalassaemia". As a consequence, sections 4.1, 4.2, 4.4, 4.5, 4.8, 5.1 and 5.2 of the SmPC are updated. The Package Leaflet is updated in accordance.

C.I.4 (Type II clinical variation): to update sections 4.2 and 5.2 of the SmPC with pharmacokinetic information based on final results from study AG348-C-024 listed as a category 3 study in the RMP; this is a Phase 1, Open-label, Single-dose, Pharmacokinetic Study of Mitapivat in Subjects with Moderate Hepatic Impairment Compared to Matched Healthy Control Subjects with Normal Hepatic Function.

The RMP (version 1.3) is updated in accordance.

The MAH applied for the following new strength for Pyrukynd: 100 mg film coated tablet

1.2. Legal basis, dossier content

The legal basis for this application refers to:

Article 7.2 of Commission Regulation (EC) No 1234/2008 – Group of variations

Pyrukynd, was designated as an orphan medicinal product EU/3/20/2270 on 22 April 2020 in the following condition: Treatment of pyruvate kinase deficiency

The new indication, which is the subject of this application, falls within a separate orphan designation EU/3/23/2827 on 13 October 2023 in the following condition: Treatment of beta-thalassaemia intermedia and major and EU/3/23/2889 on 12 January 2024 in the following condition: Treatment of alpha-thalassaemia intermedia and major.

1.3. Information on Paediatric requirements

Pursuant to Article 8 of Regulation (EC) No 1901/2006, the application included an EMA Decision EMA/PE/0000238074 on the agreement of a paediatric investigation plan (PIP).

At the time of submission of the application, the PIP EMA/PE/0000238074 was not yet completed as some measures were deferred.

1.4. Information relating to orphan market exclusivity

1.4.1. Similarity

Pursuant to Article 8 of Regulation (EC) No. 141/2000 and Article 3 of Commission Regulation (EC) No 847/2000, the MAH did submit a critical report addressing the possible similarity with authorised orphan medicinal products.

1.5. Additional Data exclusivity/Marketing protection

The MAH requested consideration of one year data exclusivity in regards of its application for a new indication in accordance with Article 14(11) of Regulation (EC) 726/2004.

1.6. Protocol assistance

The MAH received protocol assistance from the CHMP on the development for the indication from the CHMP on 17 September 2020 (EMEA/H/SA/3006/2/2020/II). The protocol assistance pertained to clinical aspects.

- If the results from the (at the time ongoing) Phase 2 study in adult subjects with NTDT (Study AG348-C-010) support continued development of the mitapivat clinical program in subjects with thalassemia with the two proposed Phase 3 studies in adult subjects with NTDT (Study AG348-C-017) and in TDT (Study AG348-C-018), respectively.
- Dose in the 2 Phase 3 studies in adult subjects with α- or β- thalassemia.
- Acceptability of the subject population, use of placebo as a comparator, primary and secondary endpoints, treatment duration, sample size, statistical assumptions and statistical analysis methods as well as the assessments for monitoring safety for Study AG348-C-017 in subjects with NTDT and for Study AG348 C 018 in subjects with TDT, respectively.
- Acceptability of the pivotal Phase 3 study designs to support MAA, along with data from Study AG348-C-010, for the treatment of adult patients with thalassemia.

1.7. Steps taken for the assessment of the product

The Rapporteur and Co-Rapporteur appointed by the CHMP were:

Rapporteur: Alexandre Moreau;Co-Rapporteur: N/A

CHMP Peer reviewer: N/A

The application was received by the EMA on	8 November 2024
The procedure started on	28 November 2024
The CHMP Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	17 February 2025
The CHMP Co-Rapporteur's first Assessment Report was circulated to all	NA

CHMP and PRAC members on	
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC and CHMP members on	24 February 2025
The CHMP agreed on the consolidated List of Questions to be sent to the MAH during the meeting on	27 March 2025
The MAH submitted the responses to the CHMP consolidated List of Questions on	22 May 2025
The following GCP inspection were requested by the CHMP and their outcome taken into consideration as part of the Quality/Safety/Efficacy assessment of the product:	
– A GCP inspection at two investigator sites located in Bulgaria (10-14/02/2025) and Thailand (10-14/03/2025) and the sponsor site in the USA (14-18/04/2025). The outcome of the inspection carried out was issued on.	07 July 2025
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Questions to all CHMP and PRAC members on	25 June 2025
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	24 February 2025
The CHMP agreed on a list of outstanding issues in writing to be sent to the MAH on	24 July 2025
The MAH submitted the responses to the CHMP List of Outstanding Issues on	19 August 2025
The CHMP Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	8 September 2025
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	4 September 2025
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Questions to all CHMP and PRAC members on	11 September 2025
The CHMP agreed on a list of outstanding issues in writing to be sent to the MAH on	18 September 2025
The MAH submitted the responses to the CHMP List of Outstanding Issues on	22 September 2025
The CHMP Rapporteurs circulated the Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	10 October 2025

The CHMP, in the light of the overall data submitted and the scientific discussion within the Committee, issued a positive opinion for granting a marketing authorisation to Pyrukynd on	16 October 2025
The CHMP adopted a report on similarity of Pyrukynd with Reblozyl and Casgevy on (see Appendix on similarity)	16 October 2025
The CHMP adopted a report on the novelty of the indication/significant clinical benefit for Pyrukynd in comparison with existing therapies. (see Appendix on Article 14(11))	16 October 2025

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

The MAH submitted an Extension Application for Pyrukynd (mitapivat), 100mg tablet associated with a new orphan indication initially proposed as "Treatment of adult patients with non-transfusion-dependent and transfusion-dependent alpha- or beta-thalassaemia".

The proposed new thalassemia-related indication is only intended for the new tablet strength being added (100 mg).

Considering that the data primarily support a significant improvement in anaemia, the CHMP agreed with the following indication wording to adequately reflect the overall treatment objective, which was agreed by the MAH: 'Pyrukynd is indicated in adults for the treatment of anaemia associated with transfusion-dependent and non-transfusion-dependent alpha- or beta-thalassaemia.

Thalassemia is an inherited blood disorder, caused by mutations in the α- and/or β-globin genes, leading to chronic haemolytic anaemia. The degree of the anaemia in thalassemia is dependent on the magnitude of the globin mismatch and imbalance with larger imbalances resulting in worse anemia, translating into a spectrum of transfusion needs encompassing all genotypes. Thalassemia requiring lifelong regular transfusion therapy is termed transfusion-dependent thalassemia (TDT), and thalassemia requiring occasional or intermittent transfusions is termed non-transfusion-dependent thalassemia (NTDT).

2.1.2. Epidemiology

Robust data on the epidemiology of thalassemia are limited, particularly region-and subtype-specific epidemiology. Globally, the estimated prevalence of thalassemia is 411,400 people, and the incidence is estimated at 106,300 people per year (GBD 2017 Disease and Injury Incidence and Prevalence Collaborators, 2018). Although a global disease, thalassemia is most common in South Asia, the Far East, the Middle East, and Mediterranean countries (Cappellini *et al,* 2018). Estimates of prevalence and incidence vary by source but collectively support the rarity of the condition. Conservative estimates indicate that more than 56,000 infants with thalassemia are born each year in the world (Modell and Darlison, 2008), while other estimates range

from an annual incidence at birth of 1/100,000 for β thalassemia (Orphanet, 2020) to 4.4/10,000 for thalassemias in general (Muncie and Campbell, 2009). Prevalence of the disease and its subtypes is also subject to variability and uncertainty. The United States Centers for Disease Control and Prevention guidelines state that β-thalassemia major affects at least 1,000 people (NCBDDD,2020), and the Thalassemia International Federation reports that only approximately 200,000 patients with β-thalassemia major are alive and registered as receiving regular treatment around the world (Galanello and Origa, 2010). Population migration has also affected the epidemiology of thalassemia in certain regions. In Europe, migrations over the last few decades from the southern states of Europe and from Asia, the Middle East, and Africa have introduced the disease in most of the northern areas of Europe (Angastiniotis *et al,* 2013).

β-thalassemia accounts for approximately 75% of affected thalassemia conceptions worldwide based on the numbers of α- or β-thalassemia-affected conceptions reported in the literature (Modell and Darlison, 2008). Studies reporting prevalence for transfusion dependency have typically focused on β-thalassemia and suggest that more than 60% of infants with β-thalassemia worldwide are transfusion dependent (Modell and Darlison, 2008).

It is estimated that 5% of the world's population is a carrier of a defective alpha-thalassaemia gene, with approximately 1 million patients affected by various alpha-thalassaemia syndromes globally. Alpha-thalassaemia occurs at a particularly high frequency in populations from sub-Saharan Africa through the Mediterranean region and Middle East, to the Indian sub-continent and East and Southeast Asia.

2.1.3. Biologic features

Thalassemia is an inherited, chronic haemolytic anaemia caused by mutations in the α- and/or β-globin genes, leading to lifelong morbidity and early mortality. Based on where the mutation occurs, thalassemia is named α- or β-thalassemia due to the reduced or absent α-globin chains or β-globin chains, respectively, leading to the toxic and unstable accumulation of the other globin chains. In β-thalassemia, α-globin aggregates, and in α-thalassemia, β-globin aggregates. Irrespective of the mutations, it is the imbalance in these globin chains that drives the pathophysiologic manifestations of thalassemia by imposing metabolic stress on the Red Blood Cells (RBCs), specifically in the form of excess generation of reactive oxygen species and an increased demand on ATP−dependent proteolytic mechanisms to clear excess globin chains. In thalassaemic RBCs, ATP supply is insufficient to maintain RBC membrane fitness and clearance of globin precipitates, leading to early and increased death of RBC precursors in the bone marrow (ineffective erythropoiesis) and in extramedullary sites (haemolysis) (Chakraborty *et al,* 2012; Shaeffer, 1988; Ting *et al,* 1994).

All patients with thalassemia, independent of transfusion requirements, are at risk of serious, irreversible morbidities and early mortality (Musallam *et al,* 2020; Taher et *al,* 2023). Over time, chronic haemolytic anaemia, the hallmark of thalassemia, can lead to widespread organ damage, dysregulated compensatory mechanisms, and premature mortality. The complications of thalassemia, in both NTDT and TDT, include extramedullary hematopoietic pseudo tumours, leg ulcers, thrombosis, pulmonary hypertension, liver fibrosis/cirrhosis and abnormal function, heart failure, osteoporosis, and metabolic and endocrine disorders (Bazarbachi *et al,* 2016; Musallam, Cappellini, Daar, *et al,* 2021; Sleiman *et al,* 2018; Taher *et al,* 2018). Patients with thalassemia also have reduced HRQOL including symptoms of fatigue, weakness, shortness of breath, and impaired physical function (Cappellini *et al,* 2019; Taher *et al,* 2019), and report negative impacts on school and career function (Drahos *et al,* 2024; Klaassen *et al,* 2014). Historically, disease severity was associated with frequency of transfusions; however, as discussed above, patients and physician choice may affect the frequency of transfusions, and recent evidence shows that patients who do not receive regular

transfusions also experience significant morbidity and increased early mortality (Musallam, Cappellini and Taher, 2021).

2.1.4. Clinical presentation, diagnosis

Reduction in β-globin production results in an accumulation of excess, un-complexed α-globin in erythroblasts. The clinical implications of this α-globin/β-globin imbalance are (Thein, 2005) haemolysis leading to a lack of sufficient erythrocytes and Hb to effectively transport oxygen throughout the body:

1) Oxidative damage of the cell membrane, thereby resulting in apoptosis of erythrocyte precursors and therefore ineffective erythropoiesis.

2) Ineffective erythropoiesis which leads to morbidities such as splenomegaly, bone marrow expansion, concomitant bone deformities, and iron overload.

2.1.5. Management

The standard of care for thalassemia remains centered on supportive care to address symptoms through transfusions, splenectomy, and/or iron chelators. Regular RBC transfusions are the cornerstone for treatment of patients with thalassemia, despite associated burden and risks. Patients requiring frequent transfusions typically receive blood every 2 to 5 weeks to maintain pretransfusion Hb levels of 9.5 to 10.5 g/dL (95 to 105 g/L). The volume of blood required to achieve this goal often requires multiple RBC units at each visit, and each unit must be infused separately. Additionally, each transfusion appointment requires a laboratory assessment and blood product compatibility testing, in addition to the time required to complete transfusion of multiple RBC units, which can span multiple days. Because of these logistical burdens, RBC transfusions frequently interfere with school, work, and social functioning. In this regard, thalassemia and its management with transfusion therapy have a substantial impact on patients' quality of life (Patterson *et al,* 2022; Shah *et al,* 2021; Shah *et al,* 2019). In addition, transfusion therapy can be complicated by hemolytic reactions, shock, severe allergic reactions, transfusion-transmitted infections, and alloimmunisation; and more critically, shortages in availability of blood supply. Finally, despite frequent transfusions, patients continue to experience higher rates of premature mortality compared with the general population (Borgna-Pignatti *et al,* 2004; Modell *et al,* 2000).

Iron overload caused by regular transfusions is a common complication of thalassemia syndromes which could lead *per se* to the development of organ damage and increased mortality. Iron overload may occur due to increased intestinal absorption (which occurs in most patients independent of transfusions) or exogenous iron from frequent RBC transfusions (Taher *et al,* 2011). Thus, iron chelation therapy to remove excess iron is a fundamental supportive therapy; however, it addresses only one of the multiple complications of thalassemia, and does not directly treat the haemolytic anaemia that is the hallmark of the disease.

With regards to splenectomy, this latter 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 surgery (Cappellini *et al,* 2000), in addition to risks of undergoing a surgical procedure.

The Thalassemia International Federation (TIF) acknowledges the need for folic acid supplementation, particularly for patients with un-transfused or low-transfusion regimens and in specific situations like pregnancy, due to increased folate demand and potential deficiency. Folic acid is a necessary coenzyme for erythropoiesis (red blood cell production), and while not a primary treatment for the disease itself, it is

recommended as a supplement to manage or prevent a relative folate deficiency, especially in those with active bone marrow.

Allogeneic haematopoietic stem cell transplantation (allo-HSCT) is recognised as a potentially curative option for patients with transfusion-dependent β-thalassaemia (TDT). However, its applicability is limited, as fewer than 30% of otherwise eligible patients have access to an HLA-matched related donor. According to TIF recommendations, allo-HSCT should be considered only in young patients with symptomatic TDT who have a matched sibling donor and ideally performed before the development of iron overload.

Foetal haemoglobin inducers (e.g. 5-azacytidine, decitabine, hydroxycarbamide, with or without erythropoiesis-stimulating agents [ESAs]), short-chain fatty acids, and thalidomide or its derivatives) have been given as off label use treatments. These agents have been evaluated off label in patients with NTD and TDT for their ability to stimulate the production of γ-globin chain, thus, ameliorating the α/β-globin chain imbalance and ineffective erythropoiesis. These agents include DNA-methylation inhibitors (5-azacytidine, decitabine), hydroxyurea (or hydroxycarbamide, with or without erythropoiesis-stimulating agents [ESAs]), short-chain fatty acids, and thalidomide or its derivatives. Collectively, the reported effects in patients were not always consistent and the studies have various limitations: small sample size, heterogenous populations studied, lack of standardised or approved controversial doses used, short duration of follow-up, and inconsistently reproducible results.

To date, there are no authorised medicines to treat α-thalassemia.

Reblozyl is a subcutaneously administered erythroid maturation agent which has been recently approved in adults for the treatment of anaemia associated with transfusion-dependent and non-transfusion-dependent beta-thalassaemia. Luspatercept reduced the transfusion burden in patients with TD β-thalassemia and increased Hb levels in NTD β-thalassemia Administration of luspatercept requires a visit to a health care provider every 3 weeks, which can be burdensome for patients and health care systems.

Casgevy a gene editing therapy recently approved is indicated for patients, 12 years of age and older with β-thalassemia but only those transfusions dependent and for whom hematopoietic stem cell (HSC) transplantation is appropriate, but a human leukocyte antigen-matched-related HSC donor is not available. Gene therapies incur acute and long-term toxicities secondary to myeloablative chemotherapy, potential off target mutations and challenges exist with widespread availability.

2.2. About the product

Mitapivat is a first-in-class, orally bioavailable, potent, allosteric activator of WT PKR and a range of PKR mutant enzymes (Kung *et al,* 2017). Mitapivat acts by allosterically binding to the PKR tetramer and enhancing its affinity for PEP, thereby increasing the ability of RBCs to convert PEP plus ADP to pyruvate plus ATP. Increasing ATP by activating the WT PKR enzyme ameliorates RBC health, thereby reducing ineffective erythropoiesis and haemolysis in thalassemia, which clinically translates into an improvement in haemolytic anaemia, irrespective of genotype and transfusion requirements.

The initially proposed indication was "Treatment of adult patients with non-transfusion-dependent and transfusion-dependent alpha- or beta-thalassaemia".

Considering that the data primarily support a significant improvement in anaemia, the CHMP agreed the following indication wording to adequately reflect the overall treatment objective, which was agreed by the MAH: 'Pyrukynd is indicated in adults for the treatment of anaemia associated with transfusion-dependent and non-transfusion-dependent alpha- or beta-thalassaemia (see section 5.1).

The new posology for this indication is as follows:

The recommended dose is 100 mg taken orally twice daily.

Treatment with Pyrukynd is intended to be long-term. Pyrukynd should be discontinued if a patient does not experience an improvement of haemolytic anaemia after 6 months for patients with non-transfusion-dependent thalassaemia or after 1 year for patients with transfusion-dependent thalassaemia based on the totality of laboratory results and clinical status of the patient, unless there is another explanation for response failure (e.g. bleeding, surgery, other concomitant illnesses).

2.3. Type of Application and aspects on development

The development programme/compliance with guidance/scientific advice

The primary studies supporting this marketing application are the 2 pivotal Phase 3 studies: Study AG348- C-017 and Study AG348- C-018. Supportive efficacy and safety data are provided from Study AG348- C-010.

The PDCO issued an opinion on compliance for the PIP (P/0150/2022). At the time of submission of the application (November 8, 2024), the PIP is not yet completed as some measures were deferred.

General comments on compliance with GMP, GLP, GCP

The MAH states that clinical study report submitted is in compliance with the guidance ICH Topic E3 Structure and Content of Clinical Study Reports as well as the Note for guidance on the inclusion of appendices to clinical study reports in marketing authorisation applications.

There were no reported serious breaches to GCP in AG348-C-017 and AG348-C-018. While during the conduct of the study there were incidents of non-compliance with GCP identified, these events were investigated and determined to not have a significant impact to subject safety or data integrity. These instances were documented and tracked via Agios established processes and included protocol deviations, deviations to IMP procedures and potential incidents of unblinding. There were no sites or clinical investigators that were excluded from the clinical trial and Agios can confirm the study adhered to GCP, maintained the ethical principles for medical research according to the Declaration of Helsinki and met the ethical requirements of Directive 2001/20/EC.

The information in relation to the manufacturing sites is correct in terms of names, addresses and manufacturing activities.

No Good Laboratory Practice (GLP) compliance statement is included in the submission, given that the two non-clinical studies supporting the new indication are primary pharmacodynamics studies, not intended to be conducted under GLP guidelines.

2.4. Quality aspects

2.4.1. Introduction

This extension application introduces a new 100 mg strength of Pyrukynd film-coated tablets in addition to the approved 5 mg, 20 mg and 50 mg film-coated tablet strengths.

The finished product is presented as film-coated tablets containing 100 mg of mitapivat (as sulfate). The product contains the mitapivat hemisulfate sesquihydrate (2:1:3) salt, abbreviated as sulfate in the SmPC.

Other ingredients are:

Tablet core: microcrystalline cellulose, croscarmellose sodium, mannitol (E421) and sodium stearyl fumarate;

Film-coating: hypromellose (E464), titanium dioxide (E171), lactose monohydrate, triacetin, indigo carmine aluminium lake (E132) and macrogol (polyethylene glycol) (E1521);

Printing ink: shellac (E904), FD&C blue #1/brilliant blue FCF aluminium lake (E133), titanium dioxide (E171) and ammonium hydroxide (E527).

The product is available in in PVC/PCTFE/Al blister wallets as described in section 6.5 of the SmPC.

2.4.2. Active Substance

No new information on the active substance mitapivat has been provided with this line extension application. The new tablet strength contains active substance of the same quality as used in the approved strengths and the active substance is manufactured by the approved manufacturing sites. The approved specification of the active substance is suitable for the new tablet strength.

2.4.3. Finished Medicinal Product

2.4.3.1. Description of the product and pharmaceutical development

The finished product is presented as oblong blue film-coated tablets for oral administration containing 100 mg mitapivat as the active substance. The tablets are approximately 16 mm long and 6.8 mm wide and are imprinted on both sides with "M100" in blue ink. The 100 mg film-coated tablets are sufficiently distinguishable from the approved tablet strengths due to their shape (the 5 mg and 20 mg film-coated tablets are round) and/or due to their colour (the 100 mg film-coated tablet is a lighter shade of blue compared to the approved strengths) as well as by the blue imprint on both sides (instead of imprint in black and only on one side for the approved presentations).

The aim of the pharmaceutical development was to develop an immediate release dosage form to deliver the therapeutic dose of 100 mg of mitapivat twice daily.

The quality target product profile (QTPP, Table 4) was defined and this allowed identification of potential critical quality attributes of the finished product (CQAs): description, identification, assay, content uniformity, dissolution, degradation products, impurity AGI-80276, water content and microbiological quality) which were then investigated during development studies.

Table 1: quality target product profile

QTPP Element	Target	Justification
Dosage form	Solid dosage form for oral administration	Ease of patient dosing
Dosage design	Immediate release tablet	Match pharmacokinetic exposure of two 50 mg Mitapivat Tablets used in Ph 1/2 clinical studies
Age population	18 yrs+	Initial market application
Route of administration	Oral	Ease of administration, compliance
Dosage strength (Free base equivalent)	100 mg	Dose supported by pivotal studies
Pharmacokinetics	Immediate release	Low solubility at higher pH, exposure risk mitigation
Stability	Storage at 2-8°C with an in-use period at room temperature	Ensure product has appropriate quality, safety and efficacy to support manufacturing, release and distribution. In-use period for patient convenience.
Container closure system	Thermoformed blisters / foil lidding	Compliance and safety risk mitigation
Administration/Concurrence with labeling	BID, dose fasted/fed	Intrinsic PK, no food effect
Alternative methods of administration	n/a	n/a

A traditional approach to pharmaceutical development was chosen, but Quality by Design elements, such as risk assessments and design of experiments were incorporated.

The physicochemical properties of the active substance which may have an impact on the finished product have been previously studied and established during development of the approved tablet strengths.

All excipients are well known pharmaceutical ingredients, and their quality is compliant with Ph. Eur. or other relevant EU standards. There are no novel excipients used in the finished product formulation. The list of excipients is included in section 6.1 of the SmPC and in paragraph 2.3.1. of this report. Lactose is an excipient with a known physiological effect and is thus also listed in section 2 of the SmPC.

The 100 mg film-coated tablet differs in several aspects from the approved presentations. A different film-coating colour in a lighter blue shade is used for the 100 mg tablets. The imprint on the tablets is different (see above).

The development of the formulation is described in sufficient detail. Different formulations were initially tested on small scale, which included variations in the intragranular as well as the extragranular composition. A prototype formulation was selected based on flowability, friability, solid fraction, compressibility, disintegration and dissolution results.

For clinical studies, the new 100 mg tablets were used for the pivotal Phase 3 clinical studies. During Phase 2 clinical studies, 2×50 mg tablets were administered.

A discussion on the bioequivalence of the 100 mg tablets proposed for marketing and the 2 x 50 mg tablets used in Phase 2 clinical studies was provided. This discussion was further supported by results from a relative bioavailability study in which the 100 mg tablet was compared with 2 x 50 mg tablets. The pharmacokinetics were found to be equivalent.

The 100 mg tablets used during clinical development and the 100 mg tablets proposed for marketing are similar except for two differences. (1) The 100 mg tablets used during clinical development were coated in the blue film-coating used for the approved strengths, and (2) the tablets were plain-faced. The 100 mg tablets proposed for marketing are coated in a lighter blue film-coating and are imprinted on both sides with blue ink. Comparative dissolution profiles in three different physiological pH media (pH 1.1, pH 4.5 and pH 6.8) were provided, and the results showed that these differences had no impact on *in vitro* dissolution. No formal *in vivo* bioequivalence studies to compare the 100 mg tablets (plain faced/blue and imprinted/lighter blue) were conducted. This is acceptable as these differences are not expected to affect performance of the finished product or bioavailability of mitapivat (the film-coating is not functional). Demonstration of equivalence through comparative dissolution studies is acceptable.

The development of the dissolution method is described and is based on the method used for the approved strengths.

The discriminatory power of the dissolution method was studied. Tablets with intentional variations in the manufacturing process and the formulation were tested. The dissolution method was discriminatory against batches which contained active substance with smaller particle size and against tablets which were manufactured under high temperature stress conditions. The information provided on the discriminatory power of the dissolution method is satisfactory.

Manufacturing process development is described in sufficient detail. The manufacturing process for the 100 mg tablets was developed from the process used for the approved 50 mg tablets. It was found that additional manufacturing steps are necessary due to the higher content of active substance in the formulation.

The primary packaging is PVC/PCTFE/Al blister wallets. The material complies with Ph. Eur. and EC requirements. The choice of the container closure system has been validated by stability data and is adequate for the intended use of the product. The same primary packaging material is already used for the approved tablet strengths (5 mg, 20 mg and 50 mg film-coated tablets).

2.4.3.2. Manufacture of the product and process controls

The finished product is manufactured at one manufacturing site (Rottendorf Pharma GmbH, Germany), which is the same as for the approved tablet strengths.

The process is considered to be a standard manufacturing process and includes the main steps of blending, dry granulation with roller compaction, final blending, compression, film coating, printing, and blister packaging.

Major steps of the manufacturing process have been investigated during development and scale-up. It has been demonstrated that the manufacturing process is capable of producing the finished product of intended quality in a reproducible manner. The in-process controls are adequate for this type of manufacturing process and pharmaceutical form. An acceptable validation scheme has been submitted, and the process will be validated on three consecutive production-scale batches prior to commercialization.

The bulk holding time (up to 12 months at 2 – 8 °C) is supported by results from a bulk holding time study. The bulk holding time is acceptable.

2.4.3.3. Product specification

The finished product release specifications shown in Table 5 include appropriate tests for this kind of dosage form: description (visual), identification (HPLC/UV and HPLC/DAD), assay (HPLC/UV), nitrosamine impurities (LC/MS/MS), degradation products (HPLC/UV), uniformity of dosage units (Ph. Eur.), dissolution (in house), water content (Ph. Eur.) and microbial limit tests (Ph. Eur.).

The finished product specification is acceptable and covers all the necessary test for the dosage form. The analytical test methods and limits have been appropriately justified.

The test methods included in the specification are the same as for the approved tablet strengths with some modifications, mainly with regard to sample preparation (for the tests for identification, assay, degradation products and uniformity of dosage units). The dissolution test method was also modified (see above).

A risk assessment concerning the potential presence of nitrosamine impurities in the finished product has been performed considering all suspected and actual root causes in line with the "Questions and answers for marketing authorisation holders/applicants on the CHMP Opinion for the Article 5(3) of Regulation (EC) No 726/2004 referral on nitrosamine impurities in human medicinal products" (EMA/409815/2020) and the "Assessment report- Procedure under Article 5(3) of Regulation EC (No) 726/2004- Nitrosamine impurities in human medicinal products" (EMA/369136/2020). During the procedure, a major objection was initially raised as results from confirmatory testing had not been provided with the initial application. Results from confirmatory testing were then provided. Results were below 10% of the acceptable intake. It has been demonstrated that the analytical method used for the approved strengths is suitable for the control of nitrosamine impurities in the 100 mg tablet. The major objection raised during the procedure included a second aspect, namely the risk of formation of two nitrosamines from the ink component Brilliant Blue FCF Aluminum Lake. In response, a suitable justification was provided concluding that there is no risk of nitrosamine formation above acceptable levels from this ink component. The major objection is resolved. The control of nitrosamine impurities is acceptable.

The potential presence of elemental impurities in the finished product was assessed following a risk-based approach in line with the ICH Q3D Guideline for Elemental Impurities. Based on the risk assessment, it can be concluded that no specific controls for elemental impurities are required in the finished product specification.

Batch analysis results are provided for three registration batches (pilot scale) confirming the consistency of the manufacturing process and its ability to manufacture to the intended product specification.

The analytical methods used have been adequately described and appropriately validated in accordance with the ICH guidelines. Satisfactory information regarding the reference standards used for assay and impurities testing has been presented.

The finished product is released on the market based on the above release specifications, through traditional finished product release testing.

2.4.3.4. Stability of the product

Stability data from three pilot-scale registration batches of 100 mg tablets manufactured at the commercial manufacturing site using the commercial manufacturing process of the finished product and using commercial active substance stored for up to 36 months under long term conditions (5 °C) and for up to six months under accelerated conditions (25 ºC / 60% RH) according to the ICH guidelines were provided. The batches of medicinal product are representative of those proposed for marketing and were packed in the primary packaging proposed for marketing. The analytical methods used for the formal stability testing are the same as those proposed for testing of commercial product as described in Module 3.2.P.5.2. Stringent storage conditions are used as indicated below to ensure control of nitrosamines below acceptable limits throughout shelf life. An increase in the level of the two specified nitrosamines was observed in all cases. However, the detected values were well below the specification limit after 24 months. All results remained within specification.

Forced degradation studies have been conducted, and these confirmed the stability-indicating nature of the analytical method used for degradation products. Samples were exposed to acidic, basic, and oxidative conditions in solution and formation of two degradation products was observed. In addition, solid finished product samples were exposed to simulated sunlight, thermal and thermal-humidity conditions and no degradation was observed.

In addition, one batch was exposed to light as defined in the ICH Guideline on Photostability Testing of New Drug Substances and Products. Results show that no special light protection precaution statement is warranted for the label.

Based on available stability data, the proposed shelf-life of 2 years and storage conditions 'Store in a refrigerator (2 °C - 8 °C). Can be stored at room temperature (below 25 °C) for a single period of up to 3 months, after which it should be discarded' as stated in the SmPC (section 6.3 and 6.4) are acceptable.

2.4.3.5. Adventitious agents

It is confirmed that the lactose is produced from milk from healthy animals in the same condition as those used to collect milk for human consumption and that the lactose has been prepared without the use of ruminant material other than calf rennet according to the Note for Guidance on Minimising the Risk of Transmitting Animal Spongiform Encephalopathy Agents Via Human and veterinary medicinal products.

2.4.4. Discussion on chemical, pharmaceutical and biological aspects

This extension application introduces a new 100 mg strength of Pyrukynd film-coated tablets. Information on development, manufacture and control of the finished product has been presented in a satisfactory manner. One quality major objection related to the control of nitrosamines was raised and resolved during the procedure. The results of tests carried out indicate consistency and uniformity of important product quality characteristics, and these in turn lead to the conclusion that the product should have a satisfactory and uniform performance in clinical use.

2.4.5. Conclusions on the chemical, pharmaceutical and biological aspects

The quality of this product is considered to be acceptable when used in accordance with the conditions

defined in the SmPC. Physicochemical and biological aspects relevant to the uniform clinical performance of the product have been investigated and are controlled in a satisfactory way. Data has been presented to give reassurance on viral/TSE safety.

2.4.6. Recommendation for future quality development

Not applicable.

2.5. Non-clinical aspects

2.5.1. Introduction

2.5.2. Pharmacology

2.5.2.1. Primary pharmacodynamic studies

A new *in vivo* pharmacodynamics model was provided: the effects of mitapivat in a murine model of beta-thalassemia. In this *in vivo* study, mice received either 50 mg/kg of the drug by oral gavage or 1,200 ppm via diet (equivalent to ~100 mg/kg/day), with plasma concentrations in Hbb^th3/+ mice reaching approximately 314 ng/mL. This exposure correlated with significant improvements in haemoglobin, RBC indices (MCV, MCH), RBC survival, and reductions in reticulocyte count and oxidative damage, supporting a robust PK/PD relationship. Duration of effect was assessed up to 56 days, indicating sustained pharmacodynamic benefits and partial reversibility when treatment ceased. clear, dose-related efficacy in mitigating β-thalassemia-associated pathology.

2.5.2.2. Secondary pharmacodynamic studies

Mitapivat and its metabolite AGI 8702 were assessed for their potential to inhibit binding and enzymatic activity in a panel of 91 receptors, ion channels, and enzymes, including Histamine receptors and CYP19 aromatase.

AGI8702 showed no inhibition of binding to histamine receptor (H1 H2 and H3) and to aromatase (highest concentration tested 10 µM).

Mitapivat inhibits by 55 and 64% the H1 and H2 receptors but with no functional activity. However, with 72% binding inhibition of H3 receptor, mitapivat has a functional antagonism activity (IC50 = 0.102)) and an inverse agonism activity (EC50 0.012 µM). As H3 receptor is present in brain, lung and gastrointestinal tract, an inhibition should have effects on behaviour or gastrointestinal effect.

Mitapivat inhibits CYP19 Aromatase in several test enzyme systems: Insects cells expressing human recombinant aromatase, human placental microsomes, and rat ovarian microsomes. The IC50 of mitapivat in human placental microsomes was higher (2.05 µM) than the positive control which were know aromatase inhibitors (IC50 from 0.00829 to 0.397µM). This inhibition potential of aromatase should be taken into account for the reproductive and developmental toxicology studies.

2.5.2.3. Safety pharmacology programme

The IC50 for both mitapivat and its metabolite AGI-8702 was > 10 µM, suggesting a low potential inhibition of hERG current. This low potential was confirmed by GLP-compliant manual patch clamp assay for potential inhibition of the hERG current at concentrations up to 226 µM. The IC50 and IC20 (concentration that produced 50 or 20% inhibition) were 29.4 and 8.6 µM, respectively, confirming that the potential for mitapivat to inhibit the hERG current is low.

Studies showed no effect of mitapivat on respiratory and cardiac system. Following observed effects in repeat dose toxicology studies in *Cynomolgus* monkeys, the emetic activity was evaluated in ferrets. Mitapivat after

oral administration at 30, 60 and 100 mg/kg were observed during 6 hours. Emetic activity was observed 30 mg/kg and was marked at 60mg/kg.

In monkeys, the highest dose level tested is projected to have resulted in an AUC(0-12) 5.0-fold the human AUC(0-12) and a Cmax 3.3-fold the human Cmax at the recommended clinical dose of 100 mg BID. In rats, the highest dose level tested in both studies is projected to have resulted in an AUC0-12 40-fold the human AUC0-12 and a Cmax 19-fold the human Cmax at the recommended clinical dose of 100 mg BID. No significant toxicity was observed in standard safety pharmacology studies (cardiovascular, CNS, and respiratory). These findings support a favourable overall safety profile at the intended clinical dose.

The previous safety pharmacology data provided for the initial application does cover the new posology of the extension.

2.5.2.4. Pharmacodynamic drug interactions

No pharmacodynamic drug interaction studies have been conducted.

2.5.3. Pharmacokinetics

The intra-run and inter-run accuracy tested in the 4 levels of analytical QCs are acceptable (under 15%RE) as well as the precision assessed in the 4 levels of analytical QCs (under 10%CV) in the 4 plasma species.

Some criteria of validation are not provided, mitapivat and AGI-8702 were determined to be stable between 144 and 197 days (depends on the species) in plasma samples when stored at -20ºC or -70ºC but no details are provided on the degradation kinetics of these products as a function of temperature or the expected storage temperature for these samples from the validation report of the stability study where these two temperatures are evaluated, we could infer that it is -20 and/or -70).

Moreover, information on the non-specific matrix-related interferences (using individual matrix lots, analysed as blanks and fortified at the LLOQ level), specific interferences (using LLOQ (and sometimes ULOQ for LBAs) QC samples), on the recovery of the analyte from the biological matrix (Extraction recovery should be reproducible), on the carry-over (blank following a ULOQ sample) were not provided. However, these studies are GLP thus, the analytical methods are acceptable. It was shown by a permeability test that mitapivat is actively transported across Caco-2—cells. After single oral and IV doses of mitapivat, the oral bioavailability was 24 % and 82% in monkey and dog, respectively. Those data were obtained in males. The mean plasma toxicokinetic parameters showed that females were more exposed than male rats at same doses; from 100 mg/kg to 500 mg/kg the exposure was 3-fold or higher in female than male rats. At 1000 to 2000 mg/kg, exposure was less than dose proportional; was only 2/2.5-fold higher in female than male rats. The T1/2 were from 3.7 to 9.5 hours after oral dose and from 4.6 to 9.6 hours after IV dose.

In male dogs, mitapivat exposure decreased with decreasing dose, and the dose-exposure relationship was proportional between 125 and 10 mg/kg. In female monkeys, the mitapivat exposure was dose proportional up to 250mg/kg. At 500 and 1000 mg/kg, the increase exposure was less dose proportional. Two formulations were assessed at pH <1 or pH = 3 and showed similar results.

The brain penetration was assessed in male rats a 300 mg/kg in single dose or during 5 days in plasma versus brain. The mitapivat concentration was present for 24 hours after 5 days of injection and single dose. The brain concentration was lower than concentration plasma (ratio 0.0912). After 24 hours, the plasma concentration of mitapivat decreased from approximately 10,000 ng/ml to 100 ng/ml in plasma, but in brain, the mitapivat

concentration seems constant (around 1000 ng/ml) and similar at single dose or after 5 days of treatment (no accumulation).

The tissues distribution was assessed by [14C] radioactivity using validated Quantitative Whole-Body Autoradiography (QWBA) techniques. The radioactivity was above the BQL in aorta, eye, abdominal fat, liver, muscle, spleen, thyroids and uveal tract. Ocular tissues, as well as tissues of the gastrointestinal tract and metabolic/excretory system, contained the highest distribution, suggesting mitapivat may bind to melanin. Moreover, the exposure in pigmented skin was higher relative to the exposure in non-pigmented skin. The radioactivity in central nervous system was very low. The plasma protein binding for mitapivat was moderate to high (89%, 93.3% and 97.7% in dog, monkey and human, respectively) and was 53.76% for the metabolite AGI-8702 in human plasma. The partitioning of mitapivat between red blood cell and plasma showed a ratio of 0.5 in mouse, 0.47 in rat, 0.62 in dog, 0.42 in monkey and 0.37 in human. This distribution suggests a low penetration of mitapivat in red blood cells (ratio <1). However, in view of pharmacology studies, this distribution is sufficient for mitapivat to be active on PKR enzymes in red blood cells.

Microsome tests have shown that mitapivat presents metabolic stability in mouse, rat, dog, monkey and human. Mitapivat is extensively metabolised in the liver with high clearance. The main cytochromes implicated in metabolism of mitapivat in human liver microsomes are CYP3A4 and CYP3A5. All metabolites found in human were found in at least one of other species *in vitro* and *in vivo*. All metabolites were characterised and were found under 10%, except metabolites M396 or AGI-8702 which were found at 33% in human hepatocytes and > 50 % in human liver microsomal incubation; in rats this metabolite was found at > 60% in liver microsomal incubation and at 23 % in male and 21% in female *in vivo*. All studies have evaluated mitapivat and its metabolite according to ICH M3 guideline.

In male rats, the dosed radioactivity was excreted via biliary, urinary, and faecal routes in similar amounts (approximately 30% each). In female rats, biliary excretion was the major route of elimination, accounting for 48.8% of the dose; in faeces and urine, the mean cumulative excretion was 30.4% and 13.9%, respectively.

2.5.4. Toxicology

2.5.4.1. Single dose toxicity

No new single-dose toxicity study was performed for this application.

2.5.4.2. Repeat dose toxicity

Mitapivat was tested in GLP studies in rats (28 days, 3 months, 6 months) and monkeys (28 days, 3 months, 9 months) to support the initial marketing authorisation. No new study was provided, however the design of these studies covers the new posology.

In rats, sex-dependent dosing was used to achieve comparable exposure, with notable organ effects (liver, adrenal, reproductive organs) often reversible upon recovery.

In the 28-day rat study, NOAELs were 600 mg/kg/day (males) and 20 mg/kg/day (females), with aromatase inhibition affecting the oestrous cycle in females.

The 3-month rat study established NOAELs at 60 mg/kg/day (males) and 50 mg/kg/day (females); effects on kidney, heart, prostate, and pituitary at higher doses in the 28-day study did not recur here. The 6-month rat

study showed similar findings (e.g., adrenal vacuolation, liver hypertrophy, reproductive organ changes), mostly reversible except certain effects at higher doses.

In monkeys, the 28-day study found the drug well tolerated up to 150 mg/kg/day, with only a reversible increase in liver weight noted. The 3-month monkey study revealed dose-related emesis at all doses and body weight loss (reversible), hepatocellular hypertrophy (partly reversible), and a NOAEL of 200 mg/kg/day.

The 9-month monkey study again showed increased emesis and mild liver changes (e.g., hepatocellular hypertrophy, pigmented macrophages), often reversible.

Across all studies, the drug was rapidly absorbed (tmax ~1–3 hours), and exposure generally increased with dose but was not always strictly dose-proportional.

Overall, these studies indicate that the drug's primary target organs are liver, adrenal, and reproductive tissues in rats, and liver changes (plus some emesis) in monkeys, with most effects reversible after treatment cessation.

2.5.4.3. Genotoxicity

All bacterial reverse mutation and micronucleus assays (*in vitro* and *in vivo*) demonstrated no genotoxic or clastogenic effects for the drug and its primary metabolite under the tested conditions. The highest achievable concentrations or doses did not induce mutagenic responses, and results were consistently negative across different test systems. These findings indicate a low genotoxicity concern for the drug in the evaluated assays.

2.5.4.4. Carcinogenicity

Results of the previous provided carcinogenicity study was provided to support the extension of indication. The safety margins remain acceptable.

In the 2-year rat carcinogenicity study, proliferative and sometimes neoplastic lesions were observed in the liver, thyroid, ovaries, and pancreas. Proliferative and neoplastic findings in the liver and thyroid likely resulted from CYP enzyme induction and chronic hepatocellular hypertrophy and were considered rodent-specific.

In the ovaries, an increased incidence and/or severity of granulosa and/or luteal/granulosa cell hyperplasia was noted at mitapivat AUC0-12 values well above the range observed in humans at 100 mg BID (86-fold).

Benign acinar hyperplasia and adenoma in the exocrine pancreas were observed at an increased incidence and/or severity in males from all dose groups (30, 100, and 300 mg/kg/day); a no-effect level was not determined. The incidence of the pancreatic findings was only outside the range observed historically in the test strain at 300 mg/kg/day (35-fold the human exposure).

2.5.4.5. Reproductive and developmental toxicity

The toxicological profile of mitapivat (target organs) in this study was similar to that defined in the adult rat studies with aromatase-related adverse effects noted on male and female animals.

In males, a delay in sexual maturation was observed at the two highest dose levels. However, the mean body weight recorded in these groups on the day of balano-preputial separation was similar to that of control animals, and body weight was shown to be decreased significantly in the high dose group from PND15. Therefore, the

biological relevance of this effect should be discussed taking also into consideration relevant data available with other aromatase inhibitors.

As regards the male reproductive system, histopathological findings in testes (dilation of seminiferous tubules) were noted at ≥30 mg/kg/day with secondary findings at ≥150 mg/kg/day in testes (degeneration/atrophy) and epididymides (cellular debris). These effects were associated with altered sperm quality at 300 mg/kg/day. In the reproductive phase males, reduced mating, fertility and pregnancy indices were observed at ≥150 mg/kg/day, as well as decreased implantations and increased post-implantation losses with subsequent reduced number of liveborn pups at 300 mg/kg/day. Following an off-dose period of 13 weeks (more than one spermatogenic cycle), histopathological findings at ≥150 mg/kg/day were only partially reversed and not associated with adverse effects on other endpoints. It is also noted that juvenile rats appear as more sensitive than adults for effects on the reproductive tract since histopathological findings were seen at lower dose/exposure levels, did not fully reverse, and were associated with adverse effects on mating and fertility indices.

In females, findings were similar to those reported in adult animals with mainly increased perinatal mortality at ≥50 mg/kg/day associated with dystocia/prolonged parturition and mortality in maternal animals. At 200 mg/kg, estrous cycle length was prolonged. These findings were shown to be reversible.

Regarding growth, the body weight of males on PND97 was decreased (-12.5%) vs. controls at 300 mg/kg/day. Despite an increase in body weight gain during the post-dosing period (+16% over PND 98-185, not statistically significant), body weight values remained significantly below those of control values on most occasions up to PND185 (-8.5%). It is therefore not fully clear that effects on body weights were fully reversible in males. It is noted that body weight values were also lower than those of control animals at 30 mg/kg/day during recovery (-10.1% vs controls on PND185) with a corresponding decrease in body weight gain (-33% over PND 98-195). In parallel, drug-related effects on bone densitometry values in femur metaphysis and diaphysis were reported at all doses, with significant differences vs. controls still noted in diaphysis at the end of recovery at ≥150 mg/kg/day. The MAH explains that these changes are not viewed as adverse due to their minimal nature and partial recovery. However, historical control values are not available for such parameters. In addition, the difference vs. control group for some parameters was even greater at the end of the non-dosing period compared to the end of dosing period (e.g. total area 14-16%, endosteal circumference 13-16%, CMSI 26-28%).

There was no reported significant drug-related effect on the % of live male pups/litter and anogenital distance of F2 pups (with limited number of litters available at ≥150 mg/kg/day in litters obtained from F1 males). The anogenital distance of female pups obtained from treated F1 females was significantly increased at 10 and 50 mg/kg/day, but this was not confirmed at 200/100 mg/kg/day.

2.5.4.6. Other toxicity studies

A non–GLP-compliant study was performed to evaluate the phototoxic potential of mitapivat as measured by the relative reduction in viability of BALB/c 3T3 mouse fibroblasts exposed to mitapivat and ultraviolet radiation as compared with the viability of fibroblasts exposed to the test article in the absence of ultraviolet radiation.

2.5.5. Ecotoxicity/environmental risk assessment

Table 6: Summary of main study results

Substance (INN/Invented Name): Mitapivat / Pyrukynd			
CAS-number (if available): Freebase: 1260075-17-9 Salt: 2151847-10-6			
PBT screening		Result	**Conclusion**
Bioaccumulation potential- log Dow (taken as log Kow equivalent)	OECD107/117/123	0.3 (pH 5), 1.8 (pH 7), 1.6 (pH 9) (all < 4.5)	Potential PBT: No
PBT-assessment			
Parameter	**Result relevant for conclusion**		**Conclusion**
Bioaccumulation	log K_{ow}	1.8 at pH 7 (max among reported values)	not B
	BCF	ND	not B
Persistence	DT50 Values are derived from the OECD 308 or OECD 307 study below and have been recalculated to 12°C or ready biodegradability	ND (no DT50 values available yet) (Phase II fate studies planned)	not P
Toxicity	NOEC or CMR	NOEC (Fish Sexual Development, OECD 234) = 100 µg/L	not T
PBT-statement :	The compound is considered to be not PBT, nor vPvB		
Phase I			
Calculation	**Value**	**Unit**	**Conclusion**
PEC_{sw}, default/refined	0.13	µg/L	≥ 0.01 threshold: Y
Other concerns (e.g. chemical class)	Off-target aromatase inhibition NOEC = = 100	µg/L	Yes (addressed by additional fish test)
Phase II Physical-chemical properties and fate			
Study type	**Test protocol**	**Results**	**Remarks**
No data yet; Phase II ongoing.			

Since the logDow values (0.3 at pH5, 1.8 at pH7 and 1.6 at pH9) are all below 4.5, there is no requirement to conduct an extensive PBT screening according to the guideline. In this respect, the conclusions of the document (non-PBT, non-vPvB substance) are in line with the regulatory requirements.

The calculated PEC surface water is 0.13µg/L, which exceeds the threshold of 0.01µg/L set to trigger Phase II. This step is in line with the guideline: the substance is therefore subject to additional Phase II studies to characterise its fate and toxicity in the environment.

Mitapivat has an aromatase inhibitory activity (off-target), which led to an advanced sexual development test in fish (OECD234).

The results of this test (overall NOEC of 100µg/L) suggest the absence of relevant effects at the population level at concentrations more than 1000 times higher than the PEC (0.13µg/L). In this respect, the approach goes beyond the basic studies commonly requested in Phase II and addresses the concern of possible endocrine effects.

In accordance with the guideline, Phase II normally includes standard tests (e.g. OECD201 on algae, OECD211 on Daphnia magna, OECD209 on activated sludge respiration, etc.), as well as environmental fate tests (OECD106 adsorption/desorption, OECD301 biodegradability, OECD308 in sediments, OECD307 in soils, etc.).

In the ERA, the MAH indicates that these studies are either planned or ongoing (but not yet completed).

2.5.6. Discussion on non-clinical aspects

This application concerns an extension of indication, encompassing an increased dose from a maximum of 50mg twice daily to 100 twice daily building upon an initial non-clinical package that was deemed acceptable for the already approved use.

Pharmacology

The *in vitro* proof of concept is translatable to thalassemia and thus is still acceptable. A new *in vivo* model was used for beta-thalassemia and shows therapeutic effects. No model was provided for alpha-thalassemia. However, both forms of the disease share key downstream pathophysiological features (such as globin chain imbalance, oxidative stress, and ATP depletion). Given the mode of action of mitapivat, which targets red blood cell metabolism independently of globin genotype, and in the absence of safety concerns specific to α-thalassaemia, no additional *in vivo* model is considered necessary.

In the context of this new indication, the higher dosage has led to reduced safety margins; however, these margins remain sufficiently positive in safety pharmacology studies. The previous safety pharmacology data provided for the initial application does cover the new posology of 100 mg film-coated tablets. For certain known toxicities (e.g., emesis), the margin is less than one, but is a known toxicity, acceptable in the clinical context.

Pharmacokinetics

Mitapivat is extensively metabolised in the liver with high clearance. All studies have evaluated mitapivat and its metabolite according to ICH M3 guideline. Mitapivat is primarily metabolised by CYP3A5 and CYP3A4 and is a substrate for P glycoprotein (P-gp). Mitapivat induces CYP3A4 and may also induce CYP2B6, CYP2C8, CYP2C9, CYP2C19 and uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1). Mitapivat may inhibit CYP3A4. Mitapivat may induce and inhibit P-gp.

In male rats, the dosed radioactivity was excreted via biliary, urinary, and faecal routes in similar amounts (approximately 30% each). In female rats, biliary excretion was the major route of elimination, accounting for 48.8% of the dose; in faeces and urine, the mean cumulative excretion was 30.4% and 13.9%, respectively.

Toxicology

Repeat-dose dose-limiting toxicities were observed at exposures ≥65-fold (rats) and ≥8.4-fold (monkeys) above human exposure. In monkeys, the main dose-limiting toxicities were emesis, inappetence, and weight loss, observed across single- and repeat-dose studies. Despite investigative off-target screening and microscopic evaluation, no specific underlying mechanism for these clinical signs was identified, and the effects were reversible. Findings consistent with aromatase inhibition occurred in rats during toxicology and reproductive/developmental studies; the drug exhibited an IC_{50} of 2.05 µM against human aromatase (human placental microsomes) and 0.493 µM against rat aromatase (rat ovarian microsomes). These effects appeared at exposures of AUC_{0-12} ≥2.3-fold and Cmax ≥3380 ng/mL relative to humans, and teratogenicity was seen at ≥48-fold human exposure. In juvenile rats, findings occurred at only 1.1-fold human exposure, likely reflecting the heightened rat sensitivity to sex hormone dysregulation and the lower rat IC_{50} value. The main metabolite

does not inhibit aromatase and thus is not implicated in reproductive or developmental effects. No comparable reproductive organ findings were noted in monkeys, albeit they did not tolerate the higher exposures reached in rats.

Both species (rats and monkeys) showed non adverse, reversible hepatocellular hypertrophy and/or increased liver weight in repeated-dose GLP studies, interpreted as an adaptive CYP induction. However, in the 9-month monkey study, this adaptation contributed to adverse subcapsular hepatocellular necrosis, unaccompanied by elevated liver enzymes. Rats also presented mild hematologic shifts (e.g., lower absolute reticulocytes, decreased HDW/RDW), likely reflecting decreased erythropoiesis secondary to prolonged RBC lifespan. Reversible adrenal gland changes included lower gland weights and zona glomerulosa vacuolation in rats, whereas monkeys occasionally showed higher adrenal weights and zona fasciculata hypertrophy.

Genotoxicity

The drug was negative for genotoxicity in bacterial reverse mutation (Ames), *in vitro* human lymphocyte micronucleus, and *in vivo* rat bone marrow micronucleus assays. The metabolite was similarly tested (including formation in S9 fractions) and found non-mutagenic and non-clastogenic.

Carcinogenicity

Benign acinar hyperplasia and adenoma in the exocrine pancreas were observed at an increased incidence and/or severity in males from all dose groups (30, 100, and 300 mg/kg/day); a no-effect level was not determined. Based on a weight of evidence assessment, the pancreatic findings do not alter the benefit-risk assessment for mitapivat. Specifically, due to the benign nature of the changes, similarity to findings observed spontaneously in the test system, differences in molecular pathogenesis of proliferative pancreatic findings in rats and humans, and the very low likelihood that on-target mechanisms are driving the findings, it is concluded that the rat pancreatic findings do not represent a significant risk.

The drug did not exhibit phototoxic potential in a GLP-compliant neutral red uptake assay.

Reproductive and developmental toxicity

In embryo-foetal development studies, foetal adverse events were observed at AUC0-12 values 48-fold (rats) and 2.4-fold (rabbits) above the human AUC0-12hr value at the MRHD. Findings are reflected in section 5.3 of the SmPC. Drug-related effects on bone densitometry values in femur metaphysis and diaphysis were reported at all doses, with significant differences vs. controls still noted in diaphysis at the end of recovery at ≥150 mg/kg/day. Overall, it is not certain that such changes should be viewed as non-adverse and non-relevant to humans considering also the adverse bone effects reported in the clinical setting with aromatase inhibitors (bone loss) and the data suggesting aromatase inhibition in patients treated with mitapivat, respectively. In female animals treated at ≥50 mg/kg, a persistent increase in body weight was reported with reversible changes in bone mass/density at femur metaphysis.

Section 4.6 of the SmPC specifically mention that Pyrukynd is not recommended during pregnancy and in women of childbearing potential not using contraception. Therefore, women of childbearing potential should avoid becoming pregnant while receiving Pyrukynd. Women of childbearing potential should use contraception during treatment with Pyrukynd and for at least 1 month after the last dose.

Environmental risk assessment

Finally, an updated ERA was provided in which phase II studies are still ongoing, but the MAH was requested to explicitly commit to conducting by December 2026 further persistence testing (OECD 307, 308 and/or 309) whether the substance is not readily biodegradable. The absence of results from these key tests means that a

comprehensive risk assessment covering all compartments (water, sediment, soil, sludge, etc.) is not yet available. The Phase II ERA studies for mitapivat are currently ongoing and the MAH commits to submitting a revised ERA to EMA in Q4 2026 following completion of these studies.

As a result of the above considerations, the available data do not allow to conclude definitively on the potential risk of mitapivat to the environment.

The MAH commits to perform the following studies by December 2026 as follow-up measures: OECD 307, 308 and/or 309.

2.5.7. Conclusion on the non-clinical aspects

A comprehensive nonclinical assessment has been conducted to support mitapivat for adult patients with thalassemia, leveraging existing data for pyruvate kinase deficiency.

Newly added *in vivo* pharmacology studies in a mouse model support Mitapivat's mechanism of action, and safety margins have been revised for the recommended dose of 100 mg BID.

All the safety findings have been adequately reflected in the SmPC. An updated environmental risk assessment, including the full set of Phase II studies and any potential required follow-up on persistence testing will be provided by December 2026 as committed by the MAH.

2.6. Clinical aspects

2.6.1. Introduction

GCP aspects

The clinical trials were performed in accordance with GCP as claimed by the MAH

The MAH has provided a statement to the effect that clinical trials conducted outside the community were carried out in accordance with the ethical standards of Directive 2001/20/EC.

- **Tabular overview of clinical studies**

Study Number/ Status	Study Description	Number of Sites/ Country(ies)	Study Drug Dose(s) and Regimen(s) (duration)	Number of Randomized (Treated) Subjects	Primary Data Included in the Evaluation
Pivotal Studies					
AG348-C-017/ Ongoing	Phase 3, multicenter, randomized, double-blind, placebo-controlled study of mitapivat in adults with non–transfusion-dependent α- or β-thalassemia	79/ Brazil, Bulgaria, Canada, Denmark, France, Germany, Greece, Italy, Lebanon, Malaysia, Netherlands, Saudi Arabia, Spain, Taiwan, Thailand, Turkey, UK, US, UAE	Mitapivat tablets 100 mg or placebo, BID (Double-blind Period: 24 weeks; OLE Period: up to 5 years, and a 1-week dose taper)	Mitapivat arm: 130 (129) subjects Placebo arm: 64 (63) subjects	Efficacy and safety data from Double-blind Period
AG348-C-018/ Ongoing	Phase 3, multicenter, randomized, double-blind, placebo-controlled study of mitapivat in adults with transfusion-dependent α- or β-thalassemia	81/ Brazil, Bulgaria, Canada, Denmark, France, Germany, Greece, Italy, Lebanon, Malaysia, Netherlands, Saudi Arabia, Spain, Taiwan, Thailand, Turkey, UAE, UK, US	Mitapivat tablets 100 mg or placebo, BID (Double-blind Period: 48 weeks; OLE Period: up to 5 years, and a 1-week dose taper)	Mitapivat arm: 171 (172)[a] subjects Placebo arm: 87 (85) subjects	Efficacy and safety data from Double-blind Period
Supportive Study					
AG348-C-010/ Ongoing	Phase 2, multicenter, open-label study evaluating the efficacy, safety, pharmacokinetics, and pharmacodynamics of mitapivat in adults with non–transfusion-dependent thalassemia	4/ US, Canada, UK	Mitapivat tablets 50 mg BID followed by optional dose increase to 100 mg, BID (Core Period: 24 weeks; Extension Period: up to 10 years; and a 2-week dose taper)	20 subjects treated (single-arm study)	Efficacy data from the Core Period and safety data from the Core and Extension Periods

2.6.2. Clinical pharmacology

2.6.2.1. Pharmacokinetics

Mitapivat (AG-348 or Pyrukynd) is a first-in-class, orally bioavailable, potent, allosteric activator of wild-type red blood cell (RBC)-specific form of pyruvate kinase (PKR) and a range of mutant PKR enzymes.

Mitapivat is currently indicated for the treatment of PK deficiency (PKD) in adult patients. In Europe three strengths of mitapivat film-coated tablets 5, 20 and 50 mg are available.

In the current submission, the MAH submitted:

- A line extension application for Pyrukynd, 100 mg tablet associated with a new orphan indication for the treatment of adult patients with non-transfusion-dependent and transfusion-dependent alpha- or beta-thalassemia (nTDT-TDT α/β-Th). The new tablet strength is not applicable for the initial PK deficiency indication.

To support this new formulation and indication, four additional studies, Population PK and ER analysis updates and PBPK simulations were submitted.

- Results of the PK study investigating the hepatic impairment effect on mitapivat PK (Study AG348-C-024) and the associated SmPC updates to fulfil a PAM from the initial MAA.

Therefore, section 4.2 and 5.2 of the currently SmPC of mitapivat for the PK deficiency indication is updated accordingly.

For the new indication nTDT-TDT α/β-Th, the recommended dose is 100 mg BID.

The pharmacokinetic (PK) properties of mitapivat were characterised in the initial MAA. In addition to study AG348-C-024, for the new indication and the new strength, four additional studies, Population PK and ER analysis updates and PBPK simulations were performed.

Pharmacokinetics

Bioanalysis

Mitapivat quantification in human plasma was performed using a validated UPLC coupled with MS/MS methods conducted. This method has been already described as part of the initial MAA and was used for studies **AG348-C-010/017/018/024** and are considered acceptable.

Absorption

Following multiple dose of mitapivat at 100 mg BID in α- or β-NTDT patient (Study **AG348-C-017**), absorption was reasonably rapid with Cmax approximately achieved at median Tmax of 1 h, geometric mean Cmax and AUCtau were 1565 ng/mL and 4262 ng.h/mL respectively.

Following multiple dose of mitapivat at 100 mg BID in α- or β-TDT patient (Study **AG348-C-018**), absorption was reasonably rapid with Cmax approximately achieved at median Tmax of 1 h, geometric mean Cmax and AUCtau were 1603 ng/mL and 4365 ng.h/mL respectively.

Relative bioavailability/ Bioequivalence

A relative bioavailability study (**AG348-C-021**) was performed in a crossover design between the new tablet dosage strength of 100 mg vs the past tablet strength 50 mg. Results of this study indicated that both formulations are bioequivalent. The absolute bioavailability after a single dose was approximately 73%.

Distribution

Mitapivat is highly protein bound (97.7%) in plasma with low RBC distribution. The mean volume of distribution (Vz) was 135 L. From the PPK analysis Vc/F was estimated at 45.7L, V3/F fixed at 105 L and V4/F at 6.9 L.

Elimination

Mitapivat has a mean t1/2 ranging from 16.2 to 79.3 hours following single oral dose administrations (5 to 2 500 mg) under fasted conditions to healthy subjects. Population pharmacokinetics derived median CL/F at steady state was 11.5, 12.7 and 14.4 L/h for the 5 mg twice daily, 20 mg twice daily, and 50 mg twice daily regimens, respectively.

After a single oral administration of radiolabelled mitapivat to healthy subjects, the total recovery of administered radioactive dose was 89.1%, with 49.6% in the urine (2.6% unchanged) and 39.6% in the faeces (less than 1% unchanged). Based on the PPK analysis, steady-state clearance was estimated at 17.7 L/h (90%CI: 9.87-32.8 L/h) with a 100 mg BID regimen in subjects with thalassemia, consistent with the estimate for PKD subject (16.4 L/h, 9.6-23.9 L/h).

Dose proportionality and time dependencies

As part of the initial MAA for PKD patients, dose proportionality has been demonstrated from 5 to 50 mg BID.

As part of this submission, a new analysis was performed integrating PK parameters pooled from 8 clinical studies (**AG348-C-002/003/006/007/011** from HV/PKD and **AG348-C-010/017/018** from Thalassemia). Results from this analysis indicate that AUC and Cmax increased in a less than dose proportional manner from 5 to 100 mg BID in HV, PKD and Thalassemia.

Pharmacokinetic in the target Population

The PK of mitapivat in patients with α- or β-NTDT was evaluated in studies **AG348-C-010** and **AG348-C-017**, and patient with α- or β-TDT in study **AG348-C-018**.

Study **AG348-C-017** was a Phase 3, double-blind, randomized placebo-controlled, multicenter study to evaluate the efficacy and safety of multiple oral doses of mitapivat in adult subjects with α- or β-NTDT. The study was divided into a 24-week Double-blind Period followed by an OLE Period (up to 5 years).

During the Double-blind Period, 194 subjects with α- or β-NTDT were randomized 2:1 to receive either 100 mg BID mitapivat or placebo for 24 weeks. Randomization was stratified by baseline Hb concentration and thalassemia genotype.

Mean Plasma concentration time profiles are presented in *Figure 1* and PK parameter estimates in *Table 1*.

Figure 1: Mean (SD) plasma concentration-time profiles of mitapivat after multiple oral dose in patients with α- or β-NTDT

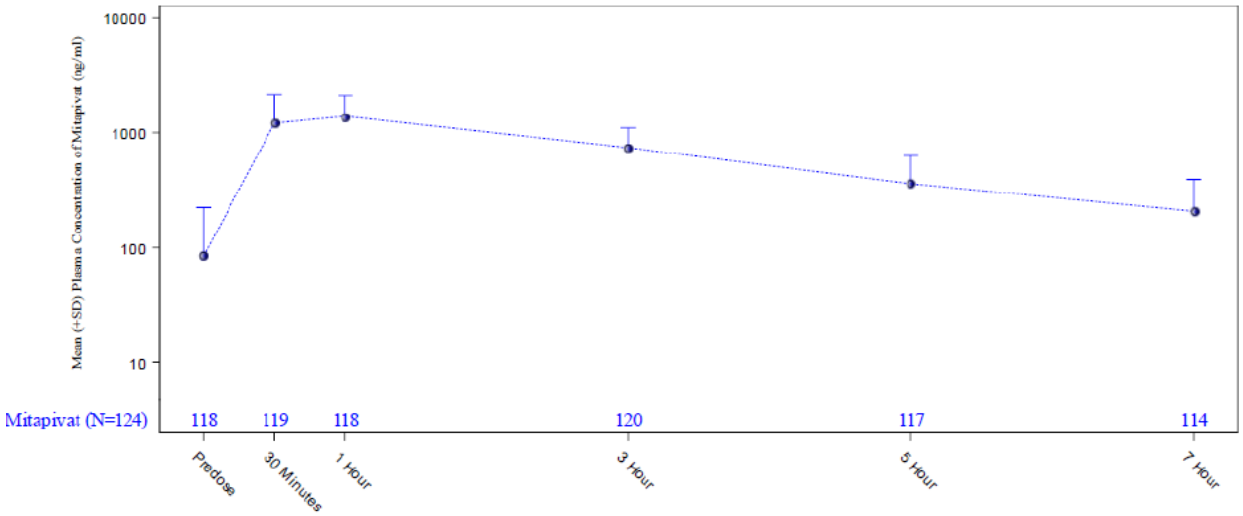


Table 2: Summary of plasma PK parameters of Mitapivat (Week 20)

Pharmacokinetic Parameters	Mitapivat (N=124)
$AUC_{0\text{-last}}$ (hr•ng/mL)[a]	4,262.57 (31.4); 114
t_{last} (hr)[b]	6.825 (6.50, 7.42); 114
C_{max} (ng/mL)[a]	1,565.69 (42.4); 121
t_{max} (hr)[b]	1.000 (0.20, 5.00); 121
C_{last} (ng/mL)[a]	166.93 (80.1); 121

Study **AG348-C-018** was a Phase 3, double-blind, randomized placebo-controlled, multicenter study to evaluate the efficacy and safety of multiple oral doses of mitapivat in adult subjects with α- or β-TDT. The study was divided into a 48-week Double-blind Period followed by an OLE Period (up to 5 years).

During the Double-blind Period, 258 subjects with α- or β-TDT were randomized 2:1 to receive either 100 mg BID mitapivat or placebo for 48 weeks. Randomization was stratified by geographical region and thalassemia genotype.

Mean Plasma concentration time profiles are presented in *Figure 2* and PK parameter estimates in *Table 2*.

Figure 2: Mean (SD) plasma concentration-time profiles of mitapivat after multiple oral dose in patients with α- or β-TDT

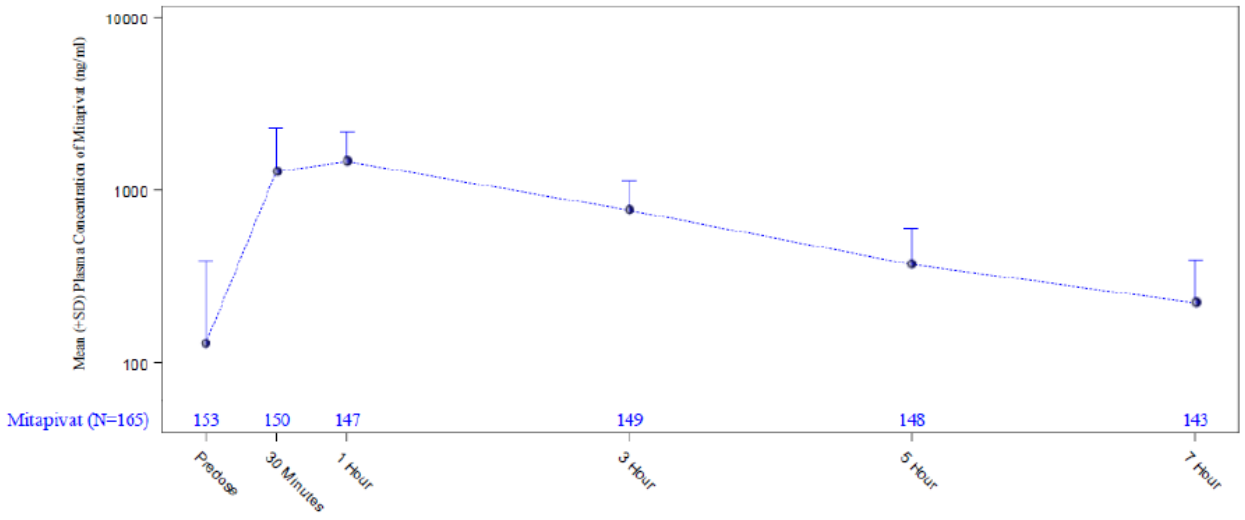


Table 7: Summary of plasma PK parameters of Mitapivat (Week 20)

Pharmacokinetic Parameters	Mitapivat (N=165)
$AUC_{0\text{-last}}$ (hr•ng/mL)[a]	4,365.84 (35.9); 141
t_{last} (hr)[b]	6.750 (4.83, 7.17); 147
C_{max} (ng/mL)[a]	1,603.00 (45.6); 149
t_{max} (hr)[b]	1.000 (0.42, 5.00); 149
C_{last} (ng/mL)[a]	175.76 (85.1); 147

Population PK analysis

One population PK analysis (PPK, Report ag348-pmx-005) aiming to characterize the PK of mitapivat in the target population (thalassemia) and identifying/quantifying source of variability was developed. From this analysis, predicted exposure metrics were used as input of subsequent ER.

The PPK of mitapivat was based on PK results pooling 3 clinical studies (**AG348-C-010**, **017** and **018**). A total of 309 subjects contributing to 2369 PK samples were used. The final PPK model has generally the same structure to the one developed for PKD subjects. The main change relies on the modelling of the autoinduction process parameterized by an Emax model where EC50 is the mitapivat concentration associated with 50% of the maximum change in elimination rate due to autoinduction. Table 8 provided the final PK parameter estimates of the final PPK model and Figure 3 and Figure 4 the associated GOF plots and pcVPC, respectively.

Table 8: Population PK parameter estimates for the final Model

	Parameter	Untransformed[a]	Transformed[a]			Shrinkage
		Estimate	Estimate	%RSE	90% CI	
1	CL/F (L/hr) on Day 1	2.40	11.0	1.30	10.3, 11.7	--
2	V_2/F (L)	3.82	45.7	0.693	43.4, 48.2	--
3	Q_3/F (L/hr)	1.26	3.54	17.5	2.30, 5.45	--
4	V_3/F (L)	4.65 (FIX)	105 (FIX)	--	--	--
5	Q_4/F (L/hr)	0.507	1.66	4.69	1.59, 1.74	--
6	V_4/F (L)	1.93	6.88	0.874	6.66, 7.11	--
7	ALAG1 (hr)	-1.39 (FIX)	0.249 (FIX)	--	--	--
8	K_a (1/hr)	1.69	5.43	12.1	3.64, 8.10	--
9	F1	-0.001 (FIX)	1.00 (FIX)	--	--	--
10	E_{max} (fraction of 1)	-0.001 (FIX)	1.00 (FIX)	--	--	--
11	EC_{50} (ng/mL)	3.26	26.1	30.6	3.68, 1.85	--
12	Male sex on V_2/F	0.166	1.18	22.1	1.10, 1.27	--
13	Weight on V_2/F	0.122	1.13	17.5	1.08, 1.18	--
14	Weight on V_3/F	1.26	3.52	32.5	1.58, 7.86	--
15	Weight on V_4/F	0.137	1.15	16.1	1.10, 1.20	--
Interindividual Variability (ω^2)						
IIV on CL/F		0.0926	12.7% CV			23.6%
IIV on V_2/F		0.00147	1.00% CV			89.2%
IIV on Q_3/F		2.85	134% CV			45.5%
IIV on V_3/F		7.22	57.7% CV			60.1%
IIV on K_a		3.44	110% CV			27.8%
IIV on EC_{50}		15.6	121% CV			42.8%
Residuals						
Proportional error		0.279	52.8%			
Additive error		1.26	1.12 ng/mL			
OFV		27369.2				
CN		65.5				

Figure 3: Diagnostic plots for the final PPK model

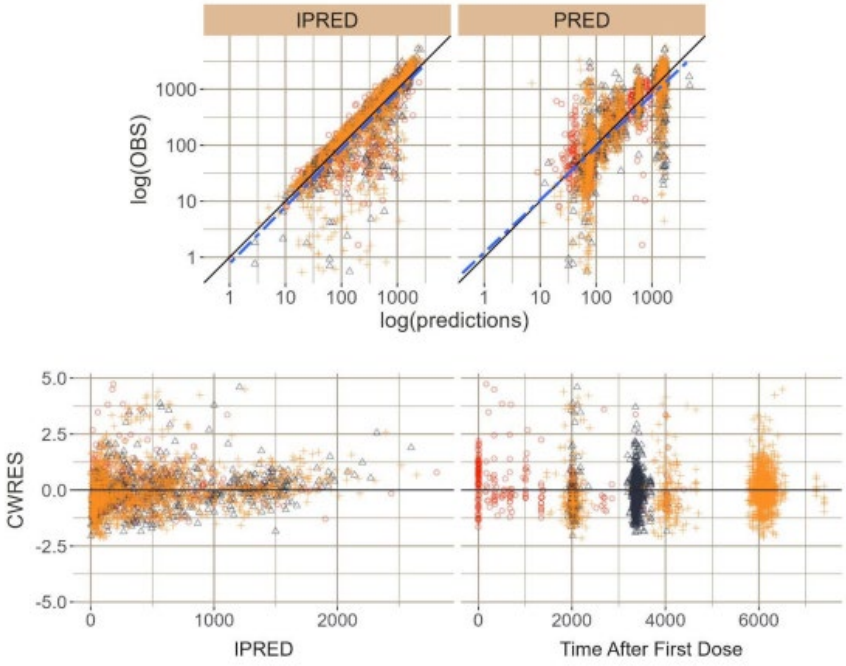
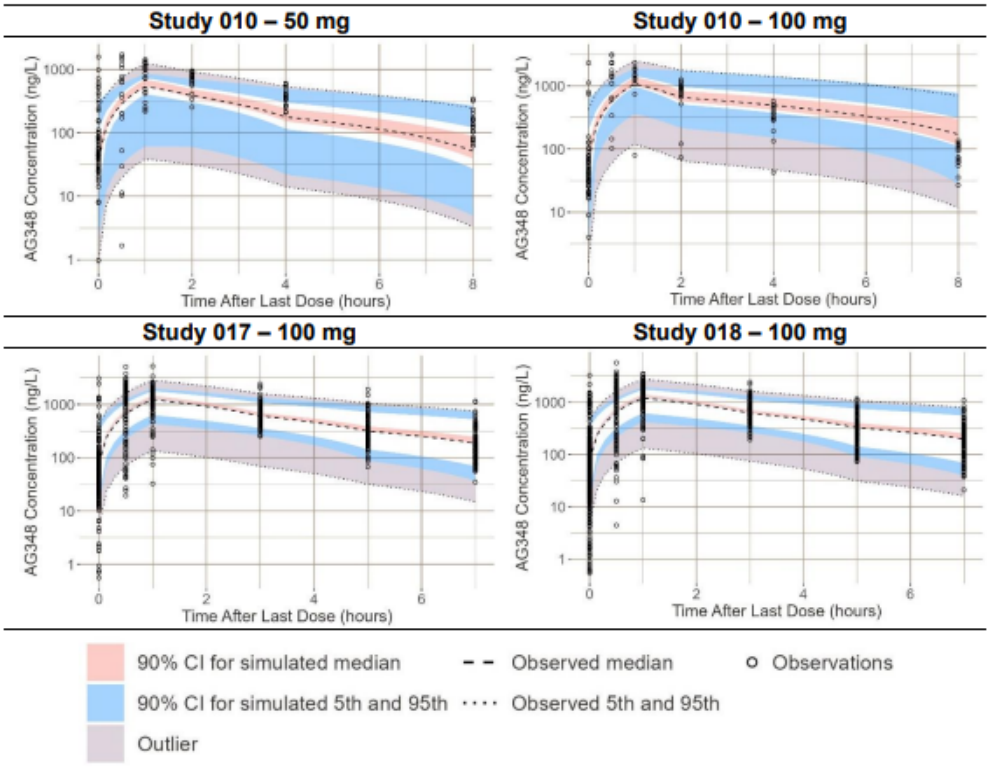


Figure 4: pcVPC for the final PPK model by study and Dose



Special populations

Renal impairment

No formal PK study investigating renal impairment on mitapivat PK was performed. Besides the effect of renal impairment on the PKs of mitapivat was investigated in patients based on the PPK model and no effect was found.

Hepatic impairment

A formal PK study (**AG348-C-024**) investigating the effect of moderate hepatic impairment on mitapivat PK was performed. In subjects with moderate impaired hepatic (MIH) function, following a 50 mg dose, Cmax was unaffected but AUCinf increased by 36% and AUCinf,u by 45% compared to normal hepatic subjects. No dose adjustment in mild or moderate impaired hepatic subjects is warranted.

A PBPK model was developed to provide an explanation to the unexpected results from the formal PK study **AG348-C-024**, which were attributed to a decrease of the fa (fraction absorbed) in moderate hepatic impairment subject compared to healthy volunteers. Then simulations were performed for alternative dosing regimen 100 mg QD, 50 mg BID or 100 mg BID, and it was predicted for a 50 mg or 100 mg BID, a 47% increase of AUCinf without a modification of Cmax in MIH vs HV.

Gender

No formal PK study investigating gender on mitapivat PK was performed. Besides the effect of gender on the PKs of mitapivat was investigated in patients based on the PPK model. Gender was found to have a significant effect on V2/F. Male have a 18% increase V2/F compared to female, however gender did not appear to have a meaningful impact on mitapivat exposure.

Race

Race effect on mitapivat PK has been investigated as part of the initial MAA for PKD subjects and no effect was found.

Weight

Weight was a statistically significantly covariate on all apparent volumes of distribution. Relative to a 70 kg subject, higher Cmax (1.24-fold) and lower Ctrough (0.65-fold) were predicted for a 45 kg subject (5th percentile). Conversely, lower Cmax (0.92-fold) and slightly higher Ctrough (1.06-fold) were predicted for an 85 kg subject (95th percentile), relative to a 70 kg subject.

When combining the effect of weight and gender, a female subject weighing 45 kg was predicted to have a 1.40-fold higher Cmax, a 0.59-fold lower Ctrough, and the same AUC as a 70 kg male subject. Conversely, a female subject weighing 85 kg had no meaningful difference in mitapivat exposure compared to a 70 kg male subject.

Age

Based on the PPK analysis, age was not found to have a significant effect on mitapivat PK. Seven subjects had an age ≥ 65 years.

Paediatric

The PK of mitapivat in children and adolescent patients less than 18 years old have not been studied.

ER-analysis

For ER-efficacy-Hb, most of the subjects from the placebo group were not responder. Without the placebo group there are no trend between PK parameter exposure and probability of response.

For ER-efficacy-TRR, 13% of the subject from the placebo group were responder vs 30.6% in the treated group with mitapivat. Without the placebo group there are no trend between PK parameter exposure and probability of response.

For ER-safety the same behaviour as already described for PKD subjects for the effect of mitapivat on male hormone (testosterone, free testosterone and estrone) was observed. However, for hot flush and insomnia which are of particular interest in the subjects treated by mitapivat vs placebo subjects no significant ER relationship was identified.

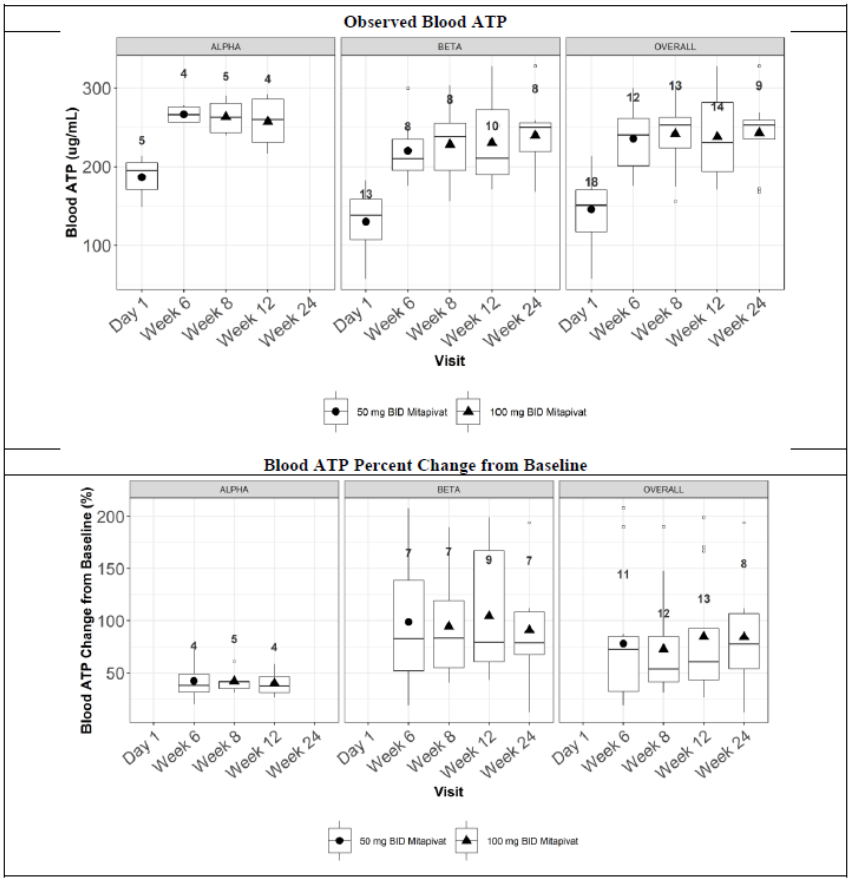
Dose rationale

In the Phase 2 study **AG348-C-010**, both the 50 mg BID and 100 mg BID dose were investigated in subjects with NTDT. The 100 mg BID dosing regimen was selected over the 50 mg BID based given its slightly better effect to increase ATP production 84.7% (Week 24) vs 78.2% (Week 6) as shown in *Figure 3* and Table 9. Furthermore, more Hb responders were observed in the 100 mg BID cohort compared to the 50 mg BID dosing cohort.

Table 9: Descriptive statistics of ATP level at baseline and following multiple dose of mitapivat, per disease status

Thalassemia Type	Statistic	Observed					Change from Baseline				
		50 mg BID		100 mg BID			50 mg BID		100 mg BID		
		Day 1	Week 6	Week 8	Week 12	Week 24	Day 1	Week 6	Week 8	Week 12	Week 24
α	N	5	4	5	4	1	NC	4	5	4	1
	Mean	186.8	266.8	263.4	257.3	269.0	NC	42.30	42.18	40.10	37.90
	SD	26.537	11.325	22.255	36.491	NC	NC	21.996	11.509	13.774	NC
β	N	13	8	8	10	8	NC	7	7	9	7
	Mean	130.1	220.3	228.3	230.2	239.6	NC	98.77	94.53	104.5	91.10
	SD	38.152	40.252	48.779	55.085	51.242	NC	73.458	55.262	58.677	55.983
Overall	N	18	12	13	14	9	NC	11	12	13	8
	Mean	145.9	235.8	241.8	237.9	242.9	NC	78.24	72.72	84.66	84.45
	SD	43.301	39.877	43.242	50.684	48.922	NC	64.765	49.402	57.435	55.137

Figure 3: Box-plots of blood pre-dose ATP (observed and percent change from baseline) concentration vs Visit at baseline (Day 1) and following Multiple dose of 50 mg BID (Week 6) or 100 mg BID (Week 8 to 24) by Disease status



PK interactions

The potential for clinical drug-drug interactions (DDIs) involving mitapivat as an object of CYP3A4, precipitant of metabolizing enzymes (CYP3A4, CYP2B6, CYP2C8, CYP2C9, CYP2C19, or UGT1A1), or precipitant of transporters (P-gp, OATP1B1, OAT3, OCT2, or MATE1) at 100 mg twice daily (BID), the recommended dose for patients with thalassemia, is expected to be similar to the DDIs at 50 mg BID, the highest recommended dose for patients with PK deficiency.

Mitapivat as a Precipitant – Effect on CYP Enzyme Substrates

In vitro studies have shown that mitapivat is a weak mechanism-based inhibitor and an inducer of CYP3A4 (Report AG348-N-054-R1 and Report AG348-N-055-R1). In addition, mitapivat appears to be an inducer of CYP2B6 (Study Report AG348-N-055-R1), CYP2C8, CYP2C9, and a weak mechanism-based inhibitor and an inducer of CYP2C19 (Report AG348-N-103 and Study Report AG348-N-054-R1); however, based on the static model, there is no risk of DDI due to inhibition of CYP2C19 at clinically relevant plasma concentrations of mitapivat, both at 50 mg BID and 100 mg BID. Results from Study 027 indicate that coadministration of mitapivat 100 mg BID and 2 mg single-dose midazolam leads to decreases in the AUC_{∞} and C_{max} of midazolam by approximately 53% and 37%, respectively, compared to midazolam administered alone (Table 5). None of the 90% CIs for the ratio of geometric least square means (GLSMs) were contained within

the predefined interval (0.8, 1.25); therefore, these differences are statistically significant with respect to the effect of mitapivat on the exposure of midazolam. Based on ICH M12, the decrease of AUC of the sensitive index CYP substrate by ≥ 50% to < 80% classifies mitapivat as a moderate CYP3A4 inducer.

Table 10: PBPK Simulations and Study 027 Geometric Mean AUC_{∞} and C_{max} Ratios for Single Oral Dose Midazolam in the Presence and Absence of Mitapivat (BID Dose) in Healthy Subjects

	Mitapivat Dose	Geometric Mean Ratio (90% Confidence Intervals)	
		$AUC_{(0-\infty)}$ (h.ng/mL)	C_{max} (ng/mL)
PBPK simulation (AG438-PBPK-001)[a]	100 mg BID	0.35 (0.33, 0.37)	0.41 (0.39,0.43)
Clinical DDI study (AG348-C-027)	100 mg BID	0.47 (0.42, 0.54)	0.63 (0.55, 0.71)

Source: AG348-PBPK-001 Table 27; Study 027 CSR Table 7.
Abbreviations: AUC_{∞}=area under the concentration-time curve extrapolated to infinity; BID=twice daily; C_{max}=maximum concentration; CYP=cytochrome P450; MBI=mechanism-based inactivation.
Notes: For PBPK simulation and clinical DDI study, mitapivat was dosed to steady state, and the CYP3A4 index substrate, midazolam single oral dose was administration with or without coadministration of mitapivat in healthy subjects. PBPK simulation used 5 mg dose of midazolam and clinical DDI study used 2 mg dose of midazolam.
[a] Simulation considered the combined CYP3A4 MBI and induction effects.

Mitapivat as a Precipitant – Effect on Transporter Substrates

Mitapivat inhibited P-gp with concentration that results in 50% inhibition (IC_{50}) of 12.8 µM in vitro (Report AG348-N-057-R1) and has a potential to induce P-gp based on the shared mechanism of induction with CYP3A4. Based on the static model (I_{gut} [intestinal concentration]/IC_{50}>10), there is a potential for DDIs with P-gp substrates, both at mitapivat 50 mg BID and mitapivat 100 mg BID. Mitapivat inhibits OATP1B1, OAT3, OCT2, and MATE1, with IC_{50} values of 29.0, 12.1, 7.76, and 7.17 µM, respectively (Report AG348-N-089 and Report AG348-N-097). Based on the static models, there is no risk of DDIs with substrates of these transporters at the clinically relevant plasma concentrations of mitapivat, both at 50 mg BID and 100 mg BID.

In vitro metabolism studies indicate that mitapivat is predominantly metabolized by CYP3A4/5. In Study AG348-C-012, itraconazole, a strong CYP3A4 inhibitor, increased mitapivat exposure (AUC_{∞} by 4.9-fold, C_{max} by 1.7-fold) after administration of a single 20 mg dose of mitapivat with itraconazole, whereas, rifampin, a strong CYP3A4 inducer, decreased mitapivat exposure (AUC_{∞} by 91%, C_{max} by 77%) after administration of a single 50 mg dose of mitapivat with rifampin (AG348-C-012 CSR).

Mitapivat as an Object – Effect of CYP3A4 Inhibitors and Inducers

In vitro metabolism studies indicate that mitapivat is predominantly metabolized by CYP3A4/5. In Study AG348-C-012, itraconazole, a strong CYP3A4 inhibitor, increased mitapivat exposure (AUC_{∞} by 4.9-fold, C_{max} by 1.7-fold) after administration of a single 20 mg dose of mitapivat with itraconazole, whereas, rifampin, a strong CYP3A4 inducer, decreased mitapivat exposure (AUC_{∞} by 91%, C_{max} by 77%) after administration of a single 50 mg dose of mitapivat with rifampin (AG348-C-012 CSR).

Results of the PBPK model predictions on the outcomes of interaction between mitapivat and fluconazole after mitapivat multiple doses (14 days) are summarised in Table 6. Moderate DDI effects (AUC_{0-T} ratio of 2.70)

were predicted with fluconazole treatment when mitapivat 100 mg twice daily (BID) was used. Reducing the mitapivat dosing frequency from 100 mg BID to 100 mg once daily (QD) when co-administered with fluconazole provided mitapivat exposures ($AUC_{0-\tau}$ and C_{max}) that were ≤1.54-fold of the mitapivat exposures achieved with 100 mg BID mitapivat without fluconazole. An increase in exposure by approximately 50% is deemed acceptable based on the exposure-safety analysis (Study Report AG348-PMX-005). Therefore, when concomitant use of a moderate CYP3A4 inhibitor with mitapivat is unavoidable, reducing mitapivat dose to 100 mg QD is recommended to minimise the risk of adverse reactions.

Table 11: Predicted Geometric Mean $AUC_{0-\tau}$ and C_{max} Ratios for Mitapivat Multiple Doses (14 days) in the Absence and Presence of a Moderate CYP3A4 Inhibitor in Healthy Subjects

CYP3A4 modulator	Treatment Simulations	$AUC_{0-\tau}$ ratio	C_{max} ratio
Fluconazole (moderate inhibitor)	100 mg **BID** mitapivat with fluconazole/ 100 mg BID mitapivat without fluconazole	2.70	1.66
Fluconazole (moderate inhibitor)	100 mg **QD** mitapivat with fluconazole/ 100 mg BID mitapivat without fluconazole	1.43	1.54

Abbreviations: $AUC_{0-\tau}$=area under the concentration-time curve from 0 to the end of the dosing interval; BID=twice daily; C_{max}=maximum concentration; CYP=cytochrome P450; QD=once daily.
Notes: Simulations used the following dosing regimens for the CYP3A4 modulator: fluconazole 200 mg QD with a loading dose of 400 mg on Day 1 (14 days). Values are ratios of geometric means.

Results of the PBPK model-based predictions on the outcomes of interaction between mitapivat and efavirenz after mitapivat multiple doses (14 days) are summarized in table 12. Moderate DDI effects ($AUC_{0-\tau}$ ratio of 0.48) were predicted when mitapivat 100 mg BID was administered with efavirenz. Increasing the dose of mitapivat from 100 mg BID to 200 mg BID when co-administered with efavirenz provided mitapivat exposures ($AUC_{0-\tau}$ and C_{max}) that were ≤1.52-fold of the mitapivat exposures achieved with 100 mg BID mitapivat without efavirenz.

Despite comparable mitapivat exposures between 100 mg BID mitapivat without efavirenz and 200 mg BID mitapivat with efavirenz, the MAH does not advise exceeding the recommended dose of 100 mg BID in any instance, including when used concomitantly with a CYP3A4 inducer, since doses above 100 mg BID of mitapivat have not been evaluated clinically in patients with thalassemia.

Table 12: Predicted Geometric Mean $AUC_{0-\tau}$ and C_{max} Ratios for Mitapivat Multiple Doses (14 days) in the Absence and Presence of a Moderate CYP3A4 Inducer in Healthy Subjects

CYP3A4 modulator	Treatment Simulations	$AUC_{0-\tau}$ ratio	C_{max} ratio
Efavirenz (moderate inducer)	**100** mg BID mitapivat with efavirenz/ 100 mg BID mitapivat without efavirenz	0.48	0.79
Efavirenz (moderate inducer)	**200** mg BID mitapivat with efavirenz/ 100 mg BID mitapivat without efavirenz	0.93	1.52

Abbreviations: $AUC_{0-\tau}$=area under the concentration-time curve from 0 to the end of the dosing interval; BID=twice daily; C_{max}=maximum concentration; CYP=cytochrome P450; QD=once daily.
Notes: Simulations used the following dosing regimens for the CYP3A4 modulator: efavirenz 600 mg QD (14 days). Values are ratios of geometric means.

2.6.2.2. Pharmacodynamics

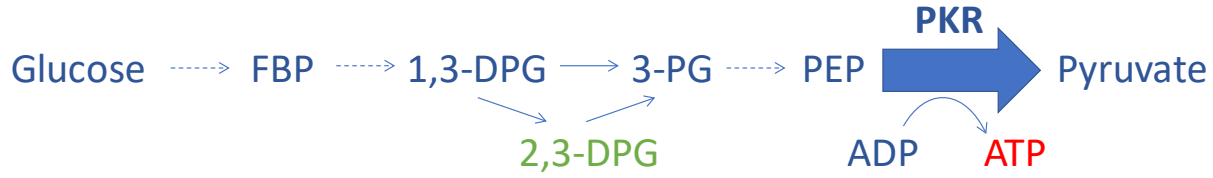
Mechanism of action

Pyruvate kinase R is one of 4 pyruvate kinase isoenzymes expressed in human tissues by 2 separate genes, *PKLR* and *PKM*. Both PKR and PKL are splice isoforms of the *PKLR* gene, while PKM1 and PKM2 are expressed from the *PKM* gene. Pyruvate kinase muscle isozyme 1 is a constitutively active isoform expressed in the brain, muscle, and heart that is not known to be allosterically regulated or influenced by posttranslational modifications (Mazurek, 2011).

The PKM2 splice isoform is expressed in the lung, adipocytes, epithelial cells, and proliferating cells, including embryonic cells and adult stem cells, and is regulated by allosteric binding to different metabolites (including FBP] [activating] and several amino acids [both activating and inhibitory]) and through posttranslational modifications (including phosphorylation and acetylation, both inhibitory) (Mazurek, 2011). Pyruvate kinase L is expressed in the liver and kidney and in parts of the small intestine, and PKR is expressed in erythroblasts and mature erythrocytes (Miwa and Takegawa, 1983). Pyruvate kinase R and PKL are known to be activated by FBP and thought to be inhibited by phosphorylation.

Pyruvate kinase enzymatically catalyzes the final step in glycolysis, the metabolic conversion of PEP and adenosine diphosphate into pyruvate and ATP (Figure 6). Mature RBCs rely on the process of glycolysis to generate ATP; thus, PKR is a key enzyme for maintaining energy homeostasis in RBCs (van Wijk and van Solinge, 2005). The increased generation of ATP resulting from mitapivat-activated PKR supports normal cellular mechanisms that reduce ROS damage in the bone marrow and circulation and improve RBC longevity and energy homeostasis, hemolysis, ineffective erythropoiesis, and iron overload associated with thalassemia.

Figure 6: Role of PKR in Glycolysis in Red Blood Cells



Abbreviations: 1,3-DPG=1,3 diphosphoglycerate; 2,3-DPG=2,3 diphosphoglycerate; 3-PG=3-phosphoglycerate; ADP=adenosine diphosphate; ATP=adenosine triphosphate; FBP=fructose 1,6-bisphosphate; PEP=phosphoenolpyruvate; PKR=red blood cell−specific form of pyruvate kinase.Note: Not all steps in glycolysis are shown.

Kinetic Characteristics

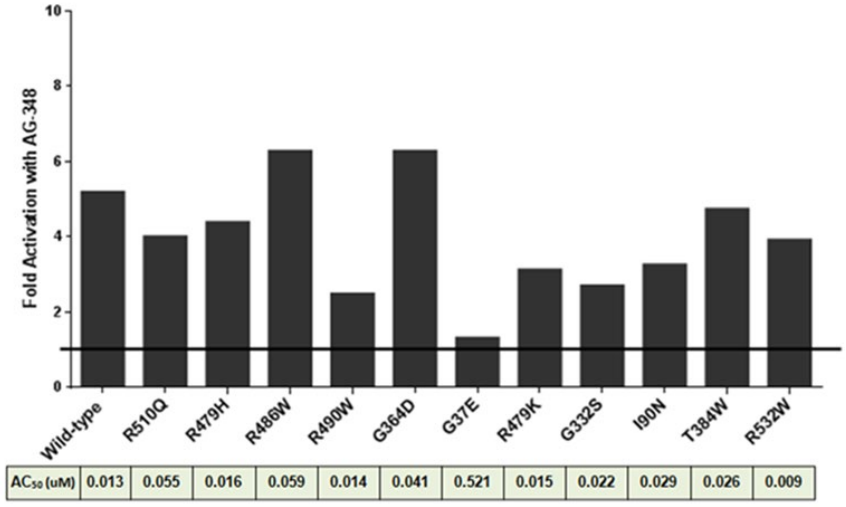
The ability of mitapivat (multiple forms, including mitapivat sulfate salt) and its metabolite AGI-8702 to activate WT PKR, 11 mutant PKR isoforms, PKM2 (mitapivat only), and PKL (mitapivat only) was evaluated using a spectrophotometric LDH coupled-enzymatic assay.To determine AC_{50}, each enzyme was preincubated with test article before initiating the enzyme reaction by adding substrate.

The effect of elevated temperatures on 5 mutant PKR isoforms and WT PKR was also investigated, along with the ability of mitapivat and AGI-8702 to protect against thermal denaturation. Before initiating the assay, the PKR isoforms, with and without test article, were incubated at room temperature. The assay was initiated by incubation at 53 °C and samples were removed at preset time points, at which time activity measurements were taken and the percentage of initial activity remaining was calculated.

Activation of PKR, PKM2, and PKL by Mitapivat and AGI-8702

Activation of PKR (WT and mutant), PKM2, and PKL by mitapivat and AGI-8702 was evaluated. Mitapivat showed >2-fold activation (versus baseline) of WT PKR and 10 of 11 mutant isoforms of PKR tested. The maximum percent activation ranged from approximately 249% to 628% and AC_{50} values ranged from 0.009 to 0.059 µM (Figure 7). Mitapivat also activated PKM2 and PKL, with AC_{50} values of 0.038 and 0.037 µM, respectively, and maximum percent activation of 587% and 293%, respectively.

Figure 7: *In Vitro* Activation of Wild-Type and Mutant PKR Isoforms by Mitapivat



AC_{50} (uM)	0.013	0.055	0.016	0.059	0.014	0.041	0.521	0.015	0.022	0.029	0.026	0.009

Source: Report AG348-N-024-R1.
Abbreviations: AC_{50}=concentration producing 50% activity; PKR=red blood cell–specific form of pyruvate kinase.
Note: The horizontal line represents the baseline activity of each PKR isoform. Mitapivat concentrations were 0.0002 to 10 µM.

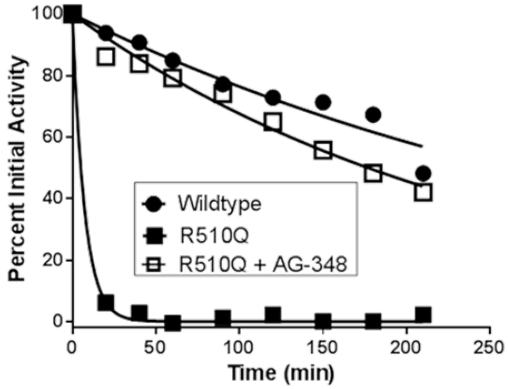
In contrast to mitapivat, AGI-8702 had low-potency mixed activity (activation, inhibition, or no effect) against mutant PKR isoforms. AGI-8702 had no effect on WT PKR.

Effect of Mitapivat and AGI-8702 on PKR Stability

The effect of mitapivat and AGI-8702 on PKR stability was evaluated. Mitapivat stabilized 3 of the 5 mutant isoforms tested as illustrated by the representative results with the R510Q mutant isoform (Figure 8). At 53 °C, the calculated half-life values were 257.5 minutes for WT PKR, 5.2 minutes for the R510Q mutant isoform without exposure to mitapivat, and 176.9 minutes for the R510Q mutant isoform after preincubation with mitapivat at concentrations ≥5 times the AC_{50} (0.055 µM). These biophysical data demonstrate that mitapivat binds to mutant isoforms of PKR.

AGI-8702 stabilised 2 of the 3 mutant isoforms tested, but with less than 10% the potency of mitapivat, which is consistent with its decreased potency against mutant PKR.

Figure 8: Stability of Wild-Type PKR and R510Q Mutant Isoform Activity, With and Without Preincubation With Mitapivat, at 53 °C



Source: Report AG348-N-024-R1.
Abbreviation: PKR=red blood cell–specific form of pyruvate kinase.

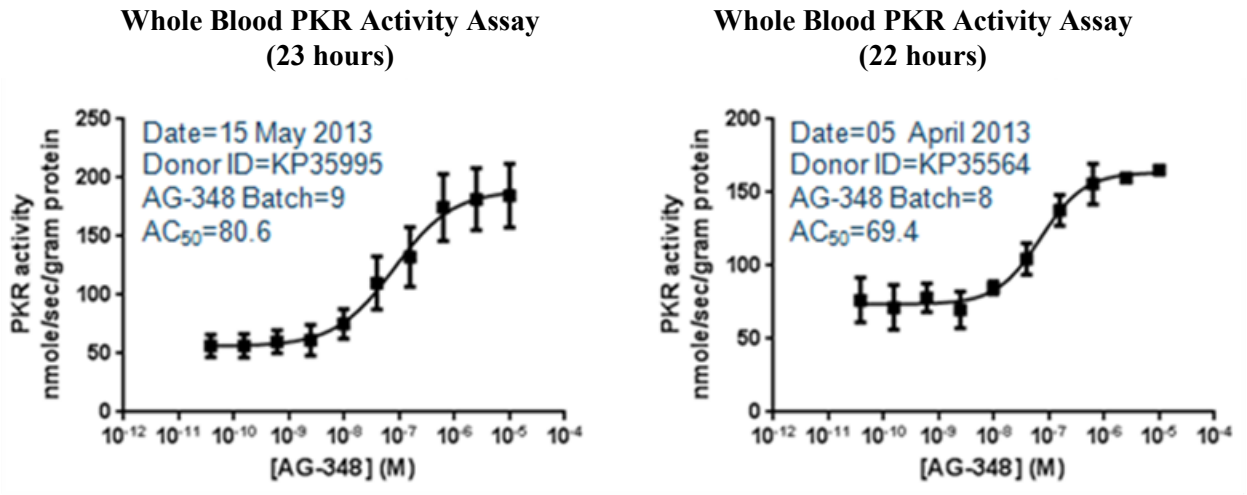
Cell-Based Activity

- Effects of Mitapivat and AGI-8702 on PKR Activity

The effect of mitapivat (concentration range typically 0.000038-10 µM; mitapivat hydrochloride salt) and AGI-8702 (concentration range typically 0.000038-10 µM) on WT PKR activity in human RBCs from healthy donors was evaluated. Fresh whole blood was incubated for 18 to 23 hours in assay plates containing serial dilutions of test article in DMSO. Red blood cells were processed, and PKR activity in the RBC lysates was determined using a spectrophotometric LDH-coupled enzymatic assay.

With mitapivat, the AC_{50} and AC_{90} for WT PKR activation were 0.0619 µM and 1.002 µM, respectively. The average maximum percent PKR activation was 274%. The representative mitapivat concentration-response curves in Figure 9 show the potent activation of PKR in human whole blood.

Figure 9: Concentration-Response Curves of Mitapivat Activation of PKR in Human Red Blood Cells From Healthy Donors



Source: Report AG348-N-026-R1. Abbreviations: AC_{50}=concentration producing 50% activity; PKR=red blood cell–specific form of pyruvate kinase. Note: Mitapivat concentration range was 0.000038 to 10 µM.

With AGI-8702, the AC_{50} and AC_{90} for WT PKR activation were 337 and 993 µM, respectively. The average maximum percent PKR activation was 145%. Thus, AGI-8702 is a weak activator of PKR in RBCs from human whole blood as compared with mitapivat, in terms of both potency (approximately 0.02-0.1% that observed with mitapivat) and maximal activation (approximately 50% that observed with mitapivat).

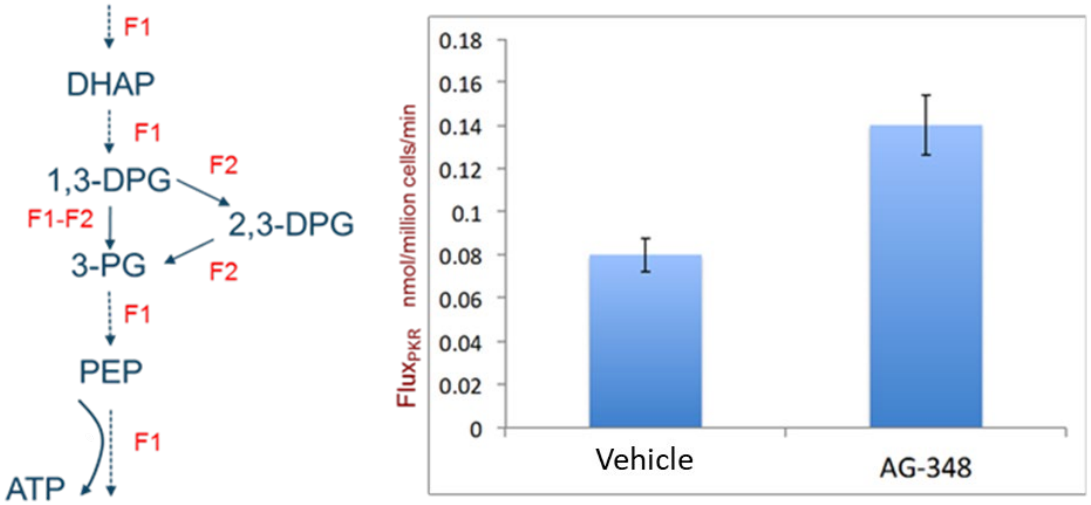
- Effect of Mitapivat on Pyruvate Kinase Flux

The effect of mitapivat on WT PKR flux (ie, the rate of carbon flow through the PKR enzyme reaction) was assessed in whole blood from mice treated with mitapivat, using a stable-isotope strategy.

Female WT C57BL/6 mice (n=5/group) were dosed by oral gavage with mitapivat (150 mg/kg BID; mitapivat hydrochloride salt) or vehicle (0.5% methylcellulose and 0.2% Tween 80) for 3 days, after which whole blood was collected. The whole blood was incubated with U-$^{13}C_6$. At various time points through 240 minutes after the addition of U-$^{13}C_6$, aliquots were collected and processed to quench metabolism, and metabolites were extracted. Metabolite pool sizes and ^{13}C-label incorporation into the glycolytic pathway pharmacodynamic markers 2,3-DPG, 3-phosphoglyceric acid, PEP, and pyruvate were monitored by LC-MS. The data were subsequently analyzed using a mathematical model to quantify flux through the PKR reaction.

Mitapivat increased PKR flux by 80% in WT C57BL/6 mouse whole blood (Figure 10). This result shows that mitapivat not only binds to and activates PKR but also induces enhanced glycolytic pathway activity in RBCs. These data support the conclusion that mitapivat activates WT PKR in vivo and effects changes in RBC metabolism.

Figure 10: RBC Glycolytic Flux Model and Results



	Vehicle	AG-348	Fold-Change
$Flux_{PKR}$	0.08 ± 0.008	0.14 ± 0.015	1.8

Source: Report AG348-N-075-R1. Abbreviations: 1,3-DPG=1,3-diphosphoglycerate; 2,3-DPG=2,3-diphosphoglycerate; 3-PG=3-phosphoglyceride; ATP=adenosine triphosphate; DHAP=dihydroxyacetone phosphate; PEP=phosphoenolpyruvate; PKR=RBC-specific form of pyruvate kinase; RBC=red blood cell; WT=wild-type. Note: F1 and F2 represent the modeled fluxes through individual steps. Whole blood was obtained from female WT C57BL/6 mice (n=5/group) dosed by oral gavage with mitapivat (150 mg/kg twice daily) or vehicle (0.5% methylcellulose and 0.2% Tween 80) for 3 days, then was incubated with uniformly labeled [^{13}C]-glucose. In the pathway schematic, the solid lines indicate a single reaction, while the dotted lines indicate that more than 1 reaction occurs to convert the upper metabolite into the lower metabolite.

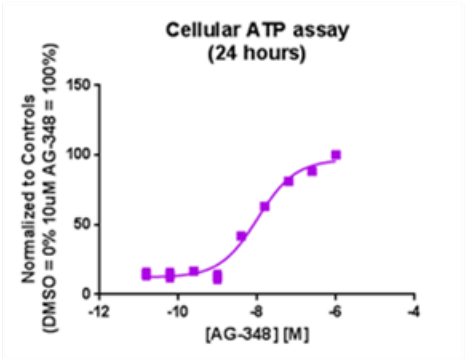
- Effect of Mitapivat and AGI-8702 on ATP Concentrations

Adenosine triphosphate is the product of the PKR reaction in RBCs. The effect of mitapivat (multiple forms; concentration range typically 0.00015-10 µM) and AGI-8702 (concentration range typically 0.00015-10 µM) on ATP levels was measured in human RBCs isolated from fresh whole blood from healthy donors. Red blood cells were diluted in AGAM media (1× phosphate-buffered saline, 1% glucose, 170 mg/L adenine, 5.25 g/L mannitol) containing 10% fetal bovine serum and added to assay plates containing serial dilutions of mitapivat in DMSO. Plates were incubated for 24 hours, after which CellTiter-Glo reagent was added to each well. Plates were then read for luminescence to determine the effect of the test article on ATP levels, and AC_{50} values were calculated.

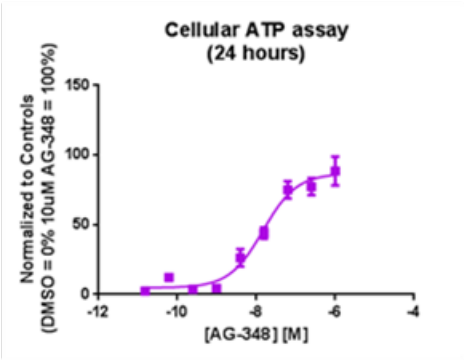
Representative examples of individual concentration-response curves with mitapivat are presented in Figure 11. The mean AC_{50} for ATP production was 0.0109 µM. The average absolute ATP fold change was 1.59. These results indicate that mitapivat potently increased ATP levels in human RBCs, consistent with activation of cellular PKR.

With AGI-8702, the mean AC_{50} for ATP production was 2.3 µM (n=4). The potency of AGI-8702 was approximately 0.5% that of mitapivat, with a maximal effect approximately 50% that of mitapivat.

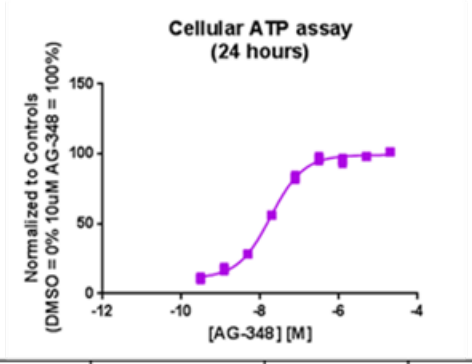
Figure 11: Normalised Concentration-Response Curves of Mitapivat Effect on ATP Levels in Healthy Donor Red Blood Cells



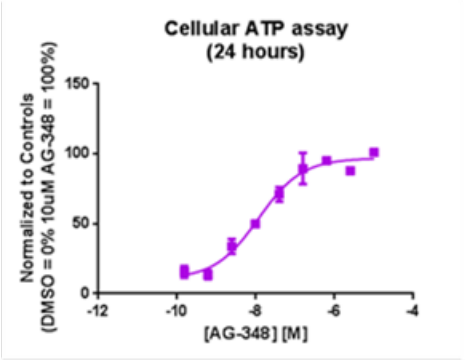
Date Run	AG-348 Batch	Donor ID	AC_{50} (nM)
10.26.2012	8	KP 34141	10.2



Date Run	AG-348 Batch	Donor ID	AC_{50} (nM)
03.21.2013	8	KP 35413	14



Date Run	AG-348 Batch	Donor ID	AC_{50} (nM)
05.30.2012	8	KP 32030	18



Date Run	AG-348 Batch	Donor ID	AC_{50} (nM)
07.25.2013	9	KP 36702	11

Source: Report AG348-N-025-R1. Abbreviations: AC_{50}=concentration producing 50% activity; ATP=adenosine triphosphate.
Note: Mitapivat concentration range was 0.00015 to 10 µM.

***In Vivo* Pharmacology**

- Studies of Pharmacokinetics/Pharmacodynamics in Female WT C57BL/6 Mice

Three pharmacology studies were conducted with mitapivat sulfate salt in female WT C57BL/6 mice (n=32/group). The objective of these studies was to investigate the relationship between mitapivat pharmacokinetics and target engagement (PKR activation) and pharmacodynamic parameters (2,3-DPG and ATP concentrations, and calculated ATP/2,3-DPG ratios) after single or multiple oral doses of mitapivat.

The 3 studies were similar in design, with animals dosed orally with single or repeated doses of vehicle control or mitapivat (1, 10, 50, or 150 mg/kg) prepared in 0.5% methylcellulose in water; in each study, a non-dosed group served as an additional control. At pre-dose and at 3, 6, 12, 24, 36, 48, and 72 hours after the last dose, blood was collected from 4 mice per time point. The blood samples were divided and processed, as necessary, to obtain plasma, whole blood, and RBC lysates. The concentrations of mitapivat in plasma and the concentrations of 2,3-DPG and ATP in whole blood were determined using quantitative LC-MS/MS methods. Pyruvate kinase R activity in RBCs was determined using a spectrophotometric LDH-coupled enzymatic assay. Pharmacokinetic analyses and pharmacokinetic/ pharmacodynamic modeling of plasma and blood concentration data were conducted using WinNonLin (PharSight Corporation, Version 6.2).

- Single-Dose Pharmacokinetic/Pharmacodynamic Study of Mitapivat (1, 10, 50, or 150 mg/kg) to Female WT C57BL/6 Mice

After a single oral dose, mitapivat exposure (AUC_{0-12}) in plasma increased with increasing dose. Compared with vehicle control, blood ATP concentration was essentially unchanged (1.78% to 3.67% increase), blood 2,3-DPG concentration decreased by 6.53% to 23.3%, and PKR activity in RBCs increased by 24.1% to 145%.

Table 13: Mean AUC_{0-12} of Mitapivat, ATP, 2,3-DPG, and PKR Activity After a Single Oral Dose of Mitapivat in Female WT C57BL/6 Mice

	AUC_{0-12}				
	Plasma	Blood			RBCs
Mitapivat dose (mg/kg)	Mitapivat (hr•ng/mL)	ATP (hr•ng/mL)	2,3-DPG (hr•ng/mL)	ATP/ 2,3-DPG	PKR activity (hr•nmol/sec/g)
0 (vehicle control)	NA	3,012,000	19,807,500	0.152	645
1	36.0	3,078,000	18,513,750	0.166	800
10	125	3,073,125	16,372,500	0.188	1,129
50	1,301	3,065,625	15,626,250	0.196	1,341
150	21,507	3,122,625	15,183,750	0.206	1,580
Not dosed	NA	2,796,750	16,980,000	0.165	625

Source: Report AG348-N-028-R1.
Abbreviations: 2,3-DPG=2,3-diphosphoglycerate; ATP=adenosine triphosphate; AUC_{0-12}=area under the concentration-time curve from 0 to 12 hours; NA=not applicable; PKR=RBC-specific form of pyruvate kinase; RBC=red blood cell.
Note: n=4/time point.

- Three-Day Repeat-Dose Pharmacokinetic/Pharmacodynamic Study After Oral BID Administration of Mitapivat (1, 10, 50, or 150 mg/kg) to Female WT C57BL/6 Mice

After BID administration of mitapivat for 3 days, mitapivat exposure (AUC_{0-12}) in plasma increased with increasing dose (Table 14). Compared with vehicle control, blood ATP concentration increased by 14.8% to 45.0% and blood 2,3-DPG concentration decreased by 0.777% to 22.5%; PKR activity in RBCs increased by 39.9% to 196%.

Table 14: Mean AUC_{0-12} of Mitapivat, ATP, 2,3-DPG, and PKR Activity After Twice-Daily Oral Dosing of Mitapivat for 3 Days (5 Doses) in Female WT C57BL/6 Mice

	AUC_{0-12}				
	Plasma	Blood			RBCs
Mitapivat dose (mg/kg)	Mitapivat (hr•ng/mL)	ATP (hr•ng/mL)	2,3-DPG (hr•ng/mL)	ATP/ 2,3-DPG	PKR activity (hr•nmol/sec/g)
0 (vehicle control)	NA	4,122,375	24,135,000	0.171	613
1	39.2	4,730,625	23,947,500	0.198	858
10	302	5,616,375	22,200,000	0.253	1,324
50	1,100	5,298,000	18,705,000	0.283	1,258
150	9,761	5,976,750	19,428,750	0.308	1,814
Not dosed	NA	4,558,125	26,636,250	0.171	618

Source: Report AG348-N-029-R1. Abbreviations: 2,3-DPG=2,3-diphosphoglycerate; ATP=adenosine triphosphate; AUC_{0-12}=area under the concentration-time curve from 0 to 12 hours; NA=not applicable; PKR=RBC-specific form of pyruvate kinase; RBC=red blood cell. Note: n=4/time point.

- Seven-Day Repeat-Dose Pharmacokinetic/Pharmacodynamic Study After Oral BID Administration of Mitapivat (1, 10, 50, or 150 mg/kg) to Female WT C57BL/6 Mice

After BID administration for 7 days, mitapivat exposure (AUC_{0-12}) in plasma increased with increasing dose (Table 15). Compared with vehicle control, blood ATP concentration increased by 15.6% to 43.7% and blood 2,3-DPG concentration decreased by 1.78% to 18.7%; PKR activity in RBCs increased by 29.4% to 141%.

Table 15: Mean AUC_{0-12} of Mitapivat, ATP, 2,3-DPG, and PKR Activity After Twice-Daily Oral Dosing of Mitapivat for 7 Days (13 Doses) in Female WT C57BL/6 Mice

	AUC_{0-12}				
	Plasma	Blood			RBCs
Mitapivat dose (mg/kg)	Mitapivat (hr•ng/mL)	ATP (hr•ng/mL)	2,3-DPG (hr•ng/mL)	ATP/ 2,3-DPG	PKR activity (hr•nmol/sec/g)
0 (vehicle control)	NA	3,827,625	23,201,250	0.165	679
1	41.3	4,424,250	22,788,750	0.194	878
10	120	4,720,125	19,991,250	0.236	1,127
50	685	5,249,250	19,327,500	0.272	1,556
150	4,076	5,500,875	18,851,250	0.292	1,638
Not dosed	NA	3,786,750	22,968,750	0.165	628

Source: Report AG348-N-030-R1.
Abbreviations: 2,3-DPG=2,3-diphosphoglycerate; ATP=adenosine triphosphate; AUC_{0-12}=area under the concentration-time curve from 0 to 12 hours; NA=not applicable; PKR=RBC-specific form of pyruvate kinase; RBC=red blood cell.
Note: n=4/time point.

Pharmacokinetic/Pharmacodynamic Relationships

Because the pharmacokinetic/pharmacodynamic relationships obtained from the 3- and 7-day repeat-dose studies were similar, the data from Day 3 and Day 7, respectively, were pooled for correlation fitting using an E_{max} model:

$$E=E_0 + (E_{max} \times C) / (EC_{50} + C)$$

where:

E represents the AUC_{0-12} resulting in X% of the maximum drug-induced effect

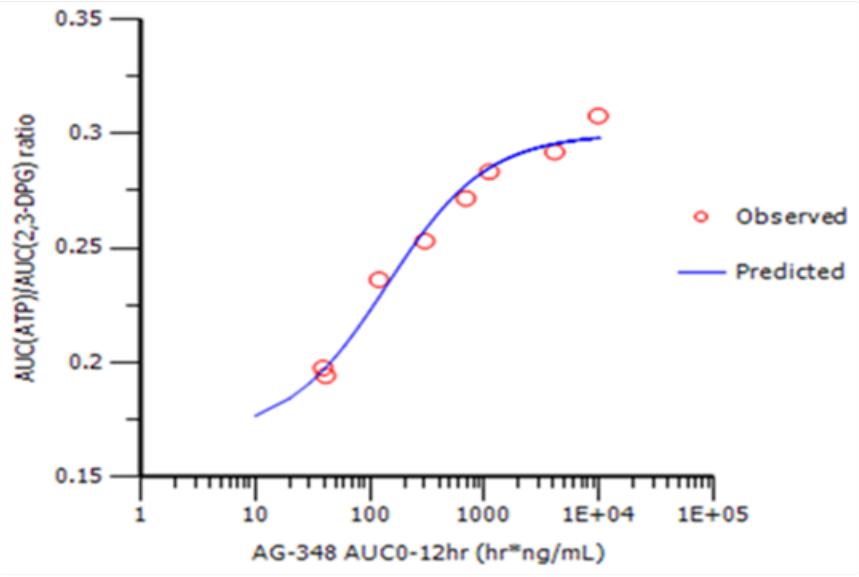
E_{max} represents the maximum drug-induced effect

E_0 represents the baseline effect

C represents the AUC_{0-12} for mitapivat at different doses

A pharmacokinetic/pharmacodynamic relationship was established between mitapivat AUC_{0-12} and the ATP–to–2,3-DPG AUC_{0-12} ratio (Figure 12).

Figure 12: Plot of Mitapivat AUC0-12 Versus ATP–to–2,3-DPG AUC0-12 Ratio Using Data From Female WT C57BL/6 Mouse Pharmacokinetic/Pharmacodynamic Studies of Oral BID Dosing of Mitapivat (1, 10, 50, or 150 mg/kg/dose) for 3 or 7 Days



Source: Report AG348-N-061-R1.
Abbreviations: 2,3-DPG=2,3-diphosphoglycerate; ATP=adenosine triphosphate; AUC_{0-12}=area under the concentration-time curve from 0 to 12 hours; BID=twice daily; WT=wild-type.

Effects of Mitapivat in a Mouse Model of β-Thalassemia

Hbb$^{th3/+}$ mice are a mouse model of β-thalassemia, in which one allele of both murine β-globin genes (β-minor and β-major), has been deleted. These mice exhibit dramatic reductions in Hb concentration, increased reticulocyte counts, and abnormal RBC morphology (Yang et al, 1995). *Hbb*$^{th3/+}$ mice show dyserythropoiesis marked by an expansion of erythroid precursors with extramedullary erythropoiesis, and their bone marrow is characterized by reduced basophilic erythroblasts, reduced polychromatic erythroblasts, and increased

orthochromatic erythroblasts (Matte *et al,* 2015; Matte *et al,* 2021). Importantly, these mice develop chronic anemia, which allows for testing drug effects in the setting of chronic RBC transfusions.

Effect of Mitapivat on Hematological Parameters and Erythropoiesis in the *Hbb*$^{th3/+}$ Mouse Model of β-Thalassemia

Female WT C57BL/6J and *Hbb*$^{th3/+}$ mice were treated with vehicle control or mitapivat administered via oral gavage or through diet. In the groups dosed via oral gavage, mitapivat sulfate salt (50 mg/kg) or vehicle control (10% propylene glycol, 10% Kolliphor® EL in water) was administered BID for 21 days. For the additional groups, mitapivat sulfate salt at 1,200 ppm (weight-to-weight ratio; estimated to be equivalent to a 100 mg/kg daily dose) was added to standard rodent diet and was administered for 21 or 56 days. Standard rodent diet was used as the control. Because of the low birthrate of female *Hbb*$^{th3/+}$ mice, the 21-day oral gavage study and the 56-day diet study were performed over several sub-studies. The same methods were used in each sub-study. The pooled numbers of mice in these sub-studies are provided in Table 16.

Table 16: Study Design

Genotype	Test article	Dose and regimen	Pooled n across substudies[a]
21-Day oral gavage study			
WT	Vehicle	0 mg/kg BID	16
Hbb$^{th3/+}$	Vehicle	0 mg/kg BID	19
WT	Mitapivat	50 mg/kg BID	12
Hbb$^{th3/+}$	Mitapivat	50 mg/kg BID	15
21-Day diet study			
WT	Vehicle	0 mg/kg ad libitum	4
Hbb$^{th3/+}$	Vehicle	0 mg/kg ad libitum	3
WT	Mitapivat	100 mg/kg[b] per day, ad libitum	3
Hbb$^{th3/+}$	Mitapivat	100 mg/kg[b] per day, ad libitum	3
56-Day diet study			
WT	Vehicle	0 mg/kg ad libitum	7
Hbb$^{th3/+}$	Vehicle	0 mg/kg ad libitum	7
WT	Mitapivat	100 mg/kg[b] per day, ad libitum	6
Hbb$^{th3/+}$	Mitapivat	100 mg/kg[b] per day, ad libitum	7

Source: Report AG348-N-110.
Abbreviations: BID=twice daily; WT=wild-type.
[a] Because of the low female birthrate of *Hbb*$^{th3/+}$ mice, the 21-day oral gavage study and the 56-day diet study were performed over several substudies. The same methods were used in each substudy. The 21-day diet study did not include any substudies.
[b] Mitapivat was added at a concentration of 1,200 ppm (weight-to-weight ratio).

Blood was collected on Days 7, 14, or 21 at 1 hour post-dose. Terminal blood was collected 4 hours following the last dose on Day 21. Hematologic parameters, RBC indices, and reticulocyte count were analyzed. On Day 21 or Day 56, mice were euthaniszed and terminal blood, bone marrow, and spleen samples were collected, processed, and analyzed.

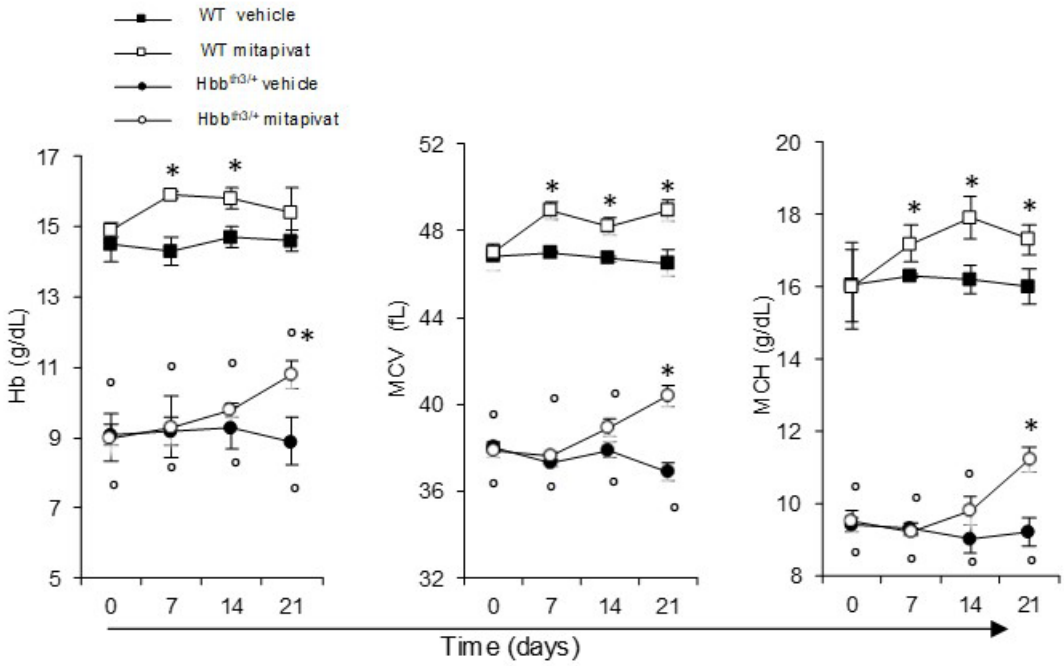
Treatment of *Hbb*$^{th3/+}$ mice with mitapivat at 50 mg/kg BID for 21 days resulted in a significant increase in Hb of approximately 2 g/dL and a significant decrease in absolute reticulocyte counts, along with a significant increase in MCV compared with the vehicle *Hbb*$^{th3/+}$ group (Figure 13). Similar results were observed in *Hbb*$^{th3/+}$mice dosed with approximately 100 mg/kg/day mitapivat for 56 days through diet.

After 21 days of treatment with 50 mg/kg BID, plasma total bilirubin was lower in mitapivat-treated *Hbb*$^{th3/+}$ mice compared to the vehicle *Hbb*$^{th3/+}$ group (Figure 14). There was also a trend toward increased ATP concentrations in the peripheral blood, suggesting increased PKR activity. At 4 hours post-dose on Day 21, the mean (±SD) plasma mitapivat concentration was 733±355 ng/mL and 314±35.3 ng/mL in the mitapivat-treated WT and *Hbb*$^{th3/+}$ groups, respectively.

RBC life span in the periphery was evaluated during 56 days of mitapivat administration through diet and was significantly increased with mitapivat treatment, with an estimated half-life of 14.4 days, compared with 9.6 days in *Hbb*$^{th3/+}$ mice dosed with vehicle control and 18.9 days in WT mice dosed with vehicle control (Figure 15).

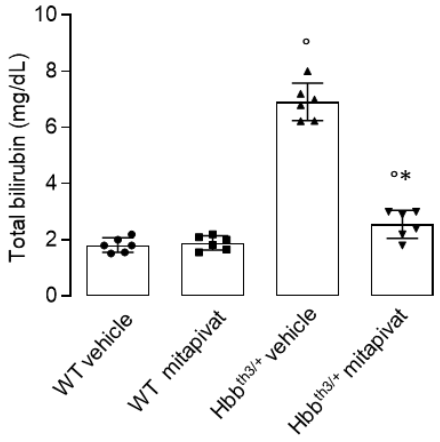
Taken together, these results indicate that mitapivat treatment improved anemia, reduced hemolysis, and improved RBC survival in *Hbb*$^{th3/+}$mice.

Figure 13: Effect of Mitapivat on Hemoglobin, Mean Corpuscular Volume, and Reticulocyte Count in Hbb$^{th3/+}$ and Wild-Type Mice Treated With Mitapivat 50 mg/kg BID or Vehicle Control for 21 Days



Source: Report AG348-N-110.
Abbreviations: ANOVA=analysis of variance; BID=twice daily; Hb=hemoglobin; MCH=mean corpuscular hemoglobin; MCV=mean corpuscular volume; WT=wild-type.
Note: Data are mean±SD (n=6/group; experiment performed in single replicate).
°p<0.05 compared with WT vehicle and *p<0.05 compared with vehicle of the same genotype by one-way ANOVA with Dunnett's test for longitudinal comparison.

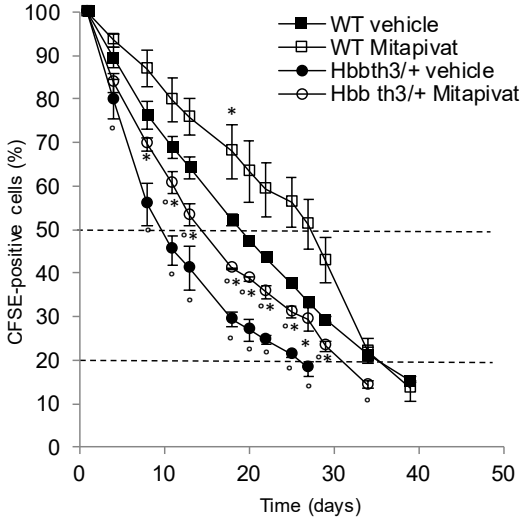
Figure 14: Plasma Total Bilirubin in Mice Treated With Vehicle or Mitapivat for 21 Days



Source: Report AG348-N-110. Abbreviations: ANOVA=analysis of variance; WT=wild-type.
Note: Data are mean±SD (n=6/group; experiment performed in single replicate). °p<0.05 compared with WT vehicle or *p<0.05 compared with vehicle-treated *Hbb*$^{th3/+}$ animals by one-way ANOVA with Bonferroni correction for multiple comparison.

Figure 15: Survival of Red Blood Cells From Hbb$^{th3/+}$ and Wild-Type Mice Treated With Mitapivat 1,200 ppm or Vehicle Control for up to 56 Days

Dose group	T_{50} (days)	T_{20} (days)
WT vehicle	18.9±0.5	34.6±1.0
WT mitapivat	27.1±1.8*	34.5±0.7
Hbb$^{th3/+}$ vehicle	9.6±0.9°	25.8±0.8°
Hbb$^{th3/+}$ mitapivat	14.4±0.4*°	31.1±0.6*°

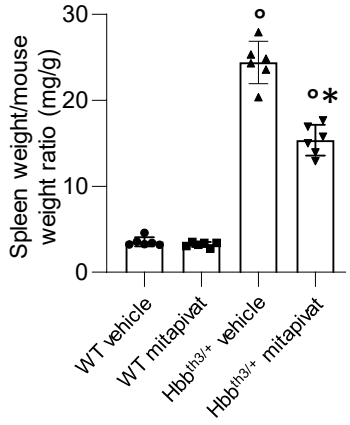


Source: Report AG348-N-110. Abbreviations: ANOVA=analysis of variance; CFSE=carboxyfluorescein succinimidyl ester; T_{20}=day at which 20% of labeled RBCs remained; T_{50}=day at which 50% of labeled RBCs remained; WT=wild-type.
Note: Data are mean±SD (n=4 for wild-type vehicle group; n=3 for WT vehicle group and *Hbb*$^{th3/+}$ and WT mitapivat groups; experiment performed in single replicate). °p<0.05 compared with WT vehicle and *p<0.05 compared with vehicle-treated animals of the same genotype by one-way ANOVA with Dunnett's test for longitudinal comparison.

After 21 days of treatment, mitapivat significantly decreased extramedullary erythropoiesis in *Hbb*$^{th3/+}$ mice as supported by a reduction in the spleen/body weight ratio relative to the vehicle *Hbb*$^{th3/+}$ group (Figure 16). Mitapivat treatment improved the bone marrow erythroblast maturation index, assessed as the ratio between early pro-erythroblasts + basophilic erythroblasts and late polychromatic erythroblasts + orthochromatic erythroblasts (Figure 17). Mitapivat administration also resulted in a marked decrease in ROS levels in maturing *Hbb*$^{th3/+}$ erythroblasts from bone marrow, consistent with reduced oxidative stress (Figure 18).

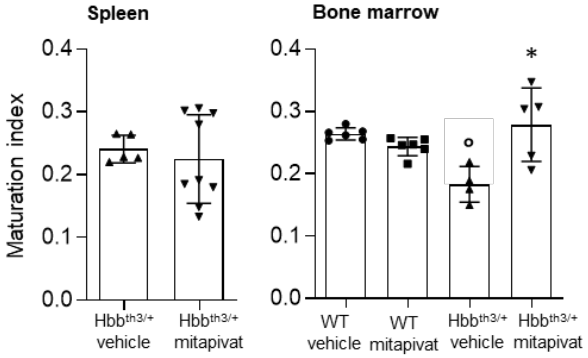
After 56 days of treatment, a reduction in plasma erythropoietin was observed in mitapivat-treated *Hbb*$^{th3/+}$ mice relative to vehicle control, which is consistent with improved erythropoiesis (Figure 19). Mitapivat also significantly reduced liver iron overload in both hepatocytes and Kupffer cells compared with vehicle-treated *Hbb*$^{th3/+}$ mice (Figure 20). Thus, compared to vehicle control, treatment with mitapivat improved dyserythropoiesis in *Hbb*$^{th3/+}$ mice and reduced iron accumulation in the liver.

Figure 16: Spleen-to-Body-Weight Ratio in Mice Treated With Vehicle or Mitapivat for 21 Days



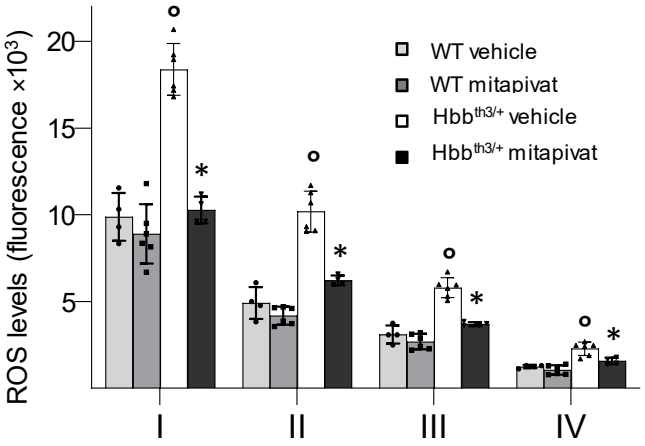
Source: Report AG348-N-110. Abbreviations: ANOVA=analysis of variance; WT=wild-type.
Note: Data are mean±SD (n=6/group; experiment performed in single replicate). °p<0.05 compared with WT vehicle mice and *p<0.05 compared with vehicle-treated animals of the same genotype by 2-way ANOVA with Bonferroni correction for multiple comparison.

Figure 17: Maturation Index of the Erythroblast Populations in the Spleen and Bone Marrow of Mice Treated With Vehicle or Mitapivat for 21 Days



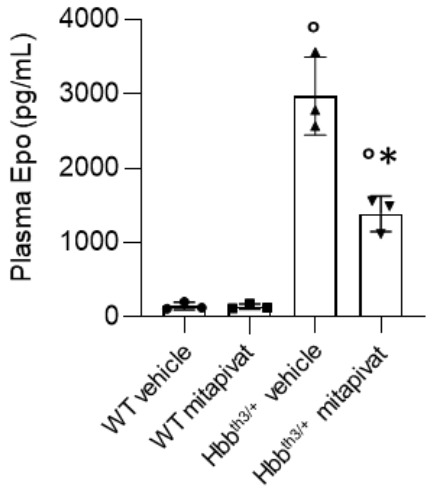
Source: Report AG348-N-110. Abbreviation: WT=wild-type.
Notes: Maturation index is defined as the ratio of pro-erythroblasts + basophilic erythroblasts to polychromatic erythroblasts + orthochromatic erythroblasts. Data are mean±SD (for bone marrow, n=6 in WT mitapivat- and vehicle-treated mice, n=4 in vehicle-treated *Hbb*$^{th3/+}$ mice, n=5 in mitapivat-treated *Hbb*$^{th3/+}$ mice; for spleen, n=5 in vehicle-treated *Hbb*$^{th3/+}$ mice, n=9 in mitapivat-treated *Hbb*$^{th3/+}$ mice; experiment performed in single replicate). Erythroblasts could not be isolated from spleens of mice in the WT vehicle or mitapivat groups.
°p<0.05 compared with WT vehicle mice and *p<0.05 compared with vehicle-treated animals of the same genotype by 2-way ANOVA with Bonferroni correction for multiple comparison.

Figure 18: Reactive Oxygen Species in Erythroblasts From Bone Marrow From Mice Treated With Mitapivat or Vehicle for 21 Days



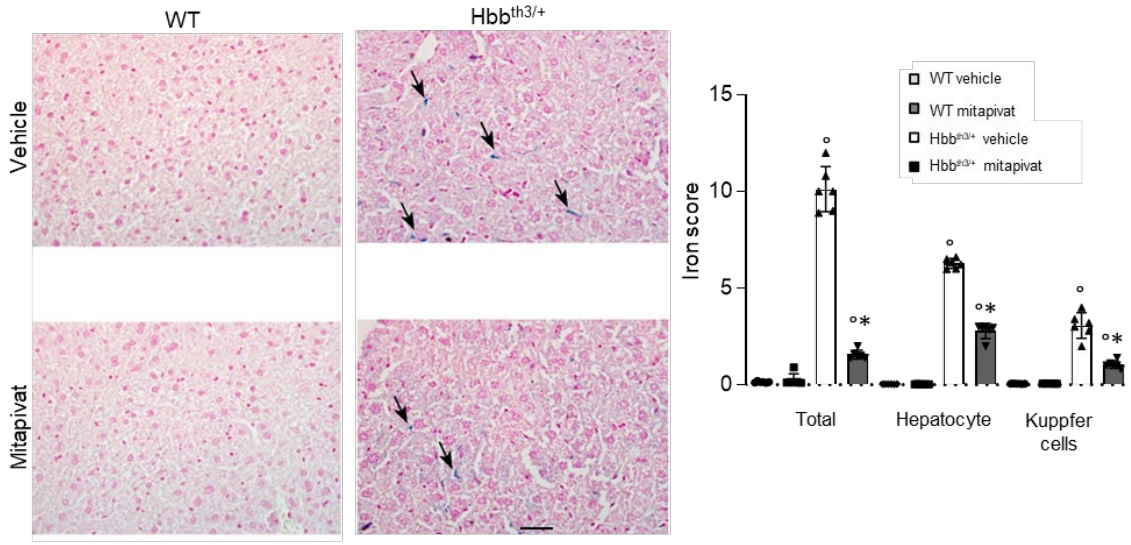
Source: Report AG348-N-110.
Abbreviations: ROS=reactive oxygen species; WT=wild-type.
Note: Data are mean±SD (n=4 in vehicle-treated WT mice, n=6 in mitapivat-treated WT mice, n=6 in vehicle-treated *Hbb*$^{th3/+}$ mice, n=4 in mitapivat-treated *Hbb*$^{th3/+}$ mice; experiment performed in single replicate).
°p<0.05 compared with WT vehicle mice and *p<0.05 compared with vehicle-treated *Hbb*$^{th3/+}$ mice by 2-way ANOVA with Bonferroni correction for multiple comparison.

Figure 19: Plasma Erythropoietin Concentration in Mice Treated With Vehicle or Mitapivat for 56 Days



Source: Report AG348-N-110.
Abbreviations: ANOVA=analysis of variance; Epo=erythropoietin; WT=wild-type.
Note: Data are mean±SD (n=3/group; experiment performed in duplicate).
°p<0.05 compared with WT vehicle mice and *p<0.05 compared with vehicle-treated mice of the same genotype by 2-way ANOVA with Bonferroni correction for multiple comparison.

Figure 20: Liver Iron Content in Mice Treated With Vehicle or Mitapivat for 56 Days



Source: Report AG348-N-110.
Abbreviation: WT=wild-type.
Note: Left panel=iron staining (Perl's Prussian blue) in liver from WT and *Hbb*$^{th3/+}$ mice treated with vehicle or mitapivat. One representative image from 6 with similar results is presented. Black arrows indicate liver iron deposits. Right panel=quantification of iron staining in liver. Data are mean±SD (n=6/group; experiment performed in single replicate).
°p<0.05 compared with WT vehicle mice and *p<0.05 compared with vehicle-treated *Hbb*$^{th3/+}$ mice by 2-way ANOVA with Bonferroni correction for multiple comparison.

Effect of Mitapivat on Chronic Transfusion Interval and Iron Overload in the Hbbth3/+ Mouse Model of β-Thalassemia

To examine the impact of mitapivat on hematologic parameters in a mouse model of transfusion-dependent β-thalassemia, *Hbb*$^{th3/+}$ mice were administered either mitapivat at 50 mg/kg or vehicle (10% propylene glycol, 10% Kolliphor EL in water) BID by oral gavage. After Day 10, mitapivat- or vehicle-treated mice were exposed to a chronic transfusion regimen of WT RBCs over a duration of 40 days (Table 9). Hemoglobin was measured 7 days after the first transfusion, and every 48 hours thereafter until it reached the prespecified transfusion threshold of >10.5 g/dL. The RBC transfusion consisted of 400 µL washed RBC at 40% to 45% hematocrit. At the end of the treatment period (Day 51), animals were euthanized and blood, bone marrow, liver, spleen, and kidney samples were collected, processed, and analysed.

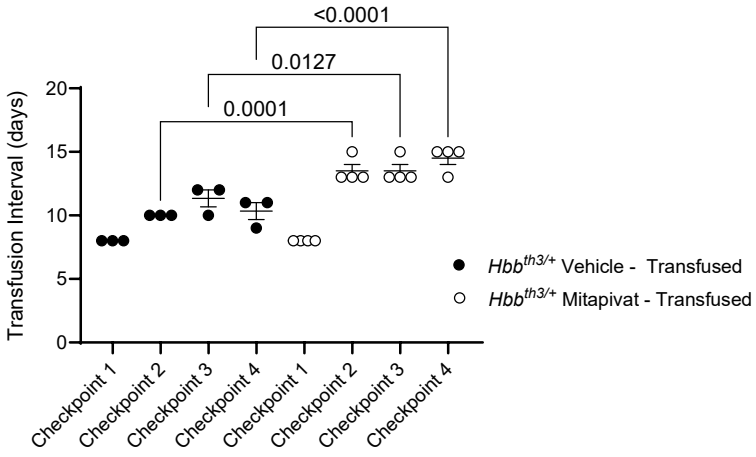
Table 9: Chronic Transfusion Study Details

Genotype	Test article	Dose level (mg/kg)	Method of administration	Regimen	RBC transfusion status	n
WT	Vehicle	0	Gavage	BID	No RBC Transfusion	4
Hbb$^{th3/+}$	Vehicle	0	Gavage	BID	No RBC Transfusion	4
Hbb$^{th3/+}$	Vehicle	0	Gavage	BID	Chronic RBC Transfusion	3
Hbb$^{th3/+}$	Mitapivat	50	Gavage	BID	Chronic RBC Transfusion	4
Hbb$^{th3/+}$	Mitapivat	50	Gavage	BID	No RBC Transfusion	4

Source: Report AG348-N-120. Abbreviations: BID=twice daily; RBC=red blood cell; WT=wild-type.

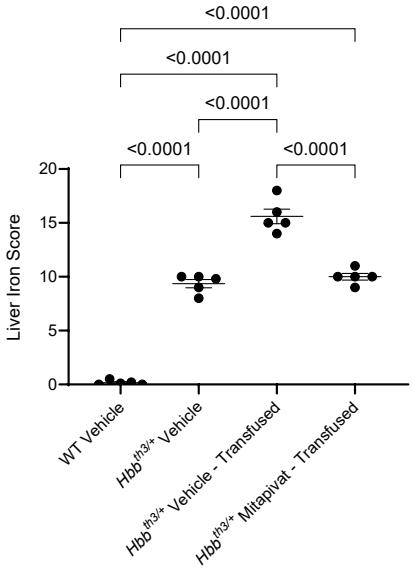
Mitapivat-treated *Hbb*$^{th3/+}$ mice exposed to chronic transfusion displayed a greater sustained rise in Hb from baseline compared to vehicle-treated transfused *Hbb*$^{th3/+}$ mice. This resulted in a longer interval between transfusions, with an interval of 13.8 days in mitapivat-treated *Hbb*$^{th3/+}$ mice versus 10.5 days in vehicle-treated Hbbth3/+ mice (Figure 21). Improvements in iron overload were also observed in this study, in which mitapivat-treated transfused Hbbth3/+ mice showed lower liver iron accumulation when compared to vehicle-treated transfused Hbbth3/+ mice (Figure 22).

Figure 21: Transfusion Time Intervals in Hbb$^{th3/+}$ Mice Treated With Either Vehicle or Mitapivat With Chronic Transfusion



Source: Report AG348-N-120.Abbreviation: ANOVA=analysis of variance. Note: Individual mice replicates plotted. Error bars are mean±SEM (n=3 or 4/group; experiment performed in single replicate). The checkpoints correspond to days in which transfusion was performed. P-values calculated by Sidak's multiple comparisons test using ordinary one-way ANOVA. Exact p-values are displayed above brackets between the indicated comparators. Comparisons with p>0.05 are not displayed.

Figure 22: Quantification of Iron Staining in Liver From Wild-Type and Hbb$^{th3/+}$ Mice Treated With Either Vehicle or Mitapivat With or Without Chronic Transfusion on Day 51



Source: Report AG348-N-120. Abbreviations: ANOVA=analysis of variance; WT=wild-type.
Note: Individual mice replicates plotted. Error bars are mean±SEM (n=5/group; experiment performed in single replicate).

P-values calculated by Tukey's multiple comparisons test using ordinary one-way ANOVA. Exact p-values are displayed above brackets between the indicated comparators. Comparisons with p>0.05 are not displayed.

Effect of Mitapivat and Iron Chelation Therapy in the Hbbth3/+ Mouse Model of β-Thalassemia

The effects of the coadministration of mitapivat and DFP on *Hbb*$^{th3/+}$ mice was investigated, since iron chelation is part of the standard treatment for transfusion-dependent thalassemia patients (Taher *et al,* 2021). To examine the impact of iron chelation on hematologic parameters in a mouse model of β-thalassemia treated with mitapivat, *Hbb*$^{th3/+}$ mice were administered either mitapivat at 50 mg/kg or vehicle (10% propylene glycol, 10% Kolliphor EL in water) BID by oral gavage for 35 days. Starting on Day 8, a subset of mice was administered DFP at 0.75 or 1.25 mg/mL in drinking water ad libitum for 28 days (table 17). At the end of the treatment periods (Day 36), animals were euthanised and blood, bone marrow, liver, spleen, and kidney samples were collected, processed, and analysed.

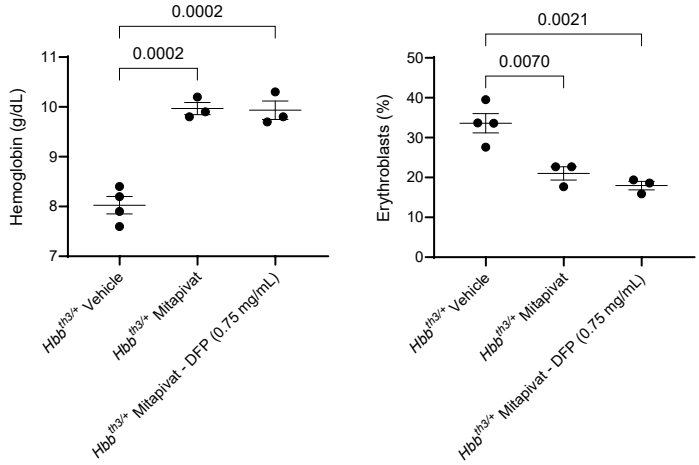
Table 17: Iron Chelator Study Details

	Test Article 1: mitapivat				Test Article 2: DFP				
Genotype	**Test article**	**Dose level (mg/kg)**	**Regimen**	**Duration (days)**	**Test article**	**Dose level (mg/mL)**	**Regimen**	**Duration (days)**	**n**
WT	Vehicle	0	BID	35	None	None	Ad libitum	28	4
Hbb$^{th3/+}$	Vehicle	0	BID	35	None	None	Ad libitum	28	4
Hbb$^{th3/+}$	Mitapivat	50	BID	35	DFP	0.75	Ad libitum	28	3
Hbb$^{th3/+}$	Mitapivat	50	BID	35	DFP	1.25	Ad libitum	28	3

Source: Report AG348-N-120. Abbreviations: BID=twice daily; DFP=deferiprone; WT=wild-type. Note: Test Article 2 was administered after the first 7 days of Test Article 1.

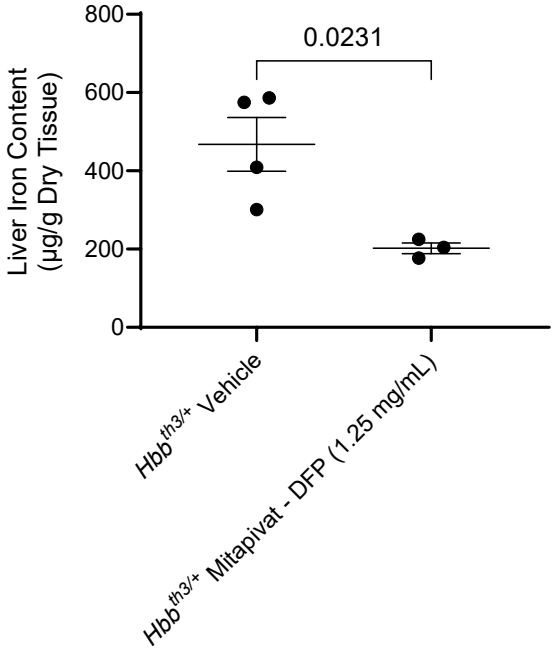
The beneficial effects of mitapivat on mouse b-thalassemia anemia were maintained when mitapivat was co-administered with DFP at both dosages, as supported by the stable and sustained increase in Hb and the reduction in circulating erythroblasts compared to baseline values (Figure 23). Deferiprone iron chelation efficacy, as represented by a change in liver iron content, was preserved in *Hbb*$^{th3/+}$ mice treated with both DFP and mitapivat (Figure 24).

Figure 23: Hemoglobin and Circulating Erythroblasts in Hbb$^{th3/+}$ Mice Treated With Either Vehicle or Mitapivat With or Without an Iron Chelator (0.75 mg/mL DFP) on Day 36



Source: Report AG348-N-120.
Abbreviations: ANOVA=analysis of variance; DFP=deferiprone.
Note: Individual mice replicates plotted. Error bars are mean±SEM (n=3 or 4/group; experiment performed in single replicate).
P-values calculated by Tukey's multiple comparisons test using ordinary one-way ANOVA. Exact p-values are displayed above brackets between the indicated comparators. Comparisons with p>0.05 are not displayed.

Figure 24: Liver Iron Content in Hbb$^{th3/+}$ Mice Treated With Either Vehicle or Mitapivat With or Without an Iron Chelator (1.25 mg/mL DFP) on Day 36



Source: Report AG348-N-120. Abbreviation: DFP=deferiprone. Note: Individual mice replicates plotted. Error bars are mean±SEM (n=3 or 4/group; experiment performed in single replicate). P-values calculated by unpaired t-test (two-tailed).

2.6.3. Discussion on clinical pharmacology

In the present application, the MAH took the option to update existing PPK, PK/PD or ER analysis developed as part of the initial MAA for PKD subjects. This approach is acceptable however, since the PK is similar between both populations (PKD, thalassemia and HV), it would have been of particular interest to pool the PK and PD data.

At the PK level, the previous PPK model developed for PKD was considered acceptable. Presently the one developed for thalassaemia clearly over-parameterised given the available PK data from the three clinical studies. Even after adjustment (fixed parameters or IIV), non-plausible IIV were estimated for both CL/F and V2/F, respectively of 12% and 1%. Covariate selection on Vds/F is questioned given the high eta-shrinkage (>50%). Furthermore, its predictive performance was not as good as claimed (worse for Study 010, underprediction of the IIV for 017 and 018).

At the PD level, given the introduction of a new dose level of 100 mg BID, from 5 to 100 mg BID there is a large range of exposure which would have been interesting to identify possible dose/exposure dependent relationship with PD endpoints.

Population PK analysis

Although the MAH considers that the developed PPK model was fit for purpose, the developed PPK model is still, not considered acceptable yet, no refinement is requested given its low impact.. The PPK model seems to be clearly over-parameterised given the available PK data from the three clinical studies. Even after adjustment (fixed parameters or IIV), non-plausible IIV were estimated for both CL/F and V2/F, respectively of 12% and 1%. Covariate selection on Vds/F is questioned given the high eta-shrinkage (>50%). Furthermore, its predictive performance was not as good as claimed (worse for Study 010, underprediction of the IIV for 017 and 018). Therefore, only a model-independent estimate of CL/F should be reported in the SmPC, and this was not performed, thus the CL/F value of 17.7 L/h (model-based) was deleted, this is acceptable.

Dosing rationale

Based on the Phase 2 study **AG348-C010**, both dosing schedule (50 mg and 100 mg BID) appears to have an equivalent effect on ATP. Furthermore, even if the MAH argue that more subjects were Hb responders with the 100 mg BID dosing schedule in this study, the reliability of a median increase of Hb by 0.7 g/dL observed in the pivotal studies (**AG348-C-017/018**) is questioned. Furthermore, based on the ER-efficacy-Hb there are no exposure dependent effect.

Primary pharmacology

The pharmacodynamics (PD) properties and PK/PD relationship of mitapivat were characterised in the initial MAA. In addition, for the new indication, β-Thalassemia Mouse Model were used to assess the effect of mitapivat on haematological Parameters and Erythropoiesis, chronic Transfusion Interval and Iron Overload, and Iron Chelation Therapy.

Treatment with mitapivat demonstrated significant hematological improvements in a mouse model of β-thalassemia compared to mice administered with a vehicle control. These include increased Hb concentration, improved glycolysis, and extended peripheral RBC life span, decreased hemolysis, decreased extramedullary hematopoiesis, improved dyserythropoiesis (marked by improvements in erythroblast maturation index and reduced ROS levels in bone marrow), and reduced iron overload.

Many of these improvements were also observed in a *Hbb*$^{th3/+}$ mouse model of transfusion-dependent β-thalassemia. In this related study, treatment with mitapivat increased the RBC transfusion interval in *Hbb*$^{th3/+}$mice compared to the vehicle-treated group. When co-administered with the iron chelator DFP, the beneficial effects of mitapivat on anemia in *Hbb*$^{th3/+}$ mice were maintained, while iron chelation remained effective. Collectively, these observations are consistent with the proposed mechanism of action of mitapivat in which pyruvate kinase activation increases ATP to support normal cellular mechanisms that decrease anemia, reduce hemolysis, improve erythropoiesis, and reduce iron overload associated with thalassemia.

PK interaction:

Since the concentrations range tested (0.1 to 100 µM) in the *in vitro* studies submitted in the original Pyrukynd MAA procedure (EMEA/H/C/005540) cover the concentrations expected in the worst-case scenario for the new higher 100 mg dose (compared to the original 50 mg, the results can be used and reinterpreted to assess the DDI risk for the 100 mg dose.

As a precipitant:

Considering the newly established cut-off values using the dose of 100mg BID, no new clinically relevant DDI risks were identified, the DDI risk profile remains consistent with that observed at 50 mg for the enzymes and transporters. Pyrukynd is considered as an *in vitro* inducer of CYP2B6, CYP3A4, CYP2C8, CYP2C9, CYP2C19,

and UGT1A1. In addition, Pyrukynd is considered as an *in vitro* inhibitor of P-gp transporter. Therefore, the potential for DDI with substrates of these enzymes cannot be ruled out and this risk is mentioned in section 4.5 of the SmPC.

During this procedure, the MAH submitted the results of a new clinical DDI study assessing the effect of steady-state Pyrukynd on the pharmacokinetics of midazolam, a sensitive CYP3A4 probe substrate, in healthy adult subjects. The results demonstrated that coadministration of Pyrukynd 100 mg BID with a single 2 mg dose of midazolam resulted in a decrease in midazolam AUC∞ and Cmax by approximately 53% and 37%, respectively, compared to midazolam administered alone. These results support the PBPK model prediction (AUC ratio of 0.35), which provided a more conservative estimate representing a worst-case DDI scenario. However, these results only validate the model's ability to predict CYP3A4 modulation. The MAH has amended the SmPC to include the results of Study 027, and this amendment is considered acceptable.

As an object:

The MAH justified the dose adjustments recommended in Section 4.5 of the SmPC for Pyrukynd when administered concomitantly with CYP3A4 inhibitors or inducers based on simulated Pyrukynd exposures at steady state ($AUC_{0-\tau}$ and Cmax) using a PBPK model and considering the drug's safety profile.

The PBPK model was built and validated using *in vitro* data and PK studies, including clinical DDI studies with a strong CYP3A4 inhibitor (itraconazole) and a strong inducer (rifampin). In the original Pyrukynd MAA procedure (EMEA/H/C/005540), the presented model was accepted by the CHMP to be used to support mitapivat interaction as victim drug of CYP3A4 inhibitors. The model was used to evaluate the DDI potential with moderate CYP3A4 inhibitor (fluconazole). The simulation predicted a moderate increase in exposure ($AUC_{0-\tau}$ ratio of 2.70) when fluconazole was administered alongside mitapivat 100 mg BID. To mitigate this effect, reducing the Pyrukynd dosing frequency from 100 mg BID to 100 mg QD was shown to result in mitapivat exposures that were no more than 1.54-fold higher than those observed with 100 mg BID mitapivat alone.

The MAH considers this approximately 50% increase in exposure to be acceptable, based on exposure–safety analyses. Therefore, when co-administration with a moderate CYP3A4 inhibitor such as fluconazole is unavoidable, to minimise the risk of adverse reactions reducing the mitapivat dose to 100 mg QD is recommended and patients should be monitored for increased risk of adverse reactions. This recommendation in section 4.5 of the SmPC is considered justified and acceptable.

Regarding CYP3A4 inducers: Although model simulations predict that a dose of 200 mg twice daily (BID) of Pyrukynd administered with a moderate CYP3A4 inducer (efavirenz) results in drug exposure approaching to 100 mg BID of Pyrukynd alone, no dose adjustment is proposed. This is because doses above 100 mg BID have not been clinically evaluated in patients with thalassemia. The MAH emphasises that patients should not exceed the recommended dose of 100 mg twice daily in Section 4.5 of the SmPC. This approach is considered acceptable.

2.6.4. Conclusions on clinical pharmacology

Overall, the PK of mitapivat has been sufficiently characterised in the target population.

Updated information based on new studies and PPBK analysis on drug/drug interactions has been adequately reflected in section 4.5 of the SmPC.

2.6.5. Clinical efficacy

2.6.5.1. Dose response studies

The recommended dose of mitapivat for the treatment of adult patients with non–transfusion dependent and transfusion -dependent and α- or β-thalassemia is 100 mg BID, taken orally with or without food.

The determination of the recommended clinical dose of mitapivat for the proposed indication was based on PK, PD, safety, and efficacy data from subjects with thalassemia (Studies 010, 017 and 018) and from healthy subjects (Studies 001 and 002).

The PK and PD effects of mitapivat were initially evaluated in healthy subjects in a single ascending dose phase 1 study (Study 001) and a multiple ascending dose (14-day) phase 1 study (Study 002).

Dose-dependent decreases in 2,3-DPG and increases in ATP consistent with the mechanism of action of mitapivat were observed across the examined dose range of 30-2,500 mg in the single ascending dose study and the examined dose range of 15-700 mg BID in the multiple ascending dose study.

Patients with thalassemia have wild-type PKR as found in healthy individuals; therefore, PK/PD results from Study 002 were used to select the dose for Phase 2 study in subjects with NTDT (Study 010). The simple Emax model used to describe the PK/PD correlation between plasma mitapivat AUC0-12 and blood AUC_Net_B0-12 for ATP observations from Study 002 showed that approximately 85% of the maximum stimulatory effect was achieved at a dose of 60 mg BID and over 96% of the maximum stimulatory effect was achieved at a dose of 360 mg BID.

The initial dose that was administered to subjects in Study 010 was 50 mg BID which was expected to result in approximately 85% of the maximum stimulatory effect on ATP. The dose of 50 mg BID, previously studied in adult subjects with pyruvate kinase deficiency, resulted in meaningful changes in Hb concentrations (Study 003) and was well-tolerated. Based on the clinical effects of mitapivat on ATP in healthy subjects and mitapivat on Hb in adult subjects with pyruvate kinase deficiency, the selected dose of mitapivat 50 mg BID was expected to increase ATP sufficiently such that it resulted in meaningful increases in Hb in adult subjects with NTDT. Adult subjects with NTDT who tolerated mitapivat at 50 mg BID and achieved an Hb concentration increase from baseline that was expected be safe and tolerable and to increase ATP to a greater extent than 50 mg BID.

The selection of 100 mg BID dosing for the Phase 3 studies (Study 017 and 018) was based on PK, PD, efficacy, and safety data from subjects in Study 010. Mitapivat exposure (as assessed by AUClast) was approximately 30% higher following multiple 100 mg BID doses compared to a single 50 mg dose. This was supported by PD data that showed the mean ATP percent change from baseline increased from 82.7% on Week 6, in which subjects were receiving 50 mg BID, to 86.7% and 92.3% on Weeks 12 and 24, respectively, in which subjects were receiving 100 mg BID. Mitapivat dosing at 100 mg BID was associated with more Hb responders than the 50 mg BID dose, and at 100 mg BID mitapivat, AEs were manageable and were consistent with those in other studies across the clinical development program.

The pivotal studies in the thalassemia program did not include a dose titration plan, unlike that implemented in the development program for pyruvate kinase deficiency. Patients with pyruvate kinase deficiency have a mutated PKR enzyme, unlike patients with thalassemia who have a wild-type enzyme. In the pyruvate kinase deficiency Phase 2 dose-ranging study (Study 003), subjects were exposed to 50 mg or 300 mg BID without a dose titration. There was considerable variability in the subjects' individual sensitivity to mitapivat treatment, most likely due to the genetic heterogeneity of the PKR enzyme, and this manifested as excessive Hb response which required dose reductions. Therefore, based on the Phase 2 experience, an individual dose optimization

regimen was incorporated into the Phase 3 pyruvate kinase deficiency studies (Studies 006, 007, and 011). In the Phase 2 study (Study 010) in subjects with thalassemia, there were no observations of excessive Hb response at the starting dose of 50 mg BID or after dose was escalated to 100 mg BID at Week 6 and as a result no dose reductions were performed; this was expected since these subjects have wild-type PKR. Therefore, dose titration was not considered necessary for patients with thalassemia.

2.6.5.2. Main study

Mitapivat was studied in a global program consisting of 3 studies that enrolled adult patients with thalassemia across genotypes and transfusion needs.

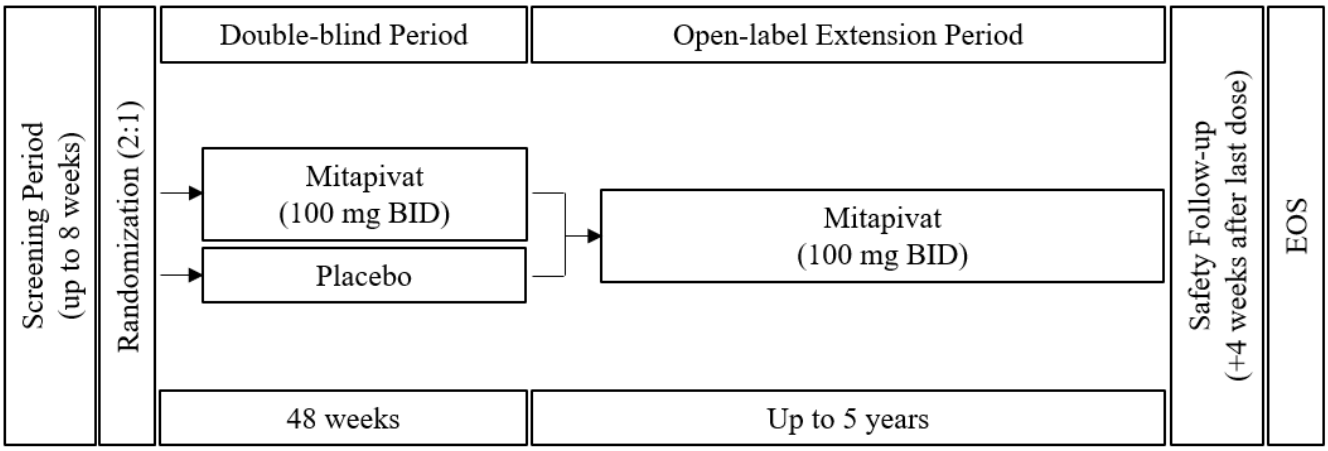
- The 2 global pivotal Phase 3 studies were randomized, double-blind, and placebo controlled and aimed to provide the primary evidence of efficacy of mitapivat in adults with thalassemia, reflective of the real-world thalassemia patient population. Study 018 enrolled subjects with TD α- or β-thalassemia and Study 017 enrolled subjects with NTD α- or β-thalassemia.
- The single-arm Phase 2 study (Study 010) aimed to provide supportive evidence of efficacy in patients with NTD α- or β-thalassemia.

Study AG348-C-018

Methods

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 transfusion-dependent α- or β-thalassemia followed by an OLE Period. Approximately 240 subjects were planned to be randomized in the study. An overview of the study design is presented in Figure 25.

Figure 25: Study Design



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

The choice of placebo control was supported by the lack of widely available and disease-specific, approved treatments for patients with TD α-or β-thalassemia. The 2:1 randomization ratio to receive mitapivat or matched placebo provided an increased chance to receive active study drug. Placebo administration was limited to a 48-week Double-blind Period, and subjects, including those who were randomized to placebo, had the opportunity to receive mitapivat during the OLE Period, allowing for a longer-term assessment of safety while providing access to active drug. The stratified randomization by thalassemia genotype ensured a balanced allocation of subjects to mitapivat or placebo to account for differences in transfusion requirements, with subjects with α-thalassemia captured primarily under the non-β^0/β^0 population. Stratification by geographical region allowed for characterization of the effect across areas with regional care variations.

Based on pharmacokinetics, PD, efficacy, and safety data from subjects with NTDT in Study 010 a dose of 100 mg BID mitapivat was selected for the Phase 3 studies.

Study Participants

The study was conducted in a population of adult subjects with TD α- or β thalassemia, defined as 6 to 20 RBC units transfused and a ≤6-week transfusion-free period during the 24-week period before randomization.

A key criterion for this study was documented diagnosis of thalassemia (β thalassemia with or without α-globin gene mutations, HbE/β-thalassemia, or α thalassemia/HbH disease).

This study did not include subjects who were homozygous or heterozygous HbS or HbC. The following treatments were excluded: prior exposure to gene therapy or prior bone marrow or stem cell transplantation. Treatment with luspatercept and hematopoietic stimulating agents also was excluded (a washout period before randomization was allowed).

Randomization and blinding

Eligible subjects were randomized in a 2:1 ratio to mitapivat or matched placebo. Randomization assignment was implemented by an IXRS and stratified by:

- Geographical region (North America and Europe; Asia Pacific; and Rest of World)
- Thalassemia genotype (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)).

The stratified randomization by thalassemia genotype ensured a balanced allocation of subjects to mitapivat or placebo within each genotype, and stratification by geographical region allowed for characterization of the effect across areas with regional care variations.

Study subjects, Investigators, clinical study center personnel, pharmacists, and the Sponsor were blinded to the subject's treatment assignment. After completing the Double-blind Period, subjects were given the opportunity to receive mitapivat in the OLE Period. At the last study visit of the Double-blind Period, subjects who continued in the OLE Period were provided with active mitapivat; however, study subjects, Investigators, clinical study center personnel, and the Sponsor continued to remain blinded to the randomized treatment assignment during the previous Double-blind Period until the study was unblinded for the analysis of the primary endpoint.

Treatments

Subjects received 100 mg BID mitapivat or matched placebo for oral administration. Mitapivat was supplied as 100-mg strength tablets and placebo was supplied as matched tablets.

The potential for acute hemolysis upon abrupt withdrawal of mitapivat treatment is anticipated from the drug's known mechanism of action to activate the erythrocyte pyruvate kinase enzyme. In pyruvate kinase deficiency, mt PK-R has low residual enzyme activity resulting in disruption of glycolytic pathway activity that leads to abnormal RBC metabolism and a shortened RBC life span. Mitapivat restores the activity of mt PK-R, thus targeting the underlying enzymatic defect that causes hemolysis. In the setting of abrupt mitapivat withdrawal, the mutant form of PK-R returns to a deficient state; therefore, in a patient with pyruvate kinase deficiency who is a responder to mitapivat treatment, abrupt withdrawal of mitapivat treatment may lead to a rapid return to pretreatment levels of hemolysis. However, in subjects with thalassemia, mitapivat activates wt PK-R rather than mt PK-R. In the setting of abrupt mitapivat withdrawal for subjects with thalassemia, wt PK-R retains baseline residual activity; therefore, acute hemolysis is unlikely to occur in this patient population as compared with pyruvate kinase deficiency.

This is further supported by pharmacodynamic data from NHVs treated with mitapivat, where wt PK-R is activated, showing extended effects on ATP after treatment discontinuation. In Study AG348-C-002, where NHVs were administered mitapivat for 14 days, the effect of mitapivat on ATP concentrations became steady within 10 days of dose administration, and mean ATP levels remained above baseline values up to 5 days (last day measured) after the final dose of mitapivat. The main process for ameliorating ineffective erythropoiesis and hemolysis in subjects with thalassemia treated with mitapivat is the generation of ATP by activating the wt PK-R enzyme. The extended pharmacodynamic effects in the form of increased ATP from wt PK-R activation further indicate that the risk of acute hemolysis is unlikely to occur in the patient population with thalassemia.

Subjects who discontinued study drug (mitapivat or placebo) were to follow the recommended dose taper (Table 18) and were to be monitored as clinically indicated for signs and symptoms of acute hemolysis and worsening anemia. If immediate or abrupt study drug discontinuation was required for an AE or medical emergency, subjects were monitored as clinically indicated for signs of acute hemolysis or worsening anemia.

Table 18: 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.

Dose modification guidelines for excessive Hb response were provided as described in Table 19 and in Table 20 for treatment-related AEs.

Table 19: 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.

Table 20: Dose Modifications for Study Drug–Related Adverse Events

AE Severity[a]	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.

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

Transfusions and other supportive care therapies were permitted as clinically indicated.

Objectives and outcomes/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	**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
• To evaluate the effect of mitapivat versus placebo on iron metabolism	• Change from baseline in iron, serum ferritin, total iron binding capacity, and transferrin saturation through Week 48
• To evaluate the safety of mitapivat	• Type, severity, and relationship of adverse events 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 and 2,3-diphosphoglycerate
Exploratory Objectives • To explore the effect of mitapivat versus placebo on iron overload	**Exploratory Endpoints** • Change from baseline in hepatic iron concentration through Week 48 • Change from baseline in daily dose of iron chelation therapy through Week 48
• To explore the effect of mitapivat versus placebo on health-related quality of life	• Change from baseline in Transfusion-Dependent Quality of Life Questionnaire score from Week 13 through Week 48 • Change from baseline in Patient Global Impression of Severity-Thalassemia symptoms and Patient Global Impression of Change-Thalassemia symptoms and the EuroQoL Group 5-Level EQ-5D questionnaires • Change from baseline in Patient Reported Outcomes Measurement Information System® Physical Function Short Form score from Week 13 through Week 48
• To explore the effect of mitapivat versus placebo on markers of hemolysis and erythropoiesis	• Change from baseline in indirect bilirubin, lactate dehydrogenase, and haptoglobin through Week 48 • Change from baseline in reticulocytes and erythropoietin through Week 48
• To explore the relationship between mitapivat pharmacokinetics and clinical response	• Exposure-response relationship between pharmacokinetic parameters and clinical response
• To explore the effect of mitapivat versus placebo on exploratory biomarkers	• Changes over time in exploratory biomarkers, including free hemoglobin (Hb), fetal Hb, hepcidin, erythroferrone, soluble transferrin receptor, estimated glomerular filtration rate, and spot urine albumin-to-creatinine ratio

• To explore the effect of open-label mitapivat on patient-reported measures and efficacy parameters	• Change from baseline in transfusion burden, patient-reported outcome measures, markers of iron overload and metabolism, and exploratory biomarkers, during the Open-label Extension Period

Sample size

The following statistical hypothesis was prespecified and 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. The TRR is defined as a ≥50% reduction in transfused RBC units with a reduction of ≥2 units of transfused RBCs in any consecutive 12-week period through Week 48 compared with the baseline transfusion burden standardized to 12 weeks.

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

Randomisation and Blinding (masking)

Eligible subjects were randomized in a 2:1 ratio to mitapivat or matched placebo. Randomization assignment was implemented by an IXRS and stratified by:

- Geographical region (North America and Europe; Asia Pacific; and Rest of World)
- Thalassemia genotype (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)).

The stratified randomization by thalassemia genotype ensured a balanced allocation of subjects to mitapivat or placebo within each genotype, and stratification by geographical region allowed for characterisation of the effect across areas with regional care variations.

Study subjects, Investigators, clinical study center personnel, pharmacists, and the Sponsor were blinded to the subject's treatment assignment. After completing the Double-blind Period, subjects were given the opportunity to receive mitapivat in the OLE Period. At the last study visit of the Double-blind Period, subjects who continued in the OLE Period were provided with active mitapivat; however, study subjects, Investigators, clinical study center personnel, and the Sponsor continued to remain blinded to the randomized treatment assignment during the previous Double-blind Period until the study was unblinded for the analysis of the primary endpoint.

Statistical methods

The following statistical hypothesis was prespecified and 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. The TRR is defined as a ≥50% reduction in transfused RBC units with a reduction of ≥2 units of

transfused RBCs in any consecutive 12-week period through Week 48 compared with the baseline transfusion burden standardized to 12 weeks.

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

Additionally, the following statistical hypotheses were 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}: $p_{t3} - p_{c3} \neq 0$

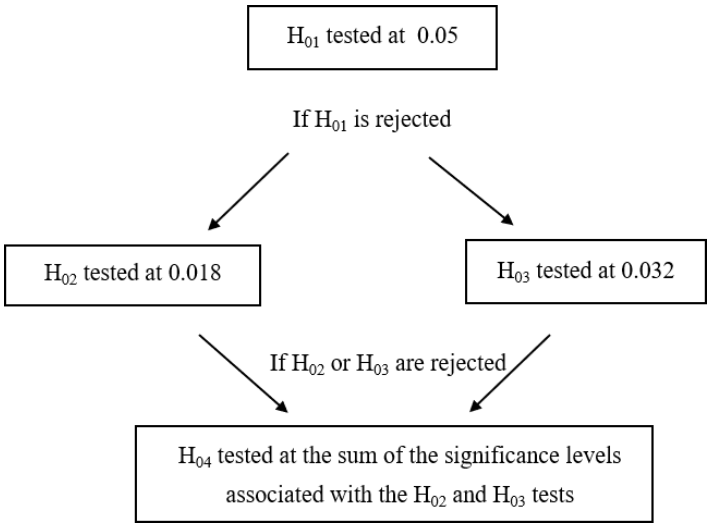
H_{04}: $p_{t4} - p_{c4} = 0$ vs H_{14}: $p_{t4} - p_{c4} \neq 0$

where

- p_{t2} and p_{c2} are the proportion of subjects achieving a TRR2 in the mitapivat arm and placebo arm, respectively. TRR2 is defined as ≥50% reduction in transfused RBC units in any consecutive 24-week period through Week 48 compared with the 24-week baseline transfusion burden.
- p_{t3} and p_{c3} are the proportion of subjects achieving a TRR3 in the mitapivat arm and placebo arm, respectively. TRR3 is defined as ≥33% reduction in transfused RBC units from Week 13 through Week 48 compared with the baseline transfusion burden standardized to 36 weeks.
- p_{t4} and p_{c4} are the proportion of subjects achieving a TRR4 in the mitapivat arm and placebo arm, respectively. TRR4 is defined as ≥50% reduction in transfused RBC units from Week 13 through Week 48 compared with the baseline transfusion burden standardized to 36 weeks.

Overall type I error was maintained at or below a 2-sided significance level of 0.05 based on a graphical gatekeeping approach as illustrated in Figure 26.

Figure 26: Statistical Testing Strategy



The primary endpoint was tested at a 2-sided α-level of 0.05. Then, the first 2 key secondary endpoints were tested at an α-level of 0.032 and 0.018, respectively, if the null hypothesis for the primary endpoint (H_{01}) was rejected. The last key secondary endpoint was 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).

Results

Participant flow

Subject disposition is summarised for all randomized subjects in Table 14. One subject in the placebo arm was randomized but did not receive study treatment. One subject in the placebo arm incorrectly received mitapivat.

Table 21: Subject Disposition-Double blind period (Full Analysis set)

	Placebo N=87 n (%)	**Mitapivat N=171 n (%)**	**Total N=258 n (%)**
Disposition: Treatment double-blind period			
Not entered [1]	1 (1.1)	0	1 (0.4)
Discontinued	3 (3.4)	16 (9.4)	19 (7.4)
Reason			
Adverse Event	1 (1.1)	9 (5.3)	10 (3.9)
Withdrawal By Subject	1 (1.1)	7 (4.1)	8 (3.1)
Pregnancy	1 (1.1)	0	1 (0.4)
Completed	83 (95.4)	155 (90.6)	238 (92.2)
Ongoing	0	0	0
Disposition: Safety follow-up [2]			
Not entered	3 (3.4)	5 (2.9)	8 (3.1)
Discontinued	0	2 (1.2)	2 (0.8)
Reason			
Withdrawal By Subject	0	2 (1.2)	2 (0.8)
Completed	1 (1.1)	12 (7.0)	13 (5.0)
Ongoing	0	0	0

The denominator used to calculate percentages is N, the number of subjects in the full analysis set within each treatment group.
[1] Subjects randomized and not dosed.
[2] Summarizes disposition for subjects who discontinued treatment during the double-blind period or completed the double-blind treatment period but did not enter the open-label extension period.

Recruitment

Study design includes a screening period up to 8 weeks before randomization. A double blind period of 48 weeks was chosen, followed by an OLE period up to 5 years. A Safety follow up is planned then 4 weeks after the last dose. Start date: (first patient enrolled): January 26, 2022. Study Completion Date (Estimated): June 2029.

Conduct of the study

The protocol was amended 2 times. The key revisions are provided in table 22.

Table 22: Summary of Substantial Changes to the Protocol

Amendment 2 (28 Sep 2022) Global	• The protocol was amended to remove the requirement for male contraception. The protocol was updated to align with current reproductive safety data. Data suggest that mitapivat has no teratogenic or fetotoxic effects in males who father a child while receiving mitapivat. • The protocol was amended to remove reporting and follow-up requirements for pregnant partners of male subjects. The protocol was updated to align with current reproductive safety data. Data suggest that mitapivat has no teratogenic or fetotoxic effects in males who father a child while receiving mitapivat.
Amendment 1 (21 Jul 2021) Global	• Mitapivat administration has not been shown to result in clinically significant changes to triglyceride levels, so the frequency of sampling for lipids was reduced. • Mitapivat administration has not been shown to result in frequent fluctuations in sex hormones, so the frequency of sampling for sex hormones during the OLE Period of the study was reduced. • Mitapivat is not hepatotoxic, so transaminase increase was removed as a potential risk and AESI. • A dedicated concentration–QT interval study showed that mitapivat administration did not result in a clinically significant increase in QT interval; therefore, the maximum heart rate–corrected QT interval using Fridericia's method value for study eligibility for female subjects was increased from 450 milliseconds to 470 milliseconds. • Language summarizing the risk assessment for concomitant use of a COVID-19 vaccine was added in response to a request from a Regulatory Agency. • Updates were made to the timing for SAE reporting to align with the recording period for AEs. • The exclusion criterion related to subjects who have received anabolic steroids was modified to allow the use of testosterone replacement therapy to treat hypogonadism. This change was incorporated as the testosterone replacement therapy to treat hypogonadism is part of the standard of care of male thalassemia subjects and it is not expected to affect the safety or efficacy of mitapivat in this study.

Changes to Planned Analyses

All analyses presented were performed. Exploratory analyses associated with daily dose of iron chelation therapy were not performed because results would not be interpretable due to the large number of combinations of route of administration and dose/dose units/frequency of administration for iron chelation therapies, both within subjects and across subjects.

Impact of COVID-19 Pandemic on the Study

No major protocol deviations were associated with COVID-19 in the study. There were 2 subjects who had a missed or altered visit due to COVID-19. The low incidence of missing assessments due to the COVID-19 pandemic did not affect the interpretation of the endpoints.

Protocol deviations

The following were subject-level major deviations assessed for the potential to impact study conclusions (Table 23):

- Informed consent
    - A total of 17 subjects had informed consent deviations: 13 subjects did not consent under the correct or current version, 2 subjects did not complete the informed consent properly, and 4

subjects were not reconsented to the correct or current version.

- IP accountability
  - Two subjects in the placebo arm and 1 subject in the mitapivat arm were dispensed the incorrect IP kit by the study site.
- Eligibility criteria not met
  - A total of 18 subjects did not meet the protocol-specified eligibility criteria of transfusion dependent, defined as 6 to 20 RBC units transfused and a ≤6-week transfusion-free period during the 24-week period before randomization (inclusion criterion 3). Among these 18 subjects, 15 subjects received >20 RBC units (11 in the mitapivat arm and 4 in the placebo arm) and 3 subjects had >6-week transfusion-free period in the 24 weeks before randomization (all in the mitapivat arm).
  - A total of 6 subjects had exclusion criteria deviations: 1 subject had a documented history of homozygous or heterozygous HbS or HbC (exclusion criterion 2), 2 subjects had a history of active/or uncontrolled cardiac or pulmonary disease ≤6 months before providing informed consent (exclusion criterion 7), 1 subject had nonfasting triglycerides >440 mg/dL (5 mmol/L) (exclusion criterion 10), 1 subject had an active infection and received systemic antimicrobial therapy <7 days before randomization (exclusion criterion 11), and 1 subject tested positive for HCV Ab with evidence of active HCV infection or tested positive for HBsAg (exclusion criterion 12).
  - As part of the protocol deviation process, each subject was evaluated and a determination made to either remain on study or discontinue based on the criterion, safety, benefit vs risk, and best clinical practice. Final determination of benefit-risk was made at the Investigator's discretion.
- SAE related deviations
  - Four subjects had SAEs that were not reported within 24 hours of the site becoming aware of the SAE.
- Study treatment deviations
  - One subject did not undergo dose modification for a Grade 3 treatment-related AE.
  - Three subjects were noncompliant with study drug administration during the Double-blind Period.
  - Three subjects were randomized under the wrong stratification factor.
- Use of prohibited concomitant treatment
  - Five subjects were taking prohibited concomitant medication, including clarithromycin (3 subjects), testosterone enanthate (1 subject), and rifampicin (1 subject)

Table 23: Summary of Major Protocol Deviations (Full Analysis Set)

Deviations	Placebo N=87 n (%)	Mitapivat N=171 n (%)	Total N=258 n (%)
Subjects with major deviations	15 (17.2)	36 (21.1)	51 (19.8)
ICH/GCP Deviation	7 (8.0)	13 (7.6)	20 (7.8)
INFORMED CONSENT	5 (5.7)	12 (7.0)	17 (6.6)
INFORMED CONSENT NOT COMPLETED CORRECTLY	0	2 (1.2)	2 (0.8)
SUBJECT NOT CONSENTED UNDER CORRECT OR CURRENT VERSION OF INFORMED CONSENT	3 (3.4)	10 (5.8)	13 (5.0)
OTHER	2 (2.3)	2 (1.2)	4 (1.6)
IP ACCOUNTABILITY	2 (2.3)	1 (0.6)	3 (1.2)
SITE DISPENSED INCORRECT IP KIT TO A SUBJECT	2 (2.3)	1 (0.6)	3 (1.2)
Protocol Deviation	8 (9.2)	27 (15.8)	35 (13.6)
SAE-RELATED DEVIATIONS	1 (1.1)	3 (1.8)	4 (1.6)
SAE NOT REPORTED WITHIN 24 HOURS FROM THE POINT IN TIME WHEN THE SITE STAFF BECAME AWARE OF THE SAE	1 (1.1)	2 (1.2)	3 (1.2)
OTHER	0	1 (0.6)	1 (0.4)
SELECTION CRITERIA NOT MET	6 (6.9)	16 (9.4)	22 (8.5)
EXCLUSION CRITERIA 2 MET	1 (1.1)	0	1 (0.4)
EXCLUSION CRITERIA 7 MET	1 (1.1)	1 (0.6)	2 (0.8)
EXCLUSION CRITERIA 10 MET	0	1 (0.6)	1 (0.4)
EXCLUSION CRITERIA 11 MET	0	1 (0.6)	1 (0.4)
EXCLUSION CRITERIA 12 MET	0	1 (0.6)	1 (0.4)
INCLUSION CRITERIA 3 NOT MET	4 (4.6)	14 (8.2)	18 (7.0)
STUDY TREATMENT DEVIATION	0	7 (4.1)	7 (2.7)
DOSE MODIFICATION CRITERIA NOT FOLLOWED FOR GRADE 3 RELATED AE	0	1 (0.6)	1 (0.4)
OVERALL STUDY DRUG NON-COMPLIANCE DURING THE DOUBLE-BLIND PERIOD	0	3 (1.8)	3 (1.2)
SUBJECT RANDOMIZED UNDER THE WRONG STRATIFICATION FACTOR(S)	0	3 (1.8)	3 (1.2)
USE OF PROHIBITED CONCOMITANT TREATMENT	2 (2.3)	3 (1.8)	5 (1.9)
SUBJECT IS TAKING A PROHIBITED CONCOMITANT MEDICATION DURING STUDY	2 (2.3)	3 (1.8)	5 (1.9)

The denominator used to calculate percentages is N, the number of subjects in the full analysis set within each treatment group.

No protocol deviations affected the study conclusions. The site-level protocol deviations reported in the study did not affect the study conclusions.

Baseline data

There were no imbalances between treatment arms in demographic characteristics or physical measurements at baseline deemed to have an impact on the interpretation of the results (Table 24).

Table 24: Summary of Demographic Characteristics and Physical Measurements at Baseline (Full Analysis Set).

Parameter	Placebo N=87	Mitapivat N=171	Total N=258
Age (yr)			
n	87	171	258
Mean (SD)	34.7 (9.77)	35.8 (11.61)	35.5 (11.02)
Median (Q1, Q3)	34.0 (26.0, 43.0)	33.0 (27.0, 45.0)	33.5 (27.0, 44.0)
Min, Max	20, 53	18, 67	18, 67
Age category 1 (yr), n (%)			
<35	44 (50.6)	91 (53.2)	135 (52.3)
≥35	43 (49.4)	80 (46.8)	123 (47.7)
Age category 2 (yr), n (%)			
<65	87 (100)	170 (99.4)	257 (99.6)
≥65	0	1 (0.6)	1 (0.4)
Sex, n (%)			
Male	44 (50.6)	78 (45.6)	122 (47.3)
Female	43 (49.4)	93 (54.4)	136 (52.7)
Childbearing Potential [1]			
Yes	33 (76.7)	73 (78.5)	106 (77.9)
No	10 (23.3)	20 (21.5)	30 (22.1)
Ethnicity, n (%)			
Hispanic or Latino	3 (3.4)	5 (2.9)	8 (3.1)
Not Hispanic or Latino	79 (90.8)	158 (92.4)	237 (91.9)
Not reported	5 (5.7)	8 (4.7)	13 (5.0)
Race, n (%)			
White	56 (64.4)	99 (57.9)	155 (60.1)
Asian	22 (25.3)	56 (32.7)	78 (30.2)
Black or African American	1 (1.1)	1 (0.6)	2 (0.8)
American Indian or Alaska Native	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0
Multiracial	0	2 (1.2)	2 (0.8)
Unknown	3 (3.4)	7 (4.1)	10 (3.9)
Not reported	5 (5.7)	6 (3.5)	11 (4.3)
Height (cm)			
n	87	171	258
Mean (SD)	162.66 (10.396)	163.30 (10.282)	163.09 (10.305)
Median (Q1, Q3)	160.30 (155.00, 172.00)	162.00 (156.00, 169.80)	161.65 (156.00, 170.00)
Min, Max	139.5, 185.0	140.0, 190.0	139.5, 190.0
Weight (kg)			
n	87	171	258
Mean (SD)	59.45 (11.547)	59.71 (11.392)	59.62 (11.422)
Median (Q1, Q3)	56.70 (50.50, 67.40)	57.80 (51.90, 67.00)	57.50 (51.70, 67.00)
Min, Max	38.7, 95.0	34.9, 93.4	34.9, 95.0
BMI (kg/m²)			
n	87	171	258
Mean (SD)	22.40 (3.457)	22.37 (3.690)	22.38 (3.606)
Median (Q1, Q3)	22.34 (19.85, 24.09)	21.92 (19.52, 24.51)	22.05 (19.69, 24.14)
Min, Max	16.8, 35.3	15.6, 37.4	15.6, 37.4

The denominator used to calculate percentages is N, the number of subjects in the full analysis set within each treatment group.
Body Mass Index (BMI)=Weight(kg)/[Height(m)²].
[1] The denominator used to calculate percentages is the number of female subjects in the full analysis set within each treatment group.

The study enrolled 258 subjects globally (Table 25).

Table 25: Summary of Accrual by Geographic Region and Country (Full Analysis Set)

Geographic Region	Country	Placebo N=87 n (%)	Mitapivat N=171 n (%)	Total N=258 n (%)
Asia	All	16 (18.4)	31 (18.1)	47 (18.2)
	Malaysia	11 (12.6)	14 (8.2)	25 (9.7)
	Taiwan	1 (1.1)	0	1 (0.4)
	Thailand	4 (4.6)	17 (9.9)	21 (8.1)
Eastern Europe	All	15 (17.2)	19 (11.1)	34 (13.2)
	Bulgaria	15 (17.2)	19 (11.1)	34 (13.2)
Latin America	All	2 (2.3)	1 (0.6)	3 (1.2)
	Brazil	2 (2.3)	1 (0.6)	3 (1.2)
Middle East	All	15 (17.2)	33 (19.3)	48 (18.6)
	Lebanon	3 (3.4)	7 (4.1)	10 (3.9)
	Saudi Arabia	0	2 (1.2)	2 (0.8)
	Turkey	11 (12.6)	23 (13.5)	34 (13.2)
	United Arab Emirates	1 (1.1)	1 (0.6)	2 (0.8)
North America	All	5 (5.7)	21 (12.3)	26 (10.1)
	Canada	2 (2.3)	9 (5.3)	11 (4.3)
	United States	3 (3.4)	12 (7.0)	15 (5.8)
Western Europe	All	34 (39.1)	66 (38.6)	100 (38.8)
	Denmark	4 (4.6)	4 (2.3)	8 (3.1)
	France	6 (6.9)	9 (5.3)	15 (5.8)
	Germany	2 (2.3)	7 (4.1)	9 (3.5)
	Greece	5 (5.7)	14 (8.2)	19 (7.4)
	Italy	6 (6.9)	8 (4.7)	14 (5.4)
	Netherlands	5 (5.7)	7 (4.1)	12 (4.7)
	Spain	5 (5.7)	12 (7.0)	17 (6.6)
	United Kingdom	1 (1.1)	5 (2.9)	6 (2.3)

The denominator used to calculate percentages is N, the number of subjects in the full analysis set within each treatment group.

Baseline Disease Characteristics

Key baseline disease characteristics were similar between treatment arms and were representative of a real-world population of patients with thalassemia who are regularly transfused (Table 26, Table 27, and Table 28). There were no imbalances between treatment arms in disease characteristics deemed to have an impact on the interpretation of the results. As expected for a regularly transfused population, most subjects in the study had a high 24-week baseline transfusion burden of >12 RBC units and a mean pretransfusion Hb threshold of 88.4 g/L (8.84 g/dL). In addition, most subjects (97.7%) had received iron chelation therapy within 1 year prior to randomization. Subjects who were assessed by MRI also had elevated hepatic iron concentrations, consistent with iron overload. Baseline markers of hemolysis and ineffective erythropoiesis were similar across treatment arms.

Table 26: Summary of Baseline Disease Characteristics (Full Analysis Set)

	Placebo N=87	Mitapivat N=171	Total N=258
24-week baseline transfusion burden [1], n (%)			
≤12 RBC Units	21 (24.1)	54 (31.6)	75 (29.1)
>12 RBC Units	66 (75.9)	117 (68.4)	183 (70.9)
Pretransfusion Hb threshold (g/L) [2]			
n	87	171	258
Mean (SD)	88.163 (11.0025)	88.533 (11.6018)	88.409 (11.3831)

	Placebo N=87	Mitapivat N=171	Total N=258
Median (Q1, Q3)	89.200 (80.000, 96.800)	89.600 (81.000, 96.400)	89.350 (80.600, 96.400)
Min, Max	51.20, 109.10	51.00, 118.40	51.00, 118.40
Splenectomy status [3], n (%)			
No	38 (43.7)	79 (46.2)	117 (45.3)
Yes	49 (56.3)	92 (53.8)	141 (54.7)
If yes, age at splenectomy (yr)			
n	43	82	125
Mean (SD)	12.4 (7.73)	15.3 (8.52)	14.3 (8.34)
Median (Q1, Q3)	11.0 (6.0, 16.0)	14.5 (8.0, 21.0)	13.0 (7.0, 20.0)
Min, Max	1, 35	2, 41	1, 41
Prior cholecystectomy status [3], n (%)			
No	63 (72.4)	129 (75.4)	192 (74.4)
Yes	24 (27.6)	42 (24.6)	66 (25.6)
If yes, age at cholecystectomy (yr)			
n	20	38	58
Mean (SD)	23.4 (6.19)	24.4 (9.35)	24.1 (8.35)
Median (Q1, Q3)	24.0 (20.0, 25.5)	25.0 (17.0, 29.0)	24.0 (17.0, 29.0)
Min, Max	12, 35	10, 43	10, 43
Prior iron chelation status [4], n (%)			
No	0	6 (3.5)	6 (2.3)
Yes	87 (100)	165 (96.5)	252 (97.7)
Prior hydroxyurea status, n (%)			
No	84 (96.6)	164 (95.9)	248 (96.1)
Yes	3 (3.4)	7 (4.1)	10 (3.9)

Table 27: Summary of Baseline Markers of Iron Metabolism and Iron Overload (Full Analysis Set)

Parameter	**Placebo N=87**	**Mitapivat N=171**	**Total N=258**
Baseline iron (umol/L)			
n	82	161	243
Mean (SD)	42.02 (13.830)	41.32 (12.483)	41.55 (12.929)
Median (Q1, Q3)	41.90 (35.40, 49.60)	40.50 (33.30, 47.26)	40.99 (33.70, 48.20)
Min, Max	7.3, 81.6	8.8, 77.1	7.3, 81.6
Baseline ferritin (ug/L)			
n	83	161	244
Mean (SD)	1928.7 (1862.11)	2020.0 (2290.86)	1988.9 (2151.15)
Median (Q1, Q3)	1365.0 (805.0, 2316.0)	1272.0 (665.0, 2314.0)	1303.5 (690.0, 2315.0)
Min, Max	240, 11217	84, 14081	84, 14081
Baseline total iron binding capacity (umol/L)			
n	52	109	161
Mean (SD)	79.02 (34.345)	82.15 (37.888)	81.14 (36.703)
Median (Q1, Q3)	76.90 (53.43, 100.00)	75.00 (50.66, 104.90)	75.70 (50.66, 101.85)
Min, Max	27.9, 157.2	26.7, 193.0	26.7, 193.0
Baseline transferrin saturation (fraction of 1)			
n	52	111	163
Mean (SD)	0.593 (0.2006)	0.580 (0.1976)	0.584 (0.1980)
Median (Q1, Q3)	0.550 (0.455, 0.740)	0.590 (0.430, 0.740)	0.560 (0.440, 0.740)
Min, Max	0.24, 1.27	0.13, 1.02	0.13, 1.27
Baseline hepatic iron concentration by MRI (mg/g)			
n	74	133	207
Mean (SD)	5.712 (4.7491)	5.731 (4.8952)	5.724 (4.8319)
Median (Q1, Q3)	4.430 (2.220, 7.640)	4.580 (2.250, 7.150)	4.550 (2.220, 7.270)

Parameter	Placebo N=87	Mitapivat N=171	Total N=258
Min, Max	0.37, 20.47	0.37, 28.21	0.37, 28.21

Abbreviations: MRI=magnetic resonance imaging; RBC=red blood cell.
Baseline is defined as the last assessment before Transfusion 0 (the last RBC transfusion before randomization).

Table 28: Summary of Baseline Markers of Hemolysis and Markers of Erythropoiesis (Full Analysis Set)

Parameter	Placebo N=87	Mitapivat N=171	Total N=258
Baseline indirect bilirubin (umol/L)			
n	86	164	250
Mean (SD)	32.93 (36.749)	31.99 (28.008)	32.31 (31.220)
Median (Q1, Q3)	24.02 (15.70, 31.30)	22.70 (14.55, 39.53)	23.00 (14.90, 36.30)
Min, Max	2.9, 220.2	0.9, 192.2	0.9, 220.2
Baseline lactate dehydrogenase (U/L)			
n	86	165	251
Mean (SD)	213.23 (143.346)	233.16 (155.214)	226.33 (151.262)
Median (Q1, Q3)	174.50 (143.00, 220.00)	178.00 (149.00, 245.00)	177.00 (146.00, 240.00)
Min, Max	91.0, 980.0	94.0, 1270.0	91.0, 1270.0
Baseline haptoglobin (g/L)			
n	82	160	242
Mean (SD)	0.388 (0.3129)	0.326 (0.2830)	0.347 (0.2943)
Median (Q1, Q3)	0.295 (0.100, 0.600)	0.205 (0.100, 0.470)	0.240 (0.100, 0.540)
Min, Max	0.10, 1.49	0.10, 1.48	0.10, 1.49
Baseline erythropoietin (IU/L)			
n	74	142	216
Mean (SD)	226.76 (500.595)	131.18 (174.367)	163.92 (327.250)
Median (Q1, Q3)	97.65 (52.10, 150.00)	83.90 (48.70, 152.00)	88.60 (49.00, 151.00)
Min, Max	12.7, 3434.0	7.1, 1490.0	7.1, 3434.0
Baseline reticulocytes (109/L)			
n	69	135	204
Mean (SD)	128.50 (140.924)	143.86 (195.903)	138.66 (178.994)
Median (Q1, Q3)	49.30 (20.00, 208.30)	74.40 (26.90, 156.40)	72.90 (25.40, 171.80)
Min, Max	10.0, 607.8	10.0, 1049.1	10.0, 1049.1
Baseline reticulocytes/erythrocytes (fraction of 1)			
n	69	140	209
Mean (SD)	0.0408 (0.04907)	0.0444 (0.05745)	0.0432 (0.05473)
Median (Q1, Q3)	0.0176 (0.0072, 0.0622)	0.0245 (0.0085, 0.0493)	0.0220 (0.0078, 0.0548)
Min, Max	0.002, 0.252	0.002, 0.323	0.002, 0.323

Abbreviation: RBC=red blood cell.
Baseline is defined as the last assessment before Transfusion 0 (the last RBC transfusion before randomization).

Numbers analysed

A total of 171 participants were randomised in the mitapivat arm and 87 were randomised in the placebo arm. Of the subjects randomised, more than 90% of participants in total were included in the PPS, i.e 157 participants in the mitapivat arm and 82 in the placebo arm.

Analysis set definitions and summaries are provided in Table 29.

Table 29: Summary of Analysis Set (All Screened subjects)

	Placebo	Mitapivat	Total
All Screened Subjects			305
Full Analysis Set	87	171	258
Per-Protocol Set, n (%) [1]	82 (94.3)	157 (91.8)	239 (92.6)
Safety Analysis Set	85	172	257
PK Analysis Set, n (%) [2]	0	165 (95.9)	165 (64.2)
PD Analysis Set, n (%) [2]	85 (100)	169 (98.3)	254 (98.8)
PK/PD Analysis Set, n (%) [2]	0	162 (94.2)	162 (63.0)

Outcomes and estimation

Mitapivat demonstrated a statistically significant reduction in transfusion burden compared with placebo (2-sided p-value=0.0003) in subjects with α- or β- thalassemia who are TD, as measured by the primary endpoint of TRR (Table 30).

Table 30: Primary Analysis for TRR (≥50% Reduction in Transfused RBC Units with a Reduction of ≥2 Transfused RBC Units in any Consecutive 12-week Period Compared with Baseline) (Full Analysis Set)

	Placebo N=87	Mitapivat N=171
TRR responders, n (%)	11 (12.6)	52 (30.4)
Adjusted difference in TRR rate (Mitapivat vs Placebo), %		17.6
95% CI		(8.0, 27.2)
2-sided p-value		0.0003

Results of prespecified sensitivity analyses for TRR were consistent with the results:

- For the analysis based on the PPS, the 2-sided p-value was 0.0004.
- For the analysis including only those subjects who completed 48 weeks of study treatment and who did not receive any concomitant medications before completion of 48 weeks of study treatment that could affect the Hb concentrations , the 2-sided p-value was 0.0007.
- For the analysis based on imputation, the 2-sided p-value was 0.0001. In this analysis, for subjects with Hb concentration below the individual transfusion threshold who did not receive an RBC transfusion for a reason other than not clinically indicated, an RBC transfusion was imputed on the day the Hb concentration was less than the individual transfusion threshold and the number of RBC

units was imputed as the number of RBC units received at the last transfusion before the Hb assessment associated with the missed transfusion.

Mitapivat further demonstrated statistically significant reduction in transfusion burden compared with placebo as measured by the 3 key secondary endpoints (TRR2, TRR3, and TRR4) with 2-sided p-values of 0.0003 (Table 31), <0.0001 (Table 32), and 0.0056 (Table 33), respectively, demonstrating the depth and durability of the effect of mitapivat in reducing transfusion burden.

Table 31: Primary Analysis for TRR2 (≥50% Reduction in Transfused RBC Units in any Consecutive 24-week Period Compared with Baseline) (Full Analysis Set).

	Placebo N=87	Mitapivat N=171
TRR2 responders, n (%)	2 (2.3)	23 (13.5)
Adjusted difference in TRR2 rate (Mitapivat vs Placebo), %		11.1
95% CI		(5.1, 17.0)
2-sided p-value		0.0003

Table 32: Primary Analysis for TRR3 (≥33% Reduction in Transfused RBC Units from Week 13 through Week 48 Compared with Baseline) (Full Analysis Set)

	Placebo N=87	Mitapivat N=171
TRR3 responders, n (%)	1 (1.1)	25 (14.6)
Adjusted difference in TRR3 rate (Mitapivat vs Placebo), %		13.4
95% CI		(7.7, 19.1)
2-sided p-value		<0.0001

Table 33: Primary Analysis for TRR4 (≥50% Reduction in Transfused RBC Units from Week 13 through Week 48 Compared with Baseline) (Full Analysis Set)

	Placebo N=87	Mitapivat N=171
TRR4 responders, n (%)	1 (1.1)	13 (7.6)
Adjusted difference in TRR4 rate (Mitapivat vs Placebo), %		6.4
95% CI		(1.9, 10.9)
2-sided p-value		0.0056

Sensitivity analyses were prespecified for the 3 key secondary endpoints, similarly to those prespecified for the primary endpoint. Results of these sensitivity analyses for the 3 key secondary endpoints were consistent with those for the primary analysis for each endpoint:

- For TRR2, the 2-sided p-values for the sensitivity analysis based on the PPS, sensitivity analysis including only subjects who completed 48 weeks of study treatment without concomitant medications that could affect Hb, and sensitivity analysis based on imputation were 0.0006, 0.0005, and <0.0001, respectively.

- For TRR3, the 2-sided p-values for the sensitivity analysis based on the PPS,, sensitivity analysis including only subjects who completed 48 weeks of study treatment without concomitant medications that could affect Hb, and sensitivity analysis based on imputation were all <0.0001.
- For TRR4, the 2-sided p-values for the sensitivity analysis based on the PPS sensitivity analysis including only subjects who completed 48 weeks of study treatment without concomitant medications that could affect Hb and sensitivity analysis based on imputation were 0.0147, 0.0239, and 0.0056, respectively.

Subjects with α-thalassemia in Study AG348-C-018 (TDT)

In Study 018, 12 of the 258 subjects enrolled had transfusion dependent α-thalassemia including, 9 in the mitapivat arm and 3 in the placebo arm. A large proportion of subjects with α-TDT in the mitapivat arm met the primary endpoint (TRR), the key secondary endpoints (TRR2, TRR3, and TRR4), and the secondary endpoint of transfusion independence compared to 0 subjects in the placebo arm (Table 34).

Table 34: Efficacy Results in Subjects with Transfusion-Dependent α-Thalassemia (Subjects with α-Thalassemia in Full Analysis Set, Study 018)

	Placebo N=3	Mitapivat N=9
TRR responders, n (%)	0	7 (77.8)
Difference in response rate (Mitapivat vs Placebo), %		77.8
95% CI		(2.6, 97.2)
TRR2 responders, n (%)	0	7 (77.8)
Difference in response rate (Mitapivat vs Placebo), %		77.8
95% CI		(2.6, 97.2)
TRR3 responders, n (%)	0	6 (66.7)
Difference in response rate (Mitapivat vs Placebo), %		66.7
95% CI		(-10.8, 92.5)
TRR4 responders, n (%)	0	6 (66.7)
Difference in response rate (Mitapivat vs Placebo), %		66.7
95% CI		(-10.8, 92.5)
Transfusion independence responders, n (%)	0	6 (66.7)
Difference in response rate (Mitapivat vs Placebo), %		66.7
95% CI		(-10.8, 92.5)

In Study 018, the treatment effect in both α- and β-thalassemia subgroups for the primary endpoint (TRR) and the key secondary endpoints (TRR2, TRR3, and TRR4) also favor mitapivat compared with placebo. The p-values for the treatment by α-/β-thalassemia subgroup interaction were large for all endpoints), strongly suggesting that the observed numerical differences in the point estimates between the α- and β-thalassemia subgroups for these endpoints are likely due to variability rather than a true differential treatment effect; this supports the interpretation that the true treatment effect of mitapivat on transfusion burden reduction endpoints is consistent for patients with α- and β-TDT.

Table 35: Heterogeneity Test for α-Thalassemia vs β-Thalassemia Subgroup - Primary and Key Secondary Efficacy Endpoints (Full Analysis Set, Study 018)

Subgroup	P-value for Heterogeneity Test [1]
Primary Endpoint	
TRR	0.1021
Key Secondary Endpoints	
TRR2	0.2090
TRR3	0.4947
TRR4	0.2929

Additional secondary efficacy endpoints

Consistent with the results demonstrated for the primary and key secondary endpoints, a lower transfusion burden and a higher percent reduction from baseline in transfusion burden from Week 13 through Week 48 was observed in the mitapivat arm compared with the placebo arm (Table 36 and Table 37).

Table 36: Analysis of Reduction in RBC Transfusion Burden from Week 13 through Week 48 Standardized to 36 Weeks - ANCOVA (Full Analysis Set)

	Placebo N=87	Mitapivat N=171
Baseline transfusion burden standardized to 36 weeks (RBC Units)		
n	87	171
Mean (SD)	23.28 (5.640)	22.25 (6.175)
Percent reduction in RBC Transfusion Burden from Week 13 Through Week 48 Standardized to 36 Weeks, %		
n	83	155
LS Mean (SE)	3.32 (2.846)	10.96 (2.149)
95% CI	(-2.28, 8.93)	(6.72, 15.19)
Difference in LS Mean (SE) (Mitapivat-Placebo)		7.63 (3.335)
95% CI		(1.06, 14.20)

Table 37: Summary of RBC Transfusion Burden from Week 13 through Week 48 Standardized to 36 Weeks (Full Analysis Set)

	Placebo N=87	Mitapivat N=171
Baseline transfusion burden standardized to 36 weeks (RBC Units)		
n	87	171
Mean (SD)	23.28 (5.640)	22.25 (6.175)
Median (Q1, Q3)	24.00 (19.50, 27.00)	22.50 (18.00, 27.00)
Min, Max	9.0, 45.0	9.0, 40.5
RBC units transfused from Week 13 through Week 48 standardized to 36 weeks		
n	83	155
Mean (SD)	22.66 (6.516)	19.64 (8.087)
Median (Q1, Q3)	22.00 (18.36, 27.56)	20.20 (14.37, 24.90)
Min, Max	1.0, 40.6	0.0, 39.8

	Placebo N=87	Mitapivat N=171
Percent reduction from baseline in RBC units transfused from Week 13 through Week 48 standardized to 36 weeks, %		
n	83	155
Mean (SD)	2.95 (15.174)	11.62 (28.472)
Median (Q1, Q3)	2.33 (-4.35, 10.40)	7.80 (-1.56, 21.86)
Min, Max	-32.8, 88.9	-122.2, 100.0
Percent reduction from baseline in RBC units transfused from Week 13 through Week 48 standardized to 36 weeks category, n (%)		
<0%	34 (39.1)	42 (24.6)
≥0 to <20%	46 (52.9)	67 (39.2)
≥20 to <33%	2 (2.3)	21 (12.3)
≥33 to <50%	0	12 (7.0)
≥50%	1 (1.1)	13 (7.6)

With continued exposure to mitapivat in the OLE Period, the mean percent reduction from baseline in RBC transfusion burden is 13.86%, increased from the 10.96% observed during the Double-blind Period.

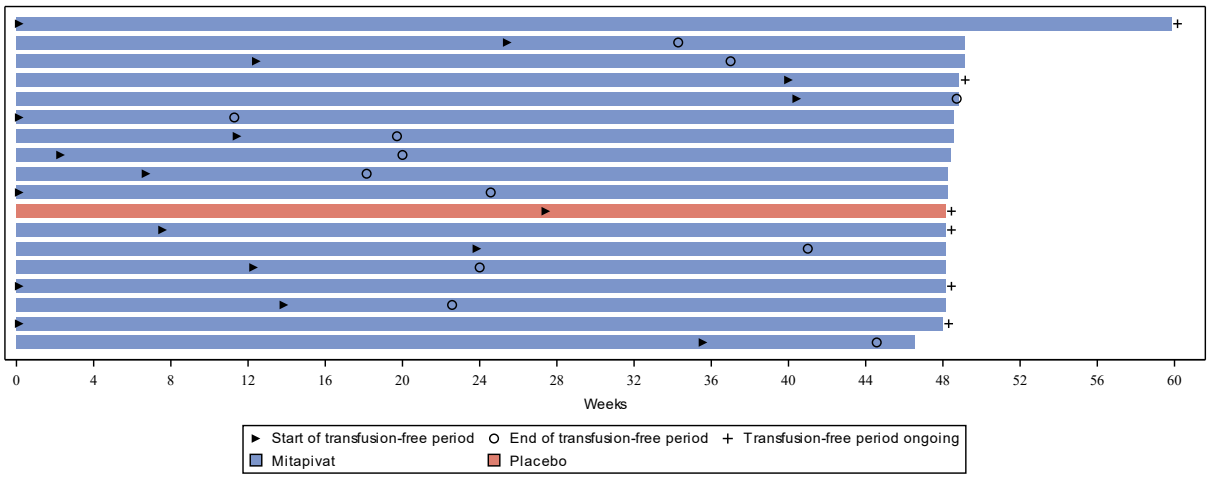
A higher proportion of subjects in the mitapivat arm compared to the placebo arm achieved transfusion independence, defined as transfusion-free for at least 8 consecutive weeks through Week 48 in the Double-blind Period (Table 38).

Table 38: Summary of Transfusion Independence Responders (Full Analysis Set)

	Placebo N=87	Mitapivat N=171
Transfusion independence responders, n (%)	1 (1.1)	17 (9.9)
Adjusted difference in response rate (Mitapivat vs Placebo), %		8.8
95% CI		(3.8, 13.8)

Three subjects in the mitapivat arm did not receive any transfusions during the 48-week Double-blind Period (Figure 27). At the end of the Double-blind Period, 4 subjects in the mitapivat arm had remained transfusion independent for >40 weeks, including 1 subject for 59.9 weeks.

Figure 27: Swimlane Plot for Duration of Transfusion Independence (Subjects in the Full Analysis Set Who Achieved Transfusion Independence)



Markers of iron metabolism were analyzed using ANCOVA, with change from baseline at Week 48 as the dependent variable, treatment arm as the independent variable, and baseline and randomisation stratification factors as covariates. The elevated mean baseline levels of serum iron, ferritin, total iron binding capacity, and transferrin saturation were consistent with those expected for subjects with thalassemia who are regularly transfused. Week 48 serum iron (Table 39), ferritin (Table 40), and total iron binding capacity (Table 41) were decreased from baseline for subjects in the mitapivat arm compared to subjects in the placebo arm as shown by the estimated difference in the LS means for the change from baseline to Week 48. There were no notable differences between the mitapivat arm and the placebo arm for transferrin saturation (Table 42).

Table 39: Analysis of Change from Baseline in Iron (umol/L) by Visit - ANCOVA (Full Analysis Set)

Visit	Placebo N=87	Mitapivat N=171
Baseline		
n	82	161
Mean (SD)	42.02 (13.830)	41.32 (12.483)
Week 24		
Change from baseline		
n	81	154
LS Mean (SE)	-1.33 (1.219)	-4.76 (0.926)
95% CI	(-3.74, 1.07)	(-6.59, -2.94)
Difference in LS Mean (SE) (Mitapivat-Placebo)		-3.43 (1.416)
95% CI		(-6.22, -0.64)
Week 48		
Change from baseline		
n	75	149
LS Mean (SE)	2.06 (1.280)	-4.57 (0.949)
95% CI	(-0.46, 4.59)	(-6.44, -2.69)
Difference in LS Mean (SE) (Mitapivat-Placebo)		-6.63 (1.474)
95% CI		(-9.53, -3.72)

Table 40: Analysis of Change from Baseline in Ferritin (ug/L) by Visit - ANCOVA (Full Analysis Set)

Visit	Placebo N=87	Mitapivat N=171
Baseline		
n	83	161
Mean (SD)	1928.7 (1862.11)	2020.0 (2290.86)
Week 24		
Change from baseline		
n	81	153
LS Mean (SE)	0.1 (96.01)	-104.6 (73.04)
95% CI	(-189.1, 189.3)	(-248.5, 39.4)
Difference in LS Mean (SE) (Mitapivat-Placebo)		-104.6 (111.59)
95% CI		(-324.5, 115.2)
Week 48		
Change from baseline		
n	75	148
LS Mean (SE)	76.3 (119.84)	45.3 (88.90)

Visit	Placebo N=87	Mitapivat N=171
95% CI	(-159.9, 312.5)	(-129.9, 220.5)
Difference in LS Mean (SE) (Mitapivat-Placebo)		-31.0 (138.00)
95% CI		(-303.0, 241.0)

Table 41: Analysis of Change from Baseline in Total Iron Binding Capacity (umol/L) by Visit - ANCOVA (Full Analysis Set)

Visit	Placebo N=87	Mitapivat N=171
Baseline		
n	52	109
Mean (SD)	79.02 (34.345)	82.15 (37.888)
Week 24		
Change from baseline		
n	41	73
LS Mean (SE)	-10.07 (4.395)	-27.98 (3.441)
95% CI	(-18.78, -1.36)	(-34.80, -21.16)
Difference in LS Mean (SE) (Mitapivat-Placebo)		-17.91 (4.654)
95% CI		(-27.13, -8.68)
Week 48		
Change from baseline		
n	41	80
LS Mean (SE)	-1.23 (5.053)	-21.80 (3.597)
95% CI	(-11.23, 8.78)	(-28.92, -14.68)
Difference in LS Mean (SE) (Mitapivat-Placebo)		-20.57 (4.992)
95% CI		(-30.46, -10.69)

Table 42: Analysis of Change from Baseline in Transferrin Saturation (fraction of 1) by Visit - ANCOVA (Full Analysis Set)

Visit	Placebo N=87	Mitapivat N=171
Baseline		
n	52	111
Mean (SD)	0.593 (0.2006)	0.580 (0.1976)
Week 24		
Change from baseline		
n	41	73
LS Mean (SE)	0.029 (0.0369)	0.108 (0.0295)
95% CI	(-0.044, 0.102)	(0.049, 0.166)
Difference in LS Mean (SE) (Mitapivat-Placebo)		0.079 (0.0393)
95% CI		(0.001, 0.157)
Week 48		
Change from baseline		
n	40	80
LS Mean (SE)	-0.013 (0.0371)	0.079 (0.0261)
95% CI	(-0.087, 0.060)	(0.027, 0.130)
Difference in LS Mean (SE) (Mitapivat-Placebo)		0.092 (0.0370)

Visit	Placebo N=87	Mitapivat N=171
95% CI		(0.019, 0.165)

Exploratory Endpoints

Iron Overload

Baseline hepatic iron concentrations were similar for both treatment arms with a mean (SD) of 5.731 mg/g (4.8952) in the mitapivat arm and 5.712 mg/g (4.7491) in the placebo arm. At Week 48, mean changes from baseline in hepatic concentration were 0.576 mg/g (2.9720) in the mitapivat arm and −0.662 mg/g (2.0469) in the placebo arm.

Markers of Hemolysis and Erythropoiesis

Mean baseline levels of indirect bilirubin, LDH, and haptoglobin were consistent with thalassemia patients who are regularly transfused. There were fluctuations in levels of markers hemolysis and erythropoiesis compared with baseline, but interpretation of these markers is limited by concomitant transfusions. Subject profiles of markers of hemolysis and erythropoiesis over time are presented in Figure 14.2-8.1. A by-subject listing of derived values for markers of hemolysis by visit is provided in Listing 16.2.6-8.1 and for markers of erythropoiesis by visit in Listing 16.2.6-8.2.

Biomarkers

Mean baseline levels of free Hb, fetal Hb, hepcidin, erythroferrone, soluble transferrin receptor, estimated glomerular filtration rate, and spot urine albumin to creatinine ratio were similar between treatment groups. There were fluctuations in levels of exploratory biomarkers compared with baseline, but interpretation of these markers is limited by concomitant transfusions.

Health-Related Quality of Life

- Transfusion-Dependent Quality of Life Questionnaire

The TranQoL is a disease-specific instrument to assess the HRQOL in patients with β-thalassemia major. The adult version of the instrument includes 36 items and provides a total score as well as 4 domain scores: physical health, emotional health, family functioning, and school and career functioning, with a fifth category that covers sexual activity (Klaassen et al, 2013). The total score ranges from 0 to 100; higher scores indicate better HRQoL. Baseline global TranQoL scores were similar for both treatment arms with a mean (SD) of 63.55 (14.104) in the mitapivat arm and 63.41 (15.350) in the placebo arm (Table 14.2-10.1). The Week 48 mean (SD) change from baseline for the global TranQoL score was 3.14 (9.623) in the mitapivat arm and 2.98 (12.074) in the placebo arm.

- PGIS-Thalassemia Symptoms and PGIC-Thalassemia Symptoms

Most subjects reported thalassemia symptoms at baseline (mild, moderate, or severe in 32.7%, 22.8%, and 4.7% of subjects in the mitapivat arm and mild, moderate, or severe in 51.7%, 18.4%, and 4.6% of subjects in the placebo arm). At baseline, 22.8% and 4.7% of subjects in the mitapivat arm and 18.4% and 4.6% of subjects in the placebo arm reported moderate or severe symptoms, respectively. At Week 48, the percentage of subjects reporting moderate or severe thalassemia symptoms was 16.4% and 1.8% respectively in the mitapivat arm, 18.4% and 2.3% in the placebo arm. The PGIC of thalassemia symptoms measured subjects' perceived change in symptoms compared with baseline. A higher frequency of subjects in the mitapivat arm compared with the placebo arm reported feeling much better (13.5% vs 4.6%) at Week 48 compared with baseline.

- EuroQoL Group 5-Level EQ-5D-5L

The EQ-5D-5L is a well-established, validated measure of general HRQOL developed by the EuroQoL group, which consists of 2 components: a descriptive system and the EQ-VAS. In the descriptive system, subjects rate their current level (1 [no problem], 2 [slight problems], 3 [moderate problems], 4 [severe problems], and 5

[extreme problems]) on each of the 5 dimensions (mobility, self-care, usual activities, pain/discomfort, and anxiety/depression). The EQ-VAS is presented as a 20-cm vertical line on which subjects are asked to mark their current health status, with scores ranging from 0 (worst imaginable health state) to 100 (best imaginable health state). Baseline EQ-VAS scores were similar for both treatment arms with a mean (SD) of 74.9 (17.27) in the mitapivat arm and 76.5 (18.43) in the placebo arm. The Week 48 mean (SD) change from baseline for the EQ-VAS score was 3.0 (16.90) in the mitapivat arm and 2.0 (15.78) in the placebo arm.

- PROMIS Physical Function 8-Item Short Form

The PROMIS Physical Function 8-Item Short Form contains 8 questions assessing limitations associated with daily physical activities. Scores are expressed as T scores, representing a standardized score with a mean of 50 and an SD of 10. Higher scores represent better physical functioning (van der Meij et al, 2018). Baseline PROMIS Physical Function T-scores were similar for both treatment arms with a mean (SD) of 45.90 (7.793) in the mitapivat arm and 47.99 (8.867) in the placebo arm (Table 14.2-10.6). The Week 48 T-score mean (SD) was 46.39 (8.196) in the mitapivat arm and 46.66 (8.380) in the placebo arm. The Week 48 mean (SD) change from baseline for the PROMIS Physical Function T-Score was 0.10 (5.948) in the mitapivat arm and −1.10 (6.394) in the placebo arm.

Ancillary analyses

Subgroup analyses were prespecified for the primary endpoint (TRR, Figure 28) and the 3 key secondary endpoints (TRR2 depicted in Figure 29, TRR3 depicted in Figure 30, TRR4 depicted in Figure 31). The results of the subgroup analyses for these endpoints further support that mitapivat is efficacious in reducing transfusion burden in the broad population of subjects with α- or β-thalassemia who are TD as the overall results observed for the primary and key secondary endpoints are not driven by any of the individual prespecified subgroups.

Figure 28: Forest Plot for Difference in TRR (≥50% Reduction in Transfused RBC Units With a Reduction of ≥2 Transfused RBC Units in any Consecutive 12-week Period Compared with Baseline) (Full Analysis Set)

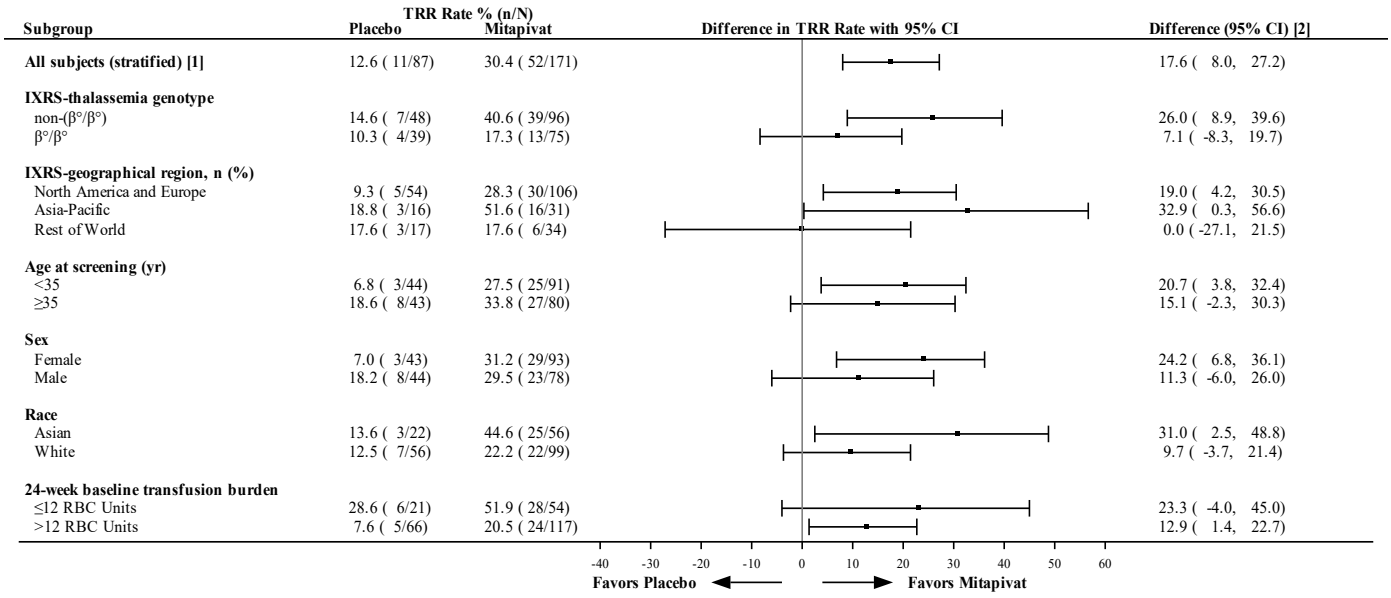


Figure 29: Forest Plot for Difference in TRR2 (≥50% Reduction in Transfused RBC Units in any Consecutive 24-week Period Compared with Baseline) (Full Analysis Set)

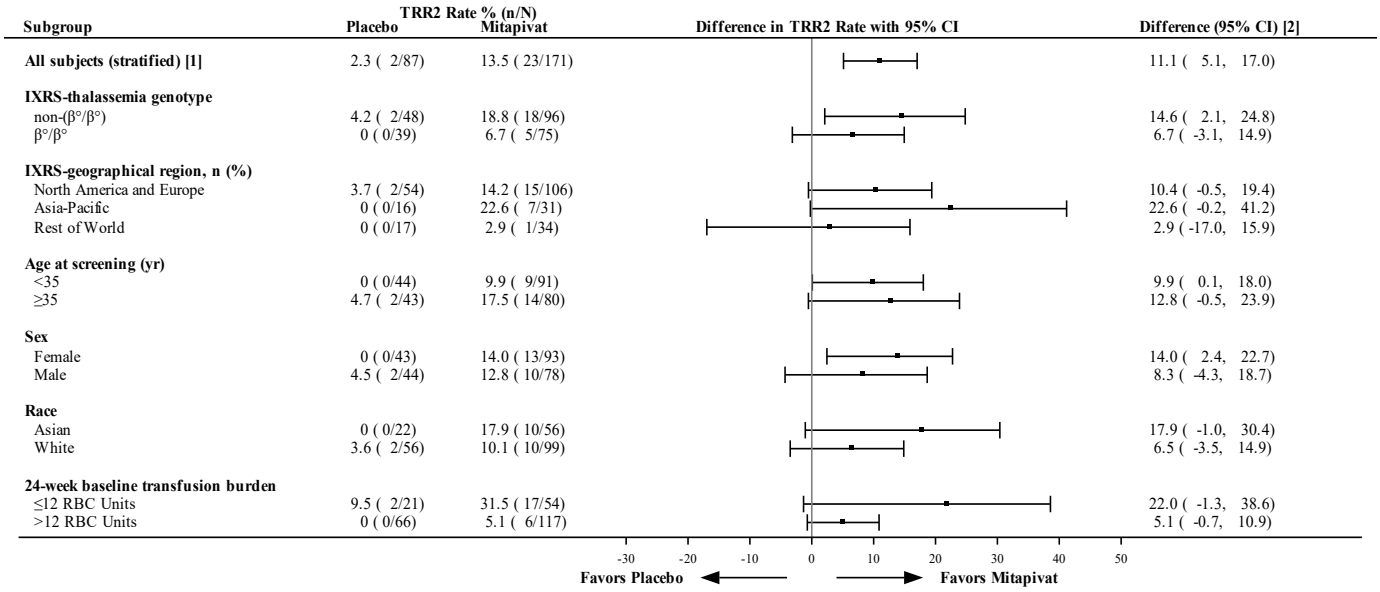


Figure 30: Forest Plot for Difference in TRR3 (≥33% Reduction in Transfused RBC Units from Week 13 through Week 48 Compared with Baseline) (Full Analysis Set)

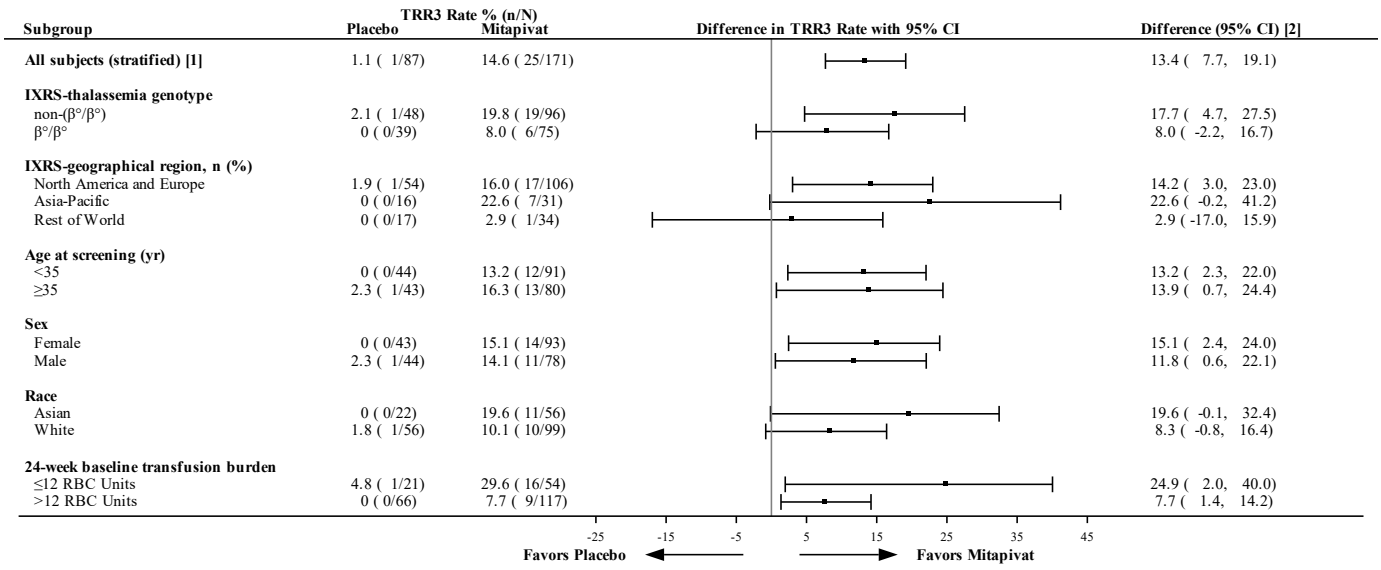
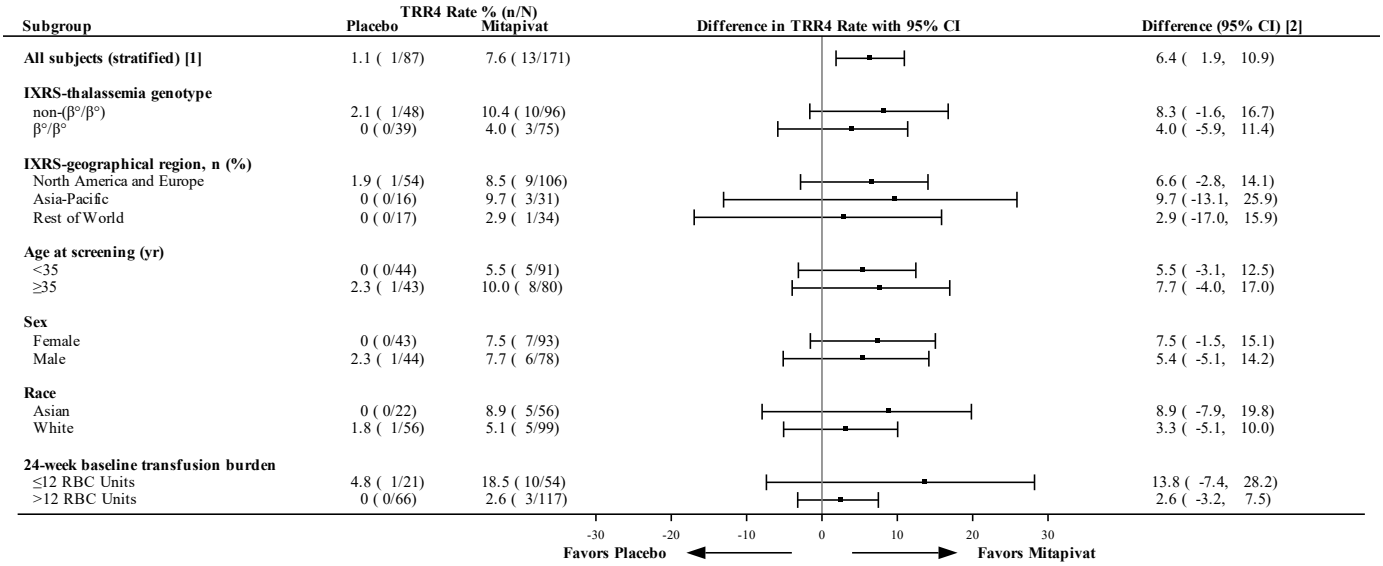


Figure 31: Forest Plot for Difference in TRR4 (≥50% Reduction in Transfused RBC Units from Week 13 through Week 48 Compared with Baseline) (Full Analysis Set)



A Breslow-Day test was performed to assess the treatment-by subgroup interaction (genotype, baseline transfusion burden, and race) (Table 43, 44 and 45).

The p-values for all endpoints were large (p-values >0.30) strongly suggesting that the observed numerical differences between thalassemia genotypes, baseline transfusion burden, race are likely due to variability rather than a true differential treatment effect.

Table 43: Heterogeneity Test for genotype Subgroup - Primary and Key Secondary Efficacy Endpoints (Full Analysis Set, Study 018).

Subgroup	P-value for Heterogeneity Test [1]
Thalassemia Genotype	
Primary Endpoint	
TRR	0.3025
Key Secondary Endpoints	
TRR2	0.4710
TRR3	0.5868
TRR4	0.5886

Table 44: Heterogeneity Test for baseline transfusion burden subgroup - Primary and Key Secondary Efficacy Endpoints (Full Analysis Set, Study 018)

Subgroup	P-value for Heterogeneity Test [1]
24-week Baseline Transfusion Burden	
Primary Endpoint	
TRR	0.8362
Key Secondary Endpoints	
TRR2	0.3693
TRR3	0.4169
TRR4	0.5422

Table 45: Heterogeneity Test for Race Subgroup - Primary and Key Secondary Efficacy Endpoints (Full Analysis Set, Study 018)

Subgroup	P-value for Heterogeneity Test [1]
Race	
Primary Endpoint	
TRR	0.4302
Key Secondary Endpoints	
TRR2	0.6060
TRR3	0.7715
TRR4	0.7756

Efficacy data from the ongoing OLE period of pivotal study AG348-C-018

Available efficacy and safety data from the OLE period of pivotal study AG348-C-018 are provided below.

This represents cumulative efficacy and safety data from the Double-blind Period and OLE Period through 90 days after the last patient's first dose in the OLE Period (this cutoff date includes an additional minimum 90 days [12.9 weeks] follow-up for subjects ongoing in Study 018).

In Study 018, transfusion independence (TI) is defined as transfusion-free for ≥8 consecutive weeks.

Cumulative efficacy data from 17 subjects in Study 018 who were randomised to mitapivat and achieved transfusion independence during the Double-blind Period are included in this update to assess the durability of the TI with longer duration of treatment with mitapivat.

For subjects who achieved transfusion independence in the mitapivat arm in Study 018, the duration of transfusion independence was calculated as the number of weeks in the longest transfusion-free period starting on or after the first dose of mitapivat through the end of the evaluation period, where the end of the evaluation period is defined as:

- The end of Double-blind treatment period, for all subjects in the summary of the Double-blind period and for subjects who did not continue onto the OLE Period in the summary of the efficacy update
- The end of Open-label extension treatment period or data cutoff date, whichever occurs earlier, for subjects who continued into the OLE Period in the summary of the efficacy update

Each transfusion-free period was derived as follows:

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

Duration of exposure to mitapivat during the Double-blind Period and the cumulative duration of exposure to mitapivat through the cutoff date in the OLE Period are summarised in Table 46 for subjects who achieved TI.

Table 46: Summary of Exposure to Study Drug (Subjects in the Full Analysis Set Who Were Randomized to Mitapivat and Achieved Transfusion Independence)

	Mitapivat	
	Double-blind Period N=17	Efficacy Update N=17
Duration of exposure (Weeks)		
n	17	17
Mean (SD)	48.95 (2.882)	75.55 (12.917)
Median (Q1, Q3)	48.29 (48.14, 48.86)	72.00 (70.29, 80.29)
Min, Max	46.6, 59.9	47.3, 99.3
Person exposure-years	15.95	24.61

The duration of TI based on the data from the Double-blind Period and the updated duration of TI incorporating data from the OLE Period are summarised in Table 47.

With continued exposure to mitapivat in the OLE Period, the mean duration of TI increased to 30.49 weeks with duration of TI up to 84.3 weeks. Further, 3 subjects in the mitapivat arm did not receive any transfusions during the 48-week Double blind Period and remain transfusion-free during the OLE Period as of the data cutoff date for this update (duration of transfusion-free of 64.3, 75.0, and 77.1 weeks)

Table 47: Summary of Duration of Transfusion Independence (Subjects in the Full Analysis Set Who Were Randomized to Mitapivat and Achieved Transfusion Independence)

	Mitapivat	
	Double-blind Period N=17	Efficacy Update N=17
Duration of transfusion independence (Weeks)		
N	17	17
Mean (SD)	21.69 (16.892)	30.49 (27.087)
Median (Q1, Q3)	11.86 (9.00, 24.71)	17.86 (11.29, 38.00)
Min, Max	8.4, 59.9+	8.4, 84.3

With continued exposure to mitapivat in the OLE Period, the mean percent reduction from baseline in RBC transfusion burden is 13.86%, increased from the 10.96% observed during the Double-blind Period.

Markers of haemolysis (indirect bilirubin [Figure 32], LDH [Figure 33], and haptoglobin [Figure 34]) were exploratory endpoints in Study 018 as the interpretation of these markers is limited by concomitant transfusions as had been previously described in the Study 018 CSR Section.

Figure 32: Mean (95% CI) of Change from Baseline in Indirect Bilirubin Over Time (Study 018 Double-blind and OLE Periods, Subjects Randomized to Mitapivat)

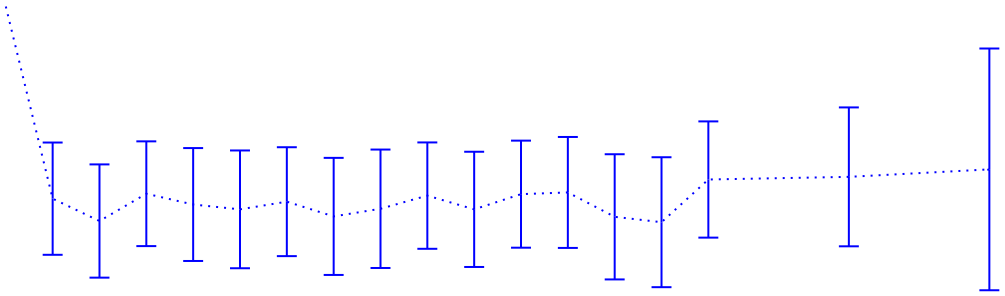


Figure 33: Mean (95% CI) of Change from Baseline in Lactate Dehydrogenase Over Time (Study 018 Double-blind and OLE Periods, Subjects Randomized to Mitapivat)

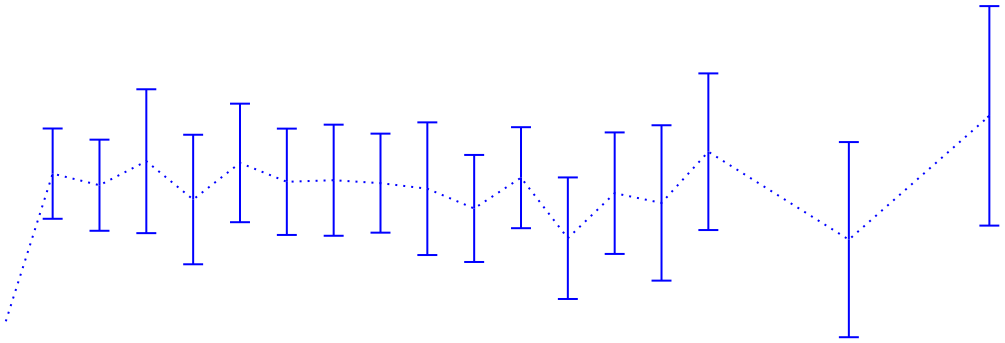
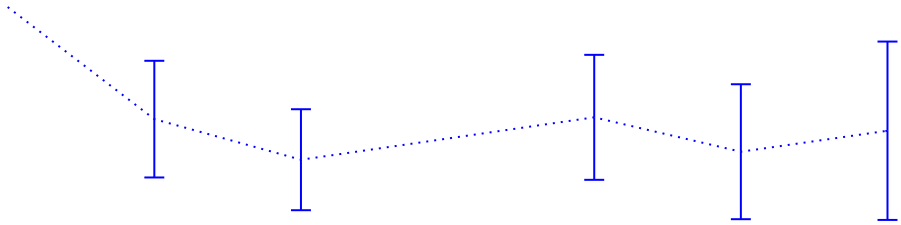
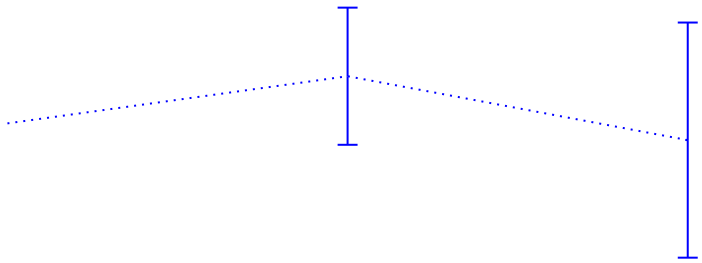


Figure 34: Mean (95% CI) of Change from Baseline in Haptoglobin Over Time (Study 018 Double-blind and OLE Periods, Subjects Randomized to Mitapivat)



Liver iron concentrations (LIC) by MRI at the end of the Double-blind Period and during the OLE Period are summarized in Figure 35. These data show that even though some subjects continued to receive transfusions, liver iron concentrations remained stable with continued mitapivat treatment.

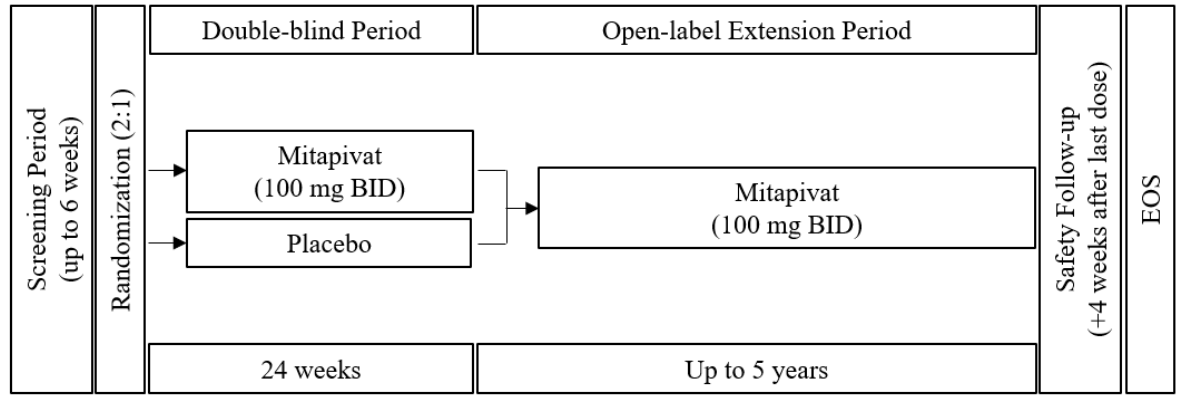
Figure 35: Mean (95% CI) of Change from Baseline in Hepatic Iron Concentration by MRI (Study 018 Double-blind and OLE Periods, Subjects Randomized to Mitapivat)



Study AG348-C-017

Study 017 is a Phase 3, double-blind, randomized, placebo-controlled, multicenter study evaluating the efficacy and safety of mitapivat versus placebo in adult subjects with NTD α- or β-thalassemia followed by an OLE Period. There were 194 subjects randomized in the study. The Double-blind Period has been completed, and the OLE is ongoing. An overview of the study design is presented in Figure 36.

Figure 36: AG348-C-017 Study Design



Placebo administration was limited to a 24-week Double-blind Period, and subjects, including those who were randomized to placebo, had the opportunity to receive mitapivat during the OLE Period, allowing for a longer-term assessment of safety while providing access to active drug.

Study participants

The study was conducted in a population of adult subjects with NTD α- or β-thalassemia, defined as ≤5 RBC units during the 24-week period before randomisation and no RBC transfusions ≤8 weeks before providing informed consent and no RBC transfusions during the Screening Period.

Additional key inclusion criteria for this study were documented diagnosis of thalassemia (β-thalassemia with or without α-globin gene mutations, HbE/β-thalassemia, or α-thalassemia/HbH disease) and an average baseline Hb concentration of ≤10 g/dL (100.0 g/L) to ensure enrollment of a patient population with moderate to severe anemia in which a benefit from an increase in Hb could be detected.

This study did not include subjects who were homozygous or heterozygous HbS or HbC. The following treatments were excluded: prior exposure to gene therapy or prior bone marrow or stem cell transplantation. Treatment with luspatercept and hematopoietic stimulating agents also was excluded (a washout period before randomization was allowed).

Treatments

Subjects received 100 mg BID mitapivat or matched placebo for oral administration. Mitapivat was supplied as 100-mg strength tablets and placebo was supplied as matched tablets.

Subjects who discontinued study drug (mitapivat or placebo) were to follow the recommended dose taper and were to be monitored as clinically indicated for signs and symptoms of acute hemolysis and worsening anemia. If immediate or abrupt study drug discontinuation was required for an AE or medical emergency, subjects were monitored as clinically indicated for signs of acute hemolysis or worsening anemia.

Objectives and Outcomes/endpoints

The study objectives and endpoints are provided in Table 48

Table 48: Objectives and Endpoints

Primary Objective	**Primary Endpoint**
• To compare the effect of mitapivat versus placebo on anemia in subjects with α- or β-non–transfusion-dependent thalassemia	• Hemoglobin (Hb) response, defined as a ≥1.0 g/dL increase in average Hb concentration from Week 12 through Week 24 compared with baseline
Key Secondary Objectives	**Key Secondary Endpoints**
• To compare the effect of mitapivat versus placebo on fatigue	• Change from baseline in average Functional Assessment of Chronic Illness Therapy-Fatigue subscale score from Week 12 through Week 24.
• To compare the effect of mitapivat versus placebo on additional measures of anemia	• Change from baseline in average Hb concentration from Week 12 through Week 24
Secondary Objectives	**Secondary Endpoints**
• To evaluate the effect of mitapivat versus placebo on anemia and markers of hemolysis and erythropoiesis	• Hb 1.5+ response, defined as a ≥1.5 g/dL increase in average Hb concentration from Week 12 through Week 24 compared with baseline
	• Change from baseline in indirect bilirubin, lactate dehydrogenase, and haptoglobin at Week 24
	• Change from baseline in reticulocytes and erythropoietin at Week 24
• To evaluate the effect of mitapivat versus placebo on additional measures of fatigue	• Improvement in the Patient Global Impression of Severity (PGIS)-Fatigue by at least 1 category at Weeks 12, 16, 20, and 24 compared with baseline, or "no change" if no or mild fatigue at baseline
	• Improvement in the Patient Global Impression of Change (PGIC)-Fatigue at Weeks 12, 16, 20, and 24, or "no change" if no or mild fatigue at baseline
• To evaluate the effect of mitapivat versus placebo on physical activity	• Change from baseline in the 6-minute walk test distance at Week 24
• To evaluate the effect of mitapivat versus placebo on iron metabolism	• Change from baseline in markers of iron metabolism, including serum ferritin and transferrin saturation, at Week 24
• To evaluate the safety of mitapivat	• Type, severity, and relationship of adverse events 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 and 2,3-diphosphoglycerate
Exploratory Objectives	**Exploratory Endpoints**
• To explore the effect of mitapivat versus placebo on exploratory biomarkers	• Change from baseline in exploratory biomarkers, including estimated glomerular filtration rate, free Hb, fetal Hb, hepcidin, erythroferrone, soluble transferrin receptor, and spot urine albumin-to-creatinine ratio
• To explore the effect of mitapivat versus placebo on number of transfusions	• Transfusion-free, defined as no transfusions for a 24-week period from randomization to Week 24
• To explore the effect of mitapivat versus placebo on health-related quality of life (HRQOL)	• Change from baseline in the Patient Reported Outcomes Measurement Information System® Physical Function 8-Item Short Form score at Week 24
	• Change from baseline in the Functional Assessment of Cancer Therapy-General score at Week 24

	• HRQOL as assessed by PGIS-Thalassemia Symptoms, PGIC-Thalassemia Symptoms, PGIS-Walking Capacity, PGIC-Walking Capacity, and the EuroQoL Group 5-Level EQ-5D questionnaire at Week 24
• To explore the relationship between mitapivat pharmacokinetics and clinical response	• Exposure-response relationship between pharmacokinetic parameters and clinical response
• To explore the effect of open-label mitapivat on efficacy and HRQOL parameters	• Change from baseline in Hb concentration, patient-reported outcome and performance outcome measures, markers of hemolysis and erythropoiesis, markers of iron overload and metabolism, and exploratory biomarkers during the Open-label Extension Period

Randomization and blinding (masking)

Eligible subjects were randomized in a 2:1 ratio to mitapivat or matched placebo. Randomization assignment was implemented by an IXRS and stratified by:

Baseline Hb concentration (≤9.0 g/dL or 9.1 to 10.0 g/dL)

Thalassemia genotype (α-thalassemia/HbH disease or β-thalassemia)

Study subjects, Investigators, clinical study center personnel, pharmacists, and the Sponsor were blinded to the subject's treatment assignment. After completing the Double-blind Period, subjects were given the opportunity to receive mitapivat in the OLE Period. At the last study visit of the Double-blind Period, subjects who continued in the OLE Period were provided with active mitapivat; however, study subjects, Investigators, clinical study center personnel, and the Sponsor continued to remain blinded to the randomized treatment assignment during the previous Double-blind Period until the study was unblinded for the analysis of the primary endpoint.

Sample size and Statistical methods

The following statistical hypothesis was prespecified and 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 with a Hb response in the mitapivat arm and placebo arm, respectively.

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

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

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

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

Where:

μ_{t1} and μ_{c1} are the mean change from baseline in average FACIT-Fatigue subscale score from Week 12 through Week 24 in the mitapivat arm and placebo arm, respectively.

μ_{t2} and μ_{c2} are the mean change from baseline in average Hb concentration from Week 12 through Week 24 in the mitapivat arm and placebo arm, respectively.

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

To control the overall type I error in the study at or below a 2-sided α-level of 0.05, a fixed-sequence testing procedure (Westfall and Krishen, 2001) was prespecified. H_{01} was tested first; if H_{01} was rejected, then H_{02} was to be tested; if H_{02} was rejected, then H_{03} was to be tested. All tests were conducted at the 2-sided 0.05 significance level.

Results

Participant flow

Subject disposition is summarized for all randomised subjects in Table 49. One subject in each treatment arm was randomised but did not receive study treatment. All treated subjects received the study treatment that was assigned at randomisation.

Subject disposition by stratification factors is summarized in Table 50. Stratification was balanced across treatment arms. Of note, for 2 subjects randomised to the mitapivat arm, the thalassemia genotype entered into the IXRS for the randomisation stratification (α-thalassemia/HbH disease) was different from that entered by the investigator in the eCRF (β-thalassemia).

Table 49: Subject Disposition - Double-blind Period (Full Analysis Set)

	Placebo N=64 n (%)	Mitapivat N=130 n (%)	Total N=194 n (%)
Disposition: Treatment double-blind period			
Not entered	1 (1.6)	1 (0.8)	2 (1.0)
Discontinued	1 (1.6)	7 (5.4)	8 (4.1)
Reason			
Withdrawal By Subject	1 (1.6)	3 (2.3)	4 (2.1)
Adverse Event	0	2 (1.5)	2 (1.0)
Pregnancy	0	1 (0.8)	1 (0.5)
Other	0	1 (0.8)	1 (0.5)
Completed	62 (96.9)	122 (93.8)	184 (94.8)
Ongoing	0	0	0
Disposition: Safety follow-up			
Not entered	3 (4.7)	4 (3.1)	7 (3.6)
Discontinued	0	0	0
Completed	0	5 (3.8)	5 (2.6)
Ongoing	0	0	0

Table 50: Subject Disposition - Stratification Factors at Randomization (Full Analysis Set)

	Placebo N=64	Mitapivat N=130	Total N=194
Source: IXRS			
Baseline Hb concentration, n (%)			
≤ 9.0 g/dL	47 (73.4)	95 (73.1)	142 (73.2)
9.1-10.0 g/dL	17 (26.6)	35 (26.9)	52 (26.8)
Thalassemia genotype, n (%)			
α-thalassemia/HbH disease	20 (31.3)	42 (32.3)	62 (32.0)
β-thalassemia	44 (68.8)	88 (67.7)	132 (68.0)
Baseline Hb concentration and Thalassemia genotype, n (%)			
≤ 9.0 g/dL and α-thalassemia/HbH disease	14 (21.9)	30 (23.1)	44 (22.7)
≤ 9.0 g/dL and β-thalassemia	33 (51.6)	65 (50.0)	98 (50.5)
9.1-10.0 g/dL and α-thalassemia/HbH disease	6 (9.4)	12 (9.2)	18 (9.3)
9.1-10.0 g/dL and β-thalassemia	11 (17.2)	23 (17.7)	34 (17.5)

Recruitment

Study design includes a screening period up to 6 weeks before randomization. A double blind period of 24 weeks was chosen, followed by an OLE period up to 5 years. A Safety follow up is planned then 4 weeks after the last dose. Study Start Date (first patient enrolled): December 20, 2021. Study Completion Date (Estimated): March 2029.

Conduct of the study

Table 51: Summary of Substantial Changes to the Protocol

Amendment 2 (28 Sep 2022) Global	• The protocol was amended to remove the requirement for male contraception. The protocol was updated to align with current reproductive safety data. Data suggest that mitapivat has no teratogenic or fetotoxic effects in males who father a child while receiving mitapivat. • The protocol was amended to remove reporting and follow-up requirements for pregnant partners of male subjects. The protocol was updated to align with current reproductive safety data. Data suggest that mitapivat has no teratogenic or fetotoxic effects in males who father a child while receiving mitapivat.
Amendment 1 (21 Jul 2021) Global	• Mitapivat administration has not been shown to result in clinically significant changes to triglyceride levels, so the frequency of sampling for lipids was reduced. • Mitapivat administration has not been shown to result in frequent fluctuations in sex steroid hormones, so the frequency of sampling for sex hormones during the OLE Period of the study was reduced. • Mitapivat is not hepatotoxic, so transaminase increase was removed as a potential risk and AESI. • A dedicated concentration–QT interval study showed that mitapivat administration did not result in a clinically significant increase in QT interval; therefore, the maximum heart rate–corrected QT interval using Fridericia's method value for study eligibility for female subjects was increased from 450 milliseconds to 470 milliseconds. • Language summarizing the risk assessment for concomitant use of a COVID-19 vaccine was added in response to a request from a Regulatory Agency. • Updates were made to the timing for SAE reporting to align with the recording period for AEs. • The exclusion criterion related to subjects who have received anabolic steroids was modified to allow the use of testosterone replacement therapy to treat hypogonadism. This change was incorporated as the testosterone replacement therapy to treat hypogonadism is part of the standard of care of male thalassemia subjects and it is not expected to affect the safety or efficacy of mitapivat in this study.

No changes were made to the planned analyses.

Major protocol deviations

The following were subject-level major deviations assessed for the potential to impact study conclusions (Table 52):

Informed consent

A total of 11 subjects had informed consent deviations: 3 subjects did not complete the informed consent properly; 4 subjects completed study-specific procedures before signing informed consent (the procedures were related to standard of care and included DXA/MRI); and 4 subjects did not consent under the correct or current version.

Eligibility criteria not met

A total of 18 subjects had inclusion criteria deviations: 1 subject did not have thalassemia (inclusion criterion 2), 12 subjects did not have 2 Hb measurements separated by 7 or more days and/or had Hb of >10 g/L (inclusion criterion 3), and 5 subjects did not meet the study definition of non–transfusion dependent (inclusion criterion 4).

A total of 7 subjects had exclusion criteria deviations: 2 subjects had active and/or uncontrolled cardiac or pulmonary disease within 6 months of informed consent (exclusion criterion 7), 2 subjects had nonfasting triglycerides >440 mg/dL (exclusion criterion 10), 1 subject had an active infection and received systemic antimicrobial therapy less than 7 days before randomization (exclusion criterion 11), and 2 subjects were taking either a strong CYP3A4/5 inhibitor or CYP3A4 inducer during the washout period before randomization (exclusion criterion 16).

As part of the protocol deviation process each subject was evaluated and a determination made to either remain on study or discontinue based on the criterion, safety, benefit vs risk, and best clinical practice. The subject found to not have thalassemia was discontinued from the study.

Study treatment deviations

Two subjects in the placebo arm and 1 subject in the mitapivat arm had Week 24 dispensation errors, and 1 subject in the mitapivat arm had a discrepancy between the kit number taken by the subject and the kit dispensed by the IRT
(the subject received a placebo kit at the Week 20 visit).

Use of prohibited concomitant treatment

One subject was taking the prohibited concomitant medication clarithromycin.

Table 53: Summary of Major Protocol Deviations (Full Analysis Set)

Deviations	**Placebo N=64 n (%)**	**Mitapivat N=130 n (%)**	**Total N=194 n (%)**
Subjects with major deviations	14 (21.9)	26 (20.0)	40 (20.6)
ICH/GCP Deviation	3 (4.7)	8 (6.2)	11 (5.7)
INFORMED CONSENT	3 (4.7)	8 (6.2)	11 (5.7)
Informed Consent not completed correctly	1 (1.6)	2 (1.5)	3 (1.5)
Protocol specific procedures conducted prior to subject/guardian signing informed consent	1 (1.6)	3 (2.3)	4 (2.1)
Subject not consented under correct or current version of informed consent	1 (1.6)	3 (2.3)	4 (2.1)

Deviations	Placebo N=64 n (%)	Mitapivat N=130 n (%)	Total N=194 n (%)
Protocol Deviation	12 (18.8)	20 (15.4)	32 (16.5)
OTHER DEVIATION	0	1 (0.8)	1 (0.5)
Other: Inclusion Criterion 3 was not met	0	1 (0.8)	1 (0.5)
SAE-RELATED DEVIATIONS	0	1 (0.8)	1 (0.5)
Other: Pregnancy Report Form was not sent to Safety within 24 hours.	0	1 (0.8)	1 (0.5)
SELECTION CRITERIA NOT MET	10 (15.6)	13 (10.0)	23 (11.9)
Exclusion Criteria 10 met	0	2 (1.5)	2 (1.0)
Exclusion Criteria 11 met	0	1 (0.8)	1 (0.5)
Exclusion Criteria 16 met	1 (1.6)	1 (0.8)	2 (1.0)
Exclusion Criteria 7 met	1 (1.6)	1 (0.8)	2 (1.0)
Inclusion Criteria 2 not met	0	1 (0.8)	1 (0.5)
Inclusion Criteria 3 not met	6 (9.4)	5 (3.8)	11 (5.7)
Inclusion Criteria 4 not met	2 (3.1)	3 (2.3)	5 (2.6)
STUDY TREATMENT DEVIATION	2 (3.1)	5 (3.8)	7 (3.6)
Other: Discrepancies between the kit Number taken by the subject and the IRT	0	1 (0.8)	1 (0.5)
Other: Error on patient 250138-001 dispensation for Week 24	0	1 (0.8)	1 (0.5)
Other: Subject Took Blinded IP following WK 24 Visit	1 (1.6)	0	1 (0.5)
Other: Subject took double blinded IP bottle after Week 24 visit was performed.	1 (1.6)	0	1 (0.5)
Subject randomized under the wrong stratification factor(s)	0	3 (2.3)	3 (1.5)
USE OF PROHIBITED CONCOMITANT TREATMENT	0	1 (0.8)	1 (0.5)
Subject is taking a prohibited concomitant medication during study	0	1 (0.8)	1 (0.5)

Number analysed

Analysis set definitions and summaries are provided in Table 54. Of the subjects randomized, more than 90% of participants in total were included in the PPS. There was 1 subject in each of the placebo and mitapivat arms who was randomised but did not receive study treatment and therefore was not included in the Safety Analysis Set.

Table 54: Summary of Analysis Sets (All Screened Subjects)

	Placebo	Mitapivat	Total
All Screened Subjects			235
Full Analysis Set	64	130	194
Per-Protocol Set, n (%) [1]	55 (85.9)	120 (92.3)	175 (90.2)
Safety Analysis Set	63	129	192
PK Analysis Set, n (%) [2]	0	124 (96.1)	124 (64.6)
PD Analysis Set, n (%) [2]	59 (93.7)	122 (94.6)	181 (94.3)
PK/PD Analysis Set, n (%) [2]	0	113 (87.6)	113 (58.9)

Baseline data

The study enrolled a higher proportion of females than males, with no meaningful differences between treatment arms. There were no imbalances between treatment arms in demographic characteristics or physical measurements at baseline deemed to have an impact on the interpretation of the results (Table 55).

Table 55: Summary of Demographic Characteristics and Physical Measurements at Baseline (Full Analysis Set)

Parameter	Placebo N=64	Mitapivat N=130	Total N=194
Age (yr)			
n	64	130	194
Mean (SD)	38.9 (12.99)	42.4 (13.03)	41.2 (13.09)
Median (Q1, Q3)	37.0 (28.0, 48.5)	43.5 (32.0, 51.0)	41.0 (30.0, 51.0)
Min, Max	18, 69	19, 68	18, 69
Age category 1 (yr), n (%)			
<35	27 (42.2)	37 (28.5)	64 (33.0)
≥35	37 (57.8)	93 (71.5)	130 (67.0)
Age category 2 (yr), n (%)			
<65	62 (96.9)	124 (95.4)	186 (95.9)
≥65	2 (3.1)	6 (4.6)	8 (4.1)
Sex, n (%)			
Male	25 (39.1)	46 (35.4)	71 (36.6)
Female	39 (60.9)	84 (64.6)	123 (63.4)
Childbearing Potential [1]			
Yes	30 (76.9)	57 (67.9)	87 (70.7)
No	9 (23.1)	27 (32.1)	36 (29.3)
Ethnicity, n (%)			
Hispanic or Latino	6 (9.4)	11 (8.5)	17 (8.8)
Not Hispanic or Latino	58 (90.6)	118 (90.8)	176 (90.7)
Not reported	0	1 (0.8)	1 (0.5)
Race, n (%)			
White	36 (56.3)	73 (56.2)	109 (56.2)
Asian	24 (37.5)	52 (40.0)	76 (39.2)
Black or African American	1 (1.6)	1 (0.8)	2 (1.0)
American Indian or Alaska Native	0	0	0
Native Hawaiian or Other Pacific Islander	0	0	0
Multiracial	0	1 (0.8)	1 (0.5)
Unknown	2 (3.1)	2 (1.5)	4 (2.1)
Not reported	1 (1.6)	1 (0.8)	2 (1.0)
Height (cm)			
n	61	125	186
Mean (SD)	165.09 (9.874)	164.58 (10.107)	164.75 (10.007)
Median (Q1, Q3)	165.00 (156.80, 173.00)	164.00 (157.00, 171.00)	164.00 (157.00, 172.00)
Min, Max	144.6, 189.0	142.2, 190.0	142.2, 190.0
Weight (kg)			
n	63	130	193
Mean (SD)	59.77 (10.688)	60.36 (12.814)	60.16 (12.136)
Median (Q1, Q3)	57.00 (51.50, 68.00)	58.65 (51.10, 66.70)	58.00 (51.30, 67.10)
Min, Max	42.9, 92.0	40.0, 110.5	40.0, 110.5
BMI (kg/m²)			
n	61	125	186
Mean (SD)	21.84 (3.113)	22.22 (3.740)	22.10 (3.543)
Median (Q1, Q3)	21.61 (19.47, 24.39)	21.65 (19.38, 24.12)	21.64 (19.38, 24.34)
Min, Max	16.2, 30.4	15.6, 37.3	15.6, 37.3

Table 56: Summary of Accrual by Geographic Region and Country (Full Analysis Set)

Geographic Region	Country	Placebo N=64 n (%)	Mitapivat N=130 n (%)	Total N=194 n (%)
Asia	All	14 (21.9)	29 (22.3)	43 (22.2)
	Malaysia	10 (15.6)	11 (8.5)	21 (10.8)
	Taiwan	1 (1.6)	1 (0.8)	2 (1.0)
	Thailand	3 (4.7)	17 (13.1)	20 (10.3)
Eastern Europe	All	2 (3.1)	1 (0.8)	3 (1.5)
	Bulgaria	2 (3.1)	1 (0.8)	3 (1.5)
Latin America	All	4 (6.3)	8 (6.2)	12 (6.2)
	Brazil	4 (6.3)	8 (6.2)	12 (6.2)
Middle East	All	7 (10.9)	15 (11.5)	22 (11.3)
	Lebanon	1 (1.6)	3 (2.3)	4 (2.1)
	Saudi Arabia	2 (3.1)	0	2 (1.0)
	Turkey	4 (6.3)	8 (6.2)	12 (6.2)
	United Arab Emirates	0	4 (3.1)	4 (2.1)
North America	All	9 (14.1)	14 (10.8)	23 (11.9)
	Canada	2 (3.1)	6 (4.6)	8 (4.1)
	United States	7 (10.9)	8 (6.2)	15 (7.7)
Western Europe	All	28 (43.8)	63 (48.5)	91 (46.9)
	Denmark	1 (1.6)	5 (3.8)	6 (3.1)
	France	2 (3.1)	4 (3.1)	6 (3.1)
	Greece	6 (9.4)	17 (13.1)	23 (11.9)
	Italy	11 (17.2)	18 (13.8)	29 (14.9)
	Netherlands	2 (3.1)	2 (1.5)	4 (2.1)
	Spain	5 (7.8)	10 (7.7)	15 (7.7)
	United Kingdom	1 (1.6)	7 (5.4)	8 (4.1)

Baseline disease Characteristics

Key baseline disease characteristics were similar between treatment arms and were representative of a real-world population of patients with thalassemia who are not regularly transfused (Table 57 and Table 58). There were no imbalances between treatment arms in disease characteristics deemed to have an impact on the interpretation of the results. As expected for subjects who were assessed by MRI, their hepatic iron concentration was elevated. Baseline Hb concentration and markers of hemolysis and ineffective erythropoiesis were similar across treatment arms.

Table 57: Summary of Baseline Disease Characteristics (Full Analysis Set)

	Placebo N=64	Mitapivat N=130	Total N=194
Baseline transfusion burden [1], n (%)			
0	54 (84.4)	114 (87.7)	168 (86.6)
1-2	7 (10.9)	10 (7.7)	17 (8.8)
3-5	3 (4.7)	6 (4.6)	9 (4.6)
>5	0	0	0
Splenectomy status [2], n (%)			
No	39 (60.9)	83 (63.8)	122 (62.9)
Yes	25 (39.1)	47 (36.2)	72 (37.1)
If yes, age at splenectomy (yr)			
n	24	47	71

	Placebo N=64	Mitapivat N=130	Total N=194
Mean (SD)	16.8 (10.98)	20.3 (11.62)	19.1 (11.44)
Median (Q1, Q3)	12.5 (8.0, 24.5)	19.0 (10.0, 27.0)	17.0 (10.0, 26.0)
Min, Max	4, 42	3, 49	3, 49
Prior cholecystectomy status [2], n (%)			
No	48 (75.0)	85 (65.4)	133 (68.6)
Yes	16 (25.0)	45 (34.6)	61 (31.4)
If yes, age at cholecystectomy (yr)			
n	16	40	56
Mean (SD)	25.1 (9.37)	27.9 (11.83)	27.1 (11.17)
Median (Q1, Q3)	24.5 (18.5, 30.0)	25.5 (19.0, 34.5)	25.5 (19.0, 33.5)
Min, Max	11, 44	10, 61	10, 61
Prior iron chelation status [3], n (%)			
No	42 (65.6)	84 (64.6)	126 (64.9)
Yes	22 (34.4)	46 (35.4)	68 (35.1)
Prior hydroxyurea status, n (%)			
No	58 (90.6)	119 (91.5)	177 (91.2)
Yes	6 (9.4)	11 (8.5)	17 (8.8)
Hepatic iron concentration by MRI (mg/g)			
n	52	98	150
Mean (SD)	4.566 (4.3062)	5.357 (4.7528)	5.083 (4.6038)
Median (Q1, Q3)	2.755 (1.370, 6.070)	3.930 (2.070, 7.460)	3.635 (1.600, 6.650)
Min, Max	0.75, 18.53	0.75, 27.19	0.75, 27.19

Table 58: Summary of Baseline Hemoglobin, Markers of Hemolysis and Markers of Erythropoiesis (Full Analysis Set)

Parameter	Placebo N=64	Mitapivat N=130	Total N=194
Baseline hemoglobin (g/L)			
n	64	130	194
Mean (SD)	83.93 (10.057)	83.04 (10.773)	83.34 (10.524)
Median (Q1, Q3)	84.17 (76.50, 91.08)	83.88 (75.00, 91.50)	84.00 (75.67, 91.50)
Min, Max	58.5, 107.0	53.0, 104.0	53.0, 107.0
Baseline indirect bilirubin (umol/L)			
n	62	130	192
Mean (SD)	27.28 (19.297)	29.03 (24.573)	28.46 (22.966)
Median (Q1, Q3)	22.63 (13.50, 34.65)	23.38 (14.30, 37.55)	23.00 (13.88, 36.86)
Min, Max	2.7, 81.6	2.2, 155.8	2.2, 155.8
Baseline lactate dehydrogenase (U/L)			
n	64	130	194
Mean (SD)	309.15 (179.966)	303.05 (157.155)	305.06 (164.585)
Median (Q1, Q3)	267.25 (190.00, 355.50)	263.50 (197.00, 367.00)	265.00 (197.00, 362.00)
Min, Max	110.0, 1009.0	107.5, 1207.5	107.5, 1207.5
Baseline haptoglobin (g/L)			
n	63	127	190
Mean (SD)	0.286 (0.4115)	0.213 (0.2599)	0.237 (0.3191)
Median (Q1, Q3)	0.100 (0.100, 0.390)	0.100 (0.100, 0.160)	0.100 (0.100, 0.230)
Min, Max	0.10, 2.83	0.10, 1.70	0.10, 2.83

Parameter	Placebo N=64	Mitapivat N=130	Total N=194
Baseline erythropoietin (IU/L)			
n	55	118	173
Mean (SD)	197.05 (639.311)	135.21 (222.629)	154.87 (403.569)
Median (Q1, Q3)	64.10 (29.50, 128.00)	65.05 (40.20, 120.00)	64.10 (36.60, 127.00)
Min, Max	15.7, 4710.0	8.3, 1587.0	8.3, 4710.0
Baseline reticulocytes (10^9/L)			
n	57	121	178
Mean (SD)	242.94 (183.964)	248.26 (168.974)	246.56 (173.410)
Median (Q1, Q3)	173.37 (131.80, 299.70)	187.90 (141.97, 315.75)	184.60 (134.53, 315.75)
Min, Max	0.0, 785.1	10.7, 835.5	0.0, 835.5
Baseline reticulocytes/erythrocytes (fraction of 1)			
n	58	122	180
Mean (SD)	0.0630 (0.05065)	0.0690 (0.05807)	0.0671 (0.05572)
Median (Q1, Q3)	0.0440 (0.0275, 0.0911)	0.0458 (0.0308, 0.0954)	0.0451 (0.0296, 0.0923)
Min, Max	0.000, 0.219	0.003, 0.298	0.000, 0.298

Numbers analysed

Analysis set definitions and summaries are provided in Table 59. Of the subjects randomized, more than 90% of participants in total were included in the PPS. There was 1 subject in each of the placebo and mitapivat arms who was randomized but did not receive study treatment and therefore was not included in the Safety Analysis Set.

Table 59: Summary of Analysis Sets (All Screened Subjects)

	Placebo	Mitapivat	Total
All Screened Subjects			235
Full Analysis Set	64	130	194
Per-Protocol Set, n (%) [1]	55 (85.9)	120 (92.3)	175 (90.2)
Safety Analysis Set	63	129	192
PK Analysis Set, n (%) [2]	0	124 (96.1)	124 (64.6)
PD Analysis Set, n (%) [2]	59 (93.7)	122 (94.6)	181 (94.3)
PK/PD Analysis Set, n (%) [2]	0	113 (87.6)	113 (58.9)

Outcomes and estimation

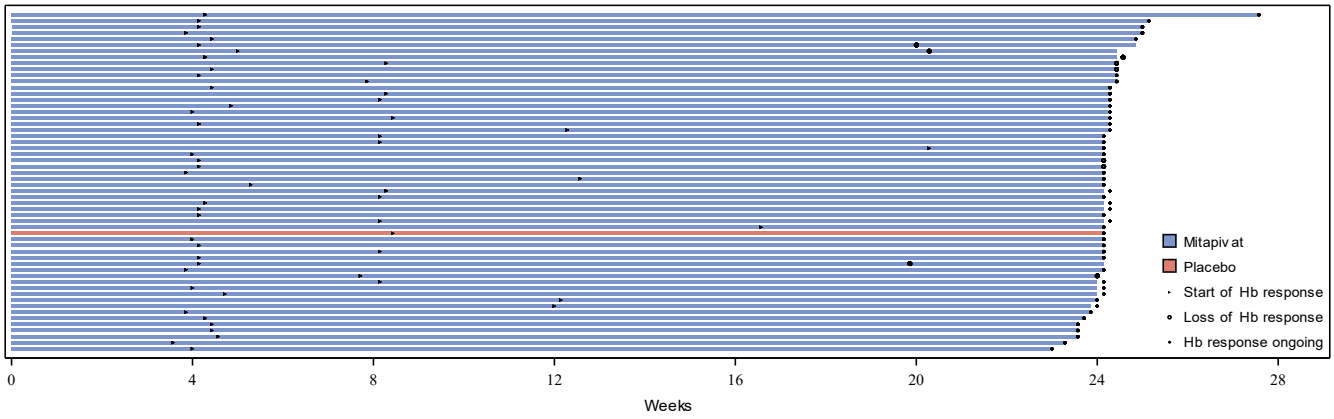
The primary endpoint was met. Mitapivat demonstrated a statistically significant improvement in Hb response compared to placebo (2-sided p-value <0.0001) in subjects with α- or β-thalassemia who are not transfusion dependent. The primary endpoint of Hb response was defined as a ≥10 g/L (1.0 g/dL) increase in average Hb concentration from Week 12 through Week 24 compared with baseline (Table 60)

Table 60: Primary Analysis of Hemoglobin Response - Mantel-Haenszel Stratum Weighted Method (Full Analysis Set)

	Placebo N=64	Mitapivat N=130
Hb responders, n (%)	1 (1.6)	55 (42.3)
Adjusted difference in response rate (Mitapivat vs Placebo), %		40.9
95% CI		(32.0, 49.8)
2-sided p-value		<0.0001

Hb responders in the mitapivat arm had a baseline mean (SD) Hb concentration of 80.3 (11.07) g/L and an average Hb concentration change from baseline from Week 12 through Week 24 of 15.55 (4.070) g/L. There was 1 Hb responder in the placebo arm with a baseline Hb of 73.0 g/L. Hb responders in the mitapivat arm had durable responses up to 23.4+ weeks and ongoing for most Hb responders at the end of the Double-blind Period (Figure 37).

Figure 37: Swimlane Plot for Duration of Hemoglobin Response (Subjects in Full Analysis Set Who Achieved Hb Response)



Results of prespecified sensitivity analyses for Hb response were consistent with the results presented in Table 45. The 2-sided p-values for the analysis based on the PPS and for the analysis including only those subjects who completed 24 weeks of study treatment and who did not receive any concomitant medications before completion of 24 weeks of study treatment that could affect the Hb concentrations were <0.0001.

Key secondary endpoints

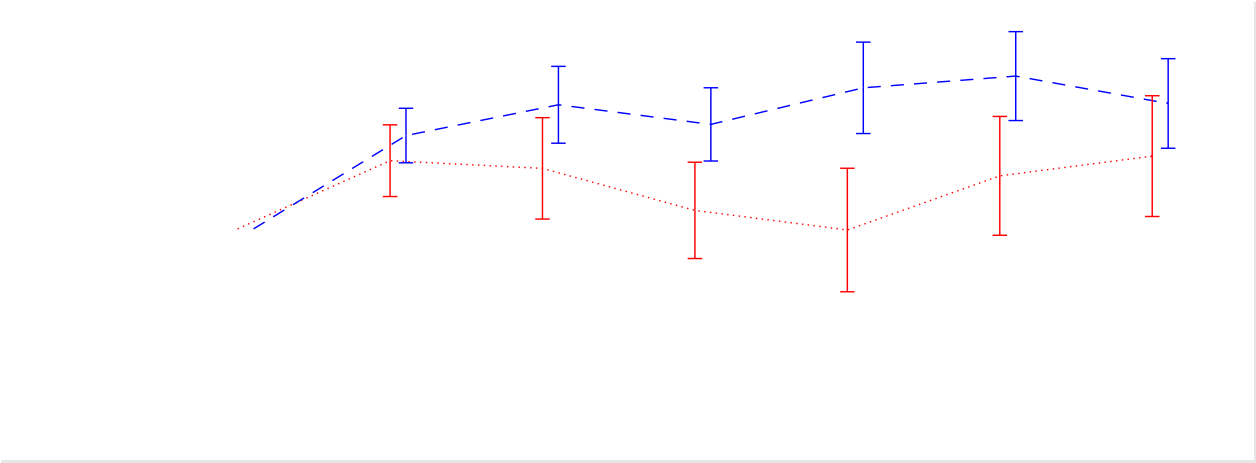
The devices used by subjects to complete patient-reported quality of life questionnaires did not provide a calculated score of their responses and did not provide a history of scores from prior assessments. Once the subject completed an assessment on the device, the subject clicked "Submit". At that point, the data was transferred to the Medidata Clinical Cloud and to the electronic data capture (EDC) system, to which the subject does not have access. Scores for any given assessment were only calculated once the data were captured in the EDC.

Mitapivat demonstrated a statistically significant (2-sided p-value=0.0026) change from baseline in average FACIT-Fatigue subscale score from Week 12 through Week 24 compared to placebo (Table 61). Mitapivat led to early and sustained improvement in FACIT-Fatigue subscale score starting at Week 12 through Week 24 (Figure 38).

Table 61: Analysis of Average Change from Baseline in FACIT-Fatigue Subscale Score from Week 12 through Week 24 - ANCOVA (Full Analysis Set)

FACIT-Fatigue Subscale Score	Placebo N=64	Mitapivat N=130
Baseline		
n	56	115
Mean (SD)	36.41 (9.407)	36.10 (11.124)
Average of Week 12 Through Week 24		
Change from baseline		
n	54	109
LS Mean (SE)	1.46 (0.955)	4.85 (0.732)
95% CI	(-0.43, 3.34)	(3.41, 6.30)
Difference in LS Mean (SE) (Mitapivat-Placebo)		3.40 (1.110)
95% CI		(1.21, 5.59)
2-sided p-value		0.0026

Figure 38: Least Squares Mean (95% CI) of Change from Baseline in FACIT-Fatigue Subscale Score Over Time - Double-blind Period (Full Analysis Set)



At baseline, the FACIT-Fatigue subscale scores were similar for both treatment arms . The mean score at baseline was approximately 36 in each treatment arm, showing that the subjects enrolled in the trial had a fatigue level at baseline consistent with other chronic anemic conditions. For comparison, FACIT-Fatigue subscale scores in a healthy population are consistent with a score of 43 or higher (Cella *et al,* 2002).

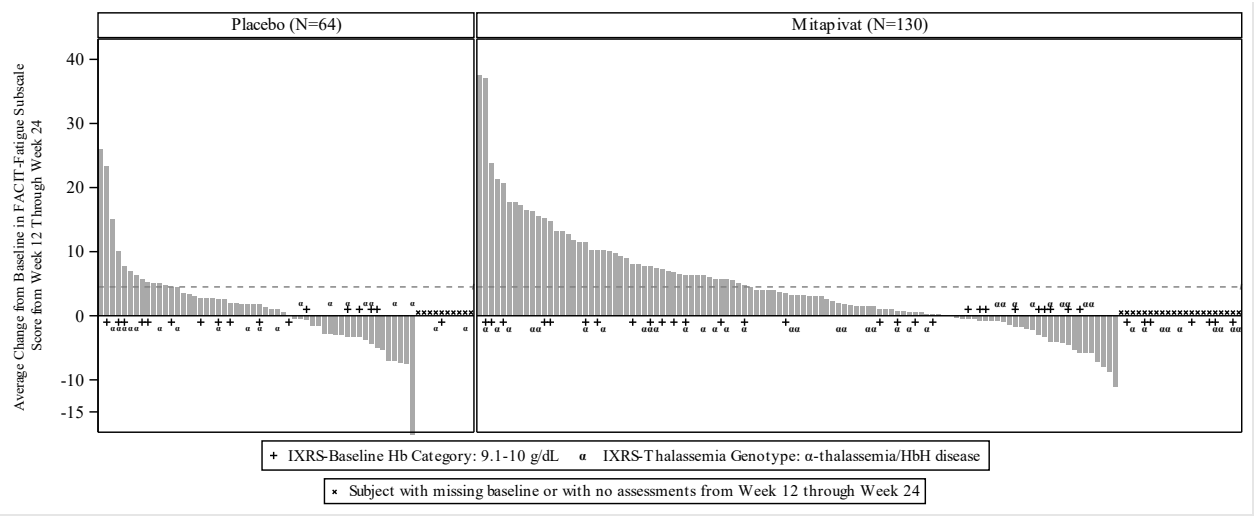
To establish a meaningful change threshold for the FACIT-Fatigue in this population, a meaningful change analysis was performed using a blinded subset from this study.

The meaningful within-person change threshold for FACIT-Fatigue was defined as ≥4.5-point improvement from baseline in average score from Week 12 through 24. The proportion of subjects who achieved the meaningful within-person change threshold was higher in the mitapivat arm than in the placebo arm (Table 62, Figure 63).

Table 62: Analysis of Meaningful Within-Patient Change (MWPC) for Average Change from Baseline in FACIT-Fatigue Subscale Score from Week 12 through Week 24 - Mantel-Haenszel Stratum Weighted Method (Full Analysis Set)

	Placebo N=64	Mitapivat N=130
Subjects with MWPC(≥ 4.5), n(%)	14 (21.9)	47 (36.2)
Adjusted difference in response rate (Mitapivat vs Placebo), %		14.2
95% CI		(1.1, 27.2)

Figure 63: Waterfall Plot of Average Change from Baseline in FACIT-Fatigue Subscale Score from Week 12 through Week 24 - Double-blind Period (Full Analysis Set)



Results of prespecified sensitivity analyses for the change from baseline in average FACIT-Fatigue subscale score from Week 12 through Week 24 were consistent with results presented above. The 2-sided p-value for the analysis based on the MMRM model was 0.0008 and the 2-sided p-value for the ANCOVA based on a pattern-mixture model with control-based pattern imputation for missing data was 0.0001.

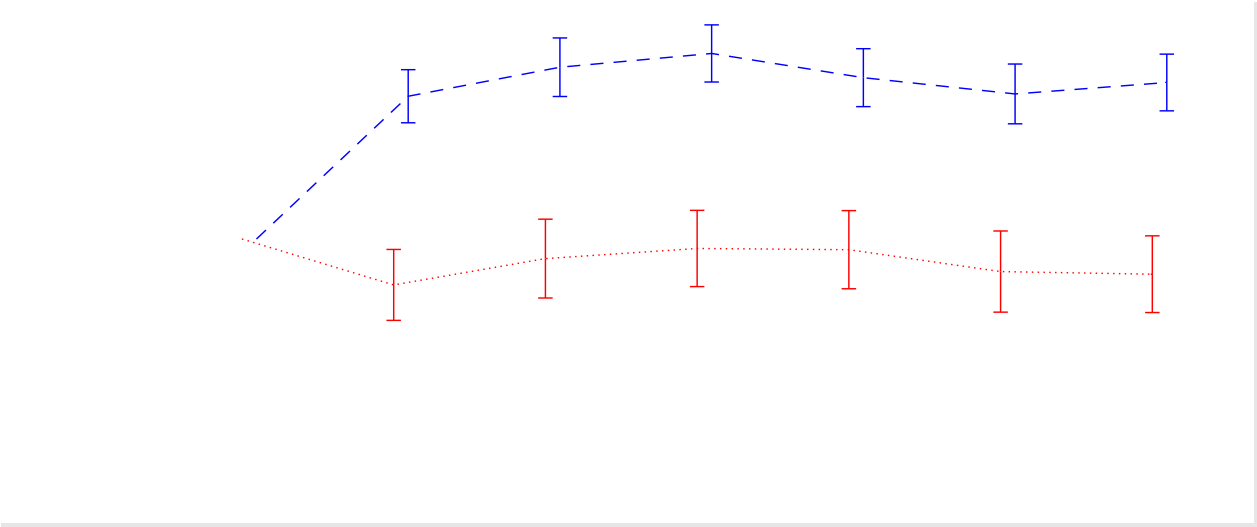
Mitapivat demonstrated a statistically significant improvement in the change from baseline in average Hb concentration from Week 12 through Week 24 compared with placebo (2-sided p-value <0.0001) (Table 64); mitapivat led to early and sustained improvement in Hb concentration starting at Week 4 through Week 24 (Figure 40).

Table 64: Analysis of Average Change from Baseline in Hemoglobin Concentrations from Week 12 Through Week 24 - ANCOVA (Full Analysis Set)

Hemoglobin (g/L)	Placebo N=64	Mitapivat N=130
Baseline		
n	64	130
Mean (SD)	83.93 (10.057)	83.04 (10.773)
Average of Week 12 Through Week 24		
Change from baseline		
n	61	123
LS Mean (SE)	-1.06 (0.867)	8.57 (0.666)
95% CI	(-2.77, 0.65)	(7.26, 9.88)
Difference in LS Mean (SE) (Mitapivat-Placebo)		9.63 (0.928)

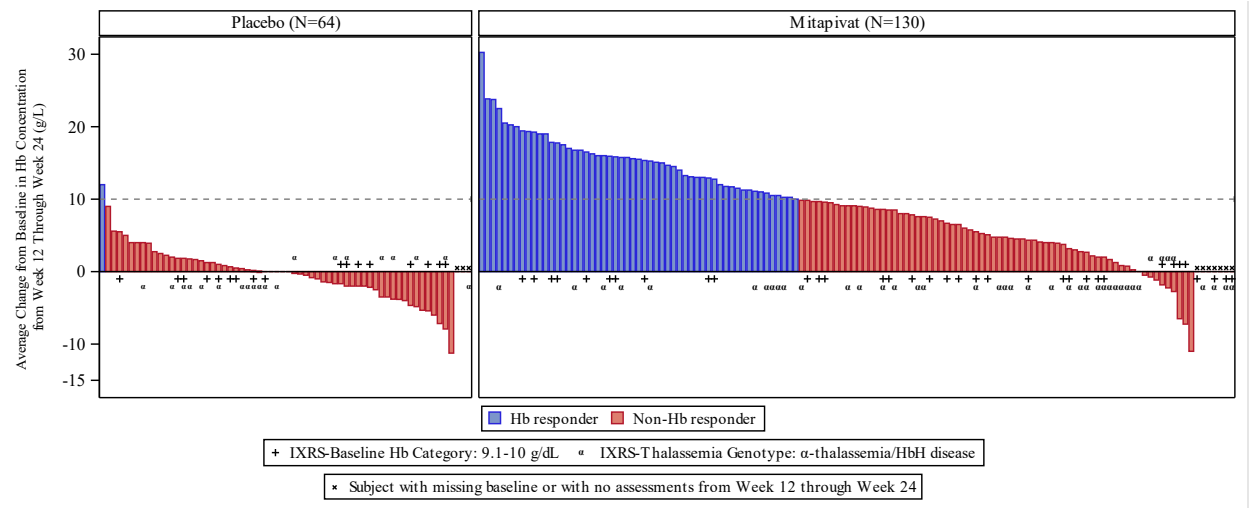
Hemoglobin (g/L)	Placebo N=64	Mitapivat N=130
95% CI		(7.80, 11.46)
2-sided p-value		<0.0001

Figure 40: Least Squares Mean (95% CI) of Change from Baseline in Hemoglobin Over Time - Double-blind Period (Full Analysis Set)



Most subjects in the mitapivat arm experienced increases in Hb concentrations as measured by the average change from baseline from Week 12 through Week 24 (Figure 41).

Figure 41: Waterfall Plot of Average Change from Baseline in Hemoglobin Concentration from Week 12 through Week 24 - Double-blind Period (Full Analysis Set)



Results of a prespecified sensitivity analysis for average change from baseline in Hb concentration was consistent with the results presented above. The 2-sided p-value for the analysis based on the MMRM model was <0.0001.

Subjects with α-thalassemia in Study AG348-C-017 (NTDT)

In Study 017, 32.0% (62/194) of the subjects enrolled had α-thalassemia including 32.3% (42/130) in the mitapivat arm and 31.3% (20/64) in the placebo arm. Among the α-NTDT population, 23.8% of subjects in the mitapivat arm met the primary Hb response endpoint compared to 0 in the placebo arm, and the least square mean difference in change from baseline in average FACIT-Fatigue score from Week 12 through Week 24 favored mitapivat compared to placebo.

Table 65: Efficacy Results in Subjects with Non-Transfusion-Dependent α-Thalassemia (Subjects with α-Thalassemia in Full Analysis Set, Study 017)

	Placebo N=20	Mitapivat N=42
Hb responders, n (%)	0	10 (23.8)
Difference in response rate (Mitapivat vs Placebo), %		23.8
95% CI		(2.2, 39.5)
Average change from baseline in Hb from Week 12 Through Week 24, (g/L)		
n	19	38
LS Mean (SE)	0.06 (1.111)	5.92 (0.780)
95% CI	(-2.17, 2.29)	(4.36, 7.49)
Difference in LS Mean (SE) (Mitapivat-Placebo)		5.86 (1.372)
95% CI		(3.11, 8.61)
Average change from baseline in FACIT-Fatigue Score from Week 12 Through Week 24		
n	18	33
LS Mean (SE)	0.78 (1.745)	5.28 (1.288)
95% CI	(-2.73, 4.29)	(2.69, 7.87)
Difference in LS Mean (SE) (Mitapivat-Placebo)		4.50 (2.172)
95% CI		(0.13, 8.87)

Additional Secondary Efficacy Endpoints

- Hb 1.5+ Response

The proportion of subjects who achieved an Hb 1.5+ response (defined as a ≥15 g/L [1.5 g/dL] increase in average Hb concentration from Week 12 through Week 24) was 24.6% (32/130) in the mitapivat arm and 0% (0/65) in the placebo arm. The adjusted difference between mitapivat and placebo arms based on Mantel-Haenszel stratum weighted method adjusting for the randomisation stratification factors was 24.7% (95% CI: 17.3, 32.1).

- Change From Baseline in Markers of Hemolysis and Erythropoiesis at Week 24

Each marker of hemolysis (indirect bilirubin, LDH, and haptoglobin) and erythropoiesis (erythropoietin, reticulocytes, and reticulocytes/erythrocytes) was analysed using ANCOVA, with treatment arm as the independent variable, and covariates for randomization stratification factors and baseline marker concentration. Indirect bilirubin LS mean (SE) change from baseline to Week 24 was −10.65 (1.047) for the mitapivat arm and −0.03 (1.403) for the placebo arm; the LS mean (SE) difference was −10.62 (1.581) (95% CI: −13.74, −7.50). LDH LS mean (SE) change from baseline to Week 24 was −30.07 (7.131) for the mitapivat arm and −5.79 (9.440) for the placebo arm; the LS mean (SE) difference was −24.28 (10.698) (95% CI: −45.40, −3.15).

Haptoglobin LS mean (SE) change from baseline to Week 24 was −0.002 (0.0145) for the mitapivat arm and 0.017 (0.0199) for the placebo arm; the LS mean difference was −0.018 (0.0223) (95% CI: −0.063, 0.026).

At baseline, subjects in both the placebo and mitapivat arms had marker levels consistent with hemolysis. At Week 24, there were reductions from baseline in indirect bilirubin and LDH, 2 markers of hemolysis, and the observed effects were greater in mitapivat arm compared with placebo. This is consistent with the mechanism of action of mitapivat and a reduction in hemolysis.

Erythropoietin LS mean (SE) change from baseline to Week 24 was 19.21 (37.773) for the mitapivat arm and 115.71 (49.413) for the placebo arm; the LS mean (SE) difference was −96.50 (57.220) (95% CI: −209.59, 16.60).

Reticulocyte LS mean (SE) change from baseline to Week 24 was −32.11 (10.600) for the mitapivat arm and −14.74 (14.932) for the placebo arm; the LS mean (SE) difference was −17.37 (16.633) (95% CI: −50.30, 15.57).

Reticulocytes/erythrocytes LS mean (SE) change from baseline to Week 24 was −0.0159 (0.00267) in the mitapivat arm and −0.0025 (0.00366) in the placebo arm; the LS mean (SE) difference was −0.0135 (0.00414) (95% CI: −0.0217, −0.0053).

At baseline, subjects in both the placebo and mitapivat arms had marker levels consistent with ineffective erythropoiesis. Overall, changes in markers of erythropoiesis were consistent with improvements in erythropoiesis. There was a decrease in the ratio of reticulocytes/erythrocytes (fraction of 1) from baseline to Week 24, and this effect was greater in mitapivat arm compared with placebo. There was also a similar trend toward decreasing from baseline at Week 24 in serum erythropoietin in the mitapivat arm compared with placebo.

- PGIS-Fatigue and PGIC-Fatigue

PGIS-Fatigue measured subjects' perception of their fatigue severity (7-day recall) on a 4-point scale ranging from 'none' to 'severe'. The PGIC-Fatigue measured subjects' perceived change in severity of fatigue compared to baseline on a 5-point scale ranging from 'much better" to 'much worse'. The distribution of the baseline PGIS-Fatigue was similar for both treatment arms.

A subject was considered to have met the PGIS-Fatigue endpoint at Weeks 12, 16, 20, or 24 if their baseline to postbaseline score met one of the following conditions: 'none' at baseline to 'none' postbaseline; 'mild' to 'mild' or 'none'; 'moderate' to 'mild' or 'none'; or 'severe' to 'moderate', 'mild', or 'none'.

The proportions of subjects who achieved a PGIS-Fatigue response at Weeks 12, 16, 20, and 24 compared to baseline were higher in the mitapivat arm compared to the placebo arm (Table 66).

Table 66: Analysis of Patient Global Impression of Severity (PGIS)-Fatigue Response by Visit - Mantel-Haenszel Stratum Weighted Method (Full Analysis Set)

Visit	Placebo N=64	Mitapivat N=130
Week 12		
PGIS-Fatigue Response, n (%)	30 (46.9)	85 (65.4)
Adjusted difference in response rate (Mitapivat vs Placebo), %		19.9
95% CI		(4.7, 35.1)
Week 16		
PGIS-Fatigue Response, n (%)	27 (42.2)	82 (63.1)
Adjusted difference in response rate (Mitapivat vs Placebo), %		18.7
95% CI		(3.2, 34.3)
Week 20		

Visit	Placebo N=64	Mitapivat N=130
PGIS-Fatigue Response, n (%)	31 (48.4)	80 (61.5)
Adjusted difference in response rate (Mitapivat vs Placebo), %		11.0
95% CI		(-4.4, 26.5)
Week 24		
PGIS-Fatigue Response, n (%)	30 (46.9)	81 (62.3)
Adjusted difference in response rate (Mitapivat vs Placebo), %		15.7
95% CI		(-0.0, 31.3)

A subject was considered to have met the PGIC-Fatigue endpoint at Weeks 12, 16, 20, or 24 if their baseline PGIS and corresponding PGIC met one of the following conditions: if the PGIS at baseline was 'none' or 'mild' and PGIC at the visit was 'no change', 'a little better', or 'much better'; or if the PGIS at baseline was 'moderate' or 'severe' and PGIC at the visit was 'a little better' or 'much better'.

The proportions of subjects who achieved a PGIC-Fatigue response at Weeks 12, 16, 20, and 24 were higher in the mitapivat arm compared to the placebo arm (Table 67).

Table 67: Analysis of Patient Global Impression of Change (PGIC)-Fatigue Response by Visit - Mantel-Haenszel Stratum Weighted Method (Full Analysis Set)

Visit	Placebo N=64	Mitapivat N=130
Week 12		
PGIC-Fatigue Response, n (%)	34 (53.1)	93 (71.5)
Adjusted difference in response rate (Mitapivat vs Placebo), %		20.1
95% CI		(5.2, 34.9)
Week 16		
PGIC-Fatigue Response, n (%)	32 (50.0)	93 (71.5)
Adjusted difference in response rate (Mitapivat vs Placebo), %		19.8
95% CI		(4.8, 34.7)
Week 20		
PGIC-Fatigue Response, n (%)	36 (56.3)	86 (66.2)
Adjusted difference in response rate (Mitapivat vs Placebo), %		8.1
95% CI		(-7.0, 23.1)
Week 24		
PGIC-Fatigue Response, n (%)	34 (53.1)	86 (66.2)
Adjusted difference in response rate (Mitapivat vs Placebo), %		12.0
95% CI		(-2.9, 26.9)

The 6MWT was analyzed using an ANCOVA, with change from baseline at Week 24 as the dependent variable, treatment arm as the independent variable, and baseline and randomisation stratification factors as covariates. An improvement in change from baseline in walking capacity at Week 24 was observed in the mitapivat arm compared to placebo arm.

At baseline, the distance walked was similar for both treatment arms. The LS mean (SE) change from baseline at Week 24 was 30.48 m (5.651) in the mitapivat arm and 7.11 m (7.346) in the placebo arm; LS mean (SE) difference was 23.36 m (8.337) (95% CI: 6.90, 39.83) (Table 68)

Table 68: Analysis of Change from Baseline in 6-Minute Walk Test Distance (meters) to Week 24 - ANCOVA (Full Analysis Set)

	Placebo N=64	Mitapivat N=130
Baseline		
n	60	119
Mean (SD)	412.43 (88.122)	422.22 (76.635)
Week 24		
Change from baseline		
n	57	107
LS Mean (SE)	7.11 (7.346)	30.48 (5.651)
95% CI	(-7.39, 21.62)	(19.31, 41.64)
Difference in LS Mean (SE) (Mitapivat-Placebo)		23.36 (8.337)
95% CI		(6.90, 39.83)

An anchor-based analysis determining a meaningful change threshold was not possible for this study, as changes in the 6MWT were not sensitive to changes in the PGIS or PGIC for walking capacity. However, previous studies among adults with chronic illness (Bohannon and Crouch, 2017) and transfusions in anemic patients (St Lezin *et al,* 2019) support a clinically meaningful improvement threshold of >20 m.

Markers of iron metabolism were analyzed using ANCOVA, with change from baseline at Week 24 as the dependent variable, treatment arm as the independent variable, and baseline and randomization stratification factors as covariates.

Baseline mean (SD) ferritin µg/L concentration was 661.59 (575.370) in the mitapivat arm and 788.17 (1119.976) in the placebo arm. The LS mean (SE) change from baseline to Week 24 was −34.75 (32.710) (95% CI: −99.33, 29.83) in the mitapivat arm and −32.48 (43.090) in the placebo arm; the LS mean (SE) difference was −2.26 (49.529) (95% CI: −100.04, 95.52).

Baseline mean (SD) transferrin saturation (fraction of 1) was 0.519 (0.2101) in the mitapivat arm and 0.518 (0.2164) in the placebo arm. LS mean (SE) change from baseline to Week 24 was −0.029 (0.0212) in the mitapivat arm and −0.047 (0.0262) in the placebo arm; the LS mean (SE) difference was 0.017 (0.0322) (95% CI: −0.047, 0.081).

The elevated mean baseline levels of ferritin and transferrin saturation were consistent with thalassemia patients who are not regularly transfused. Compared with baseline, treatment with mitapivat or placebo for 24 weeks did not lead to changes in mean serum ferritin or mean serum transferrin saturation.

Exploratory Endpoints

Biomarkers

Observations in soluble transferrin receptor and erythroferrone were consistent with the proposed mechanism of action of mitapivat, specifically improvements in erythropoiesis.

Transfusion-Free

The proportion of transfusion-free subjects, defined as no transfusions for a 24-week period from randomization to Week 24, was similar in both treatment arms. The baseline and Double-blind Period transfusion burden is defined as the total number of RBC units transfused in the 24-week period before randomisation or the 24-week Double-blind Period, respectively. Transfusion burden at baseline and its change from baseline were similar in both treatment arms.

HRQOL

- PROMIS® SF-8 Physical Function

The PROMIS Physical Function 8-Item Short Form contains 8 questions assessing limitations associated with daily physical activities that are relevant for a specific patient population. Scores are expressed as T-scores, representing a standardized score with a mean of 50 and an SD of 10.

Higher scores represent better physical functioning (van der Meij et al, 2018). Baseline PROMIS Physical Function T-scores were similar for both treatment arms with a mean (SD) of 46.15 (8.300) in the mitapivat arm and 46.22 (7.800) in the placebo arm. The Week 24 T-score mean (SD) was 48.59 (9.191) in the mitapivat arm and 46.41 (7.648) in the placebo arm. The Week 24 mean (SD) change from baseline for the PROMIS Physical Function T-Score was also higher in the mitapivat arm (2.40 [7.087]) than in the placebo arm (1.07 [6.033]).

- FACT-G

The FACT-G assesses the effects of underlying disease and its treatment on non–disease-specific HRQOL (Cella et al, 1993). The FACT-G includes 27 items to evaluate symptoms and treatment-related effects on physical well-being (7 items), social/family well-being (7 items), emotional well-being (6 items), and functional well-being (7 items). The recall period is 1 week, and each question is assessed on a 5-point Likert scale: 0 (not at all), 1 (a little bit), 2 (somewhat), 3 (quite a bit), and 4 (very much). Subscale scores range from 0 to 28 for physical, social/family, and functional well-being and from 0 to 24 for emotional well-being.

Subscale scores can be combined to produce a FACT-G total score, ranging from 0 to 108. Higher scores represent better HRQOL. Baseline FACT-G total scores were similar for both treatment arms with a mean (SD) of 82.75 (15.173) in the mitapivat arm and 81.66 (14.509) in the placebo arm. The Week 24 mean (SD) change from baseline for the FACT-G total score was higher in the mitapivat arm (3.25 [11.343]) than in the placebo arm (0.84 [13.190]).

- PGIS-Thalassemia Symptoms and PGIC-Thalassemia Symptoms

PGIS of thalassemia symptoms measured subjects' perception of their symptom severity (7-day recall) The PGIC of thalassemia symptoms measured subjects' perceived change in symptoms compared with baseline. A higher frequency of subjects in the mitapivat arm compared with the placebo arm reported feeling much better (28.5% vs 12.5%) or a little better (39.2% vs 20.3%) at Week 24 compared with baseline.

- PGIS-Walking Capacity and PGIC-Walking Capacity

PGIS of walking capacity measured subjects' perception of difficulty in walking capacity (today) The PGIC of walking capacity measured subjects' perceived change in walking difficulty compared with baseline. A higher frequency of subjects in the mitapivat arm compared with the placebo arm reported their walking capacity as much better (24.6% vs 12.5%) or a little better (30.8% vs 15.6%) at Week 24 compared with baseline.

- EuroQoL Group 5-Level EQ-5D (EQ-5D-5L)

The EQ-5D-5L is a well-established, validated measure of general HRQOL developed by the EuroQoL group, which consists of 2 components: a descriptive system and a EuroQoL-Visual Analogue Scale (EQ-VAS). In the descriptive system, subjects rate their current level (1 [no problem], 2 [slight problems], 3 [moderate problems], 4 [severe problems], and 5 [extreme problems]) on each of the 5 dimensions (mobility, self-care, usual activities, pain/discomfort, and anxiety/depression). The EQ-VAS is presented as a 20-cm vertical line on which subjects are asked to mark their current health status, with scores ranging from 0 (worst imaginable

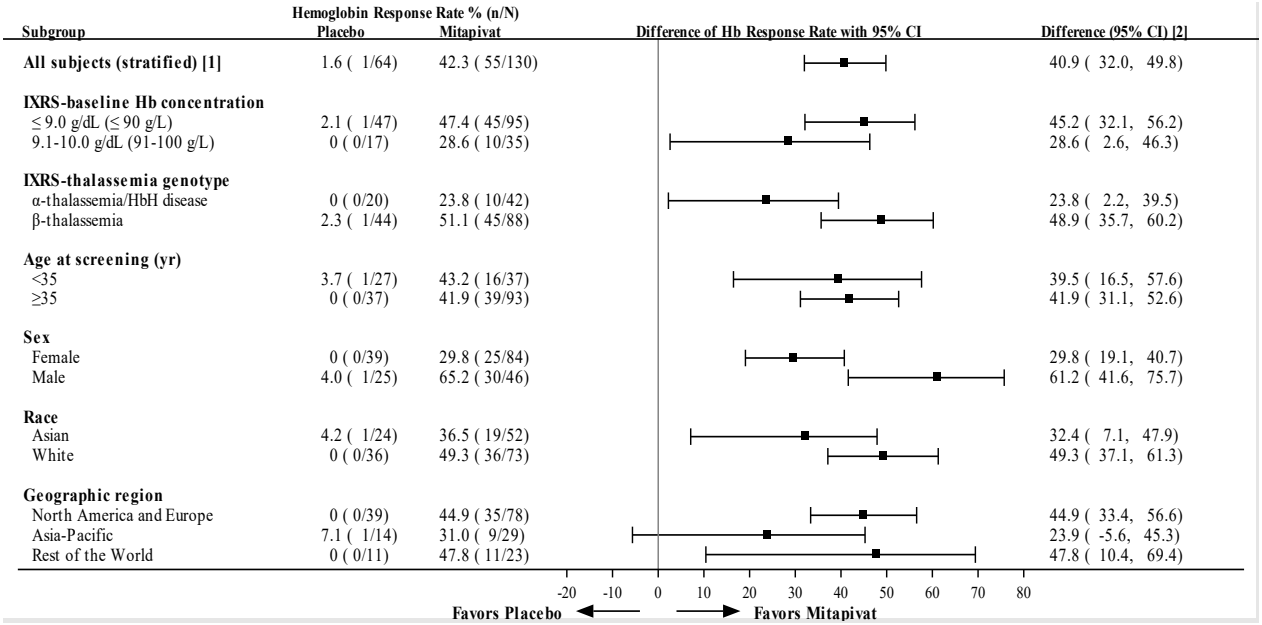
health state) to 100 (best imaginable health state Baseline EQ-5D-5L VAS scores were similar for both treatment arms with a mean (SD) of

74.7 (16.67) in the mitapivat arm and 75.5 (17.48) in the placebo arm. The Week 24 mean (SD) change from baseline for the EQ-5D-5L VAS score was higher in the mitapivat arm (4.2 [15.78]) than in the placebo arm (1.6 [14.70]).

Ancillary analyses

Subgroup analyses were prespecified for the primary endpoint (Hb response) and the key secondary endpoints (change from baseline in average FACIT-Fatigue subscale score from Week 12 through Week 24 and change from baseline in average Hb concentrations from Week 12 through Week 24). Hb response rates (Figure 42), mean change from baseline in average Hb concentration from Week 12 through Week 24 (Figure 43), and mean change from baseline in average FACIT-Fatigue scores from Week 12 through Week 24 (Figure 44) were higher in the mitapivat arm than the placebo arm across all prespecified subgroups.

Figure 42: Forest Plot for Difference in Hemoglobin Response Rate (Full Analysis Set)



Breslow-Day test was performed to assess the interaction between treatment group and race. The p-value was not statistically significant at the 0.05 nominal significance level (p-value =0.09)

Figure 43: Forest Plot of Change from Baseline in Average FACIT-Fatigue Subscale Score from Week 12 through Week 24 (Full Analysis Set)

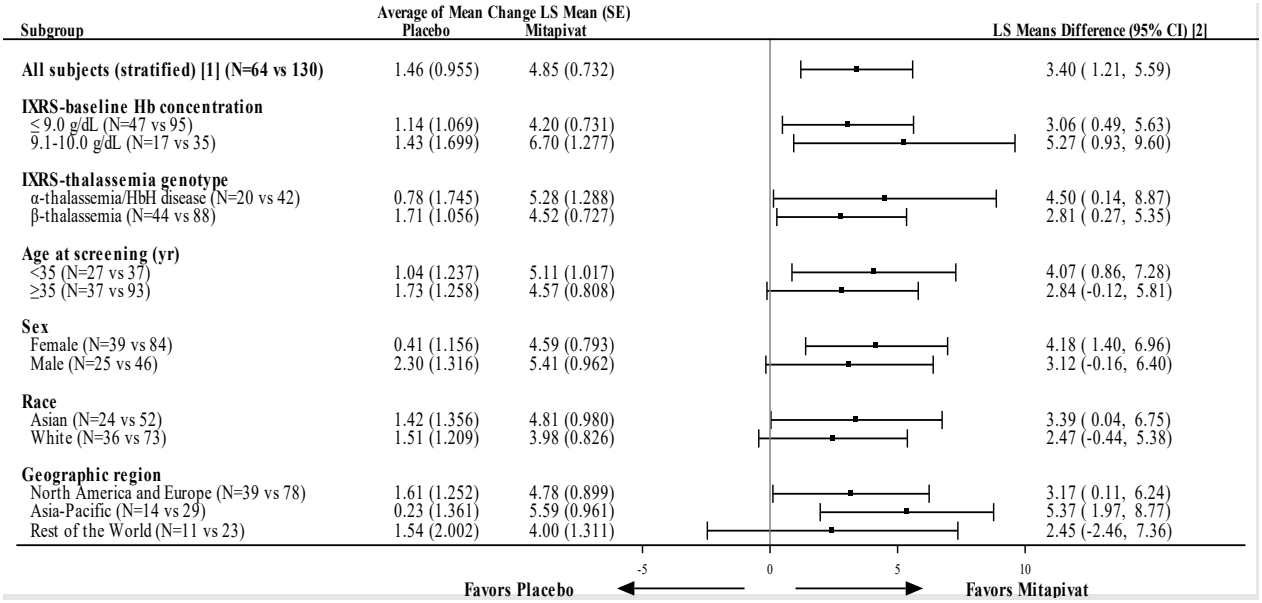
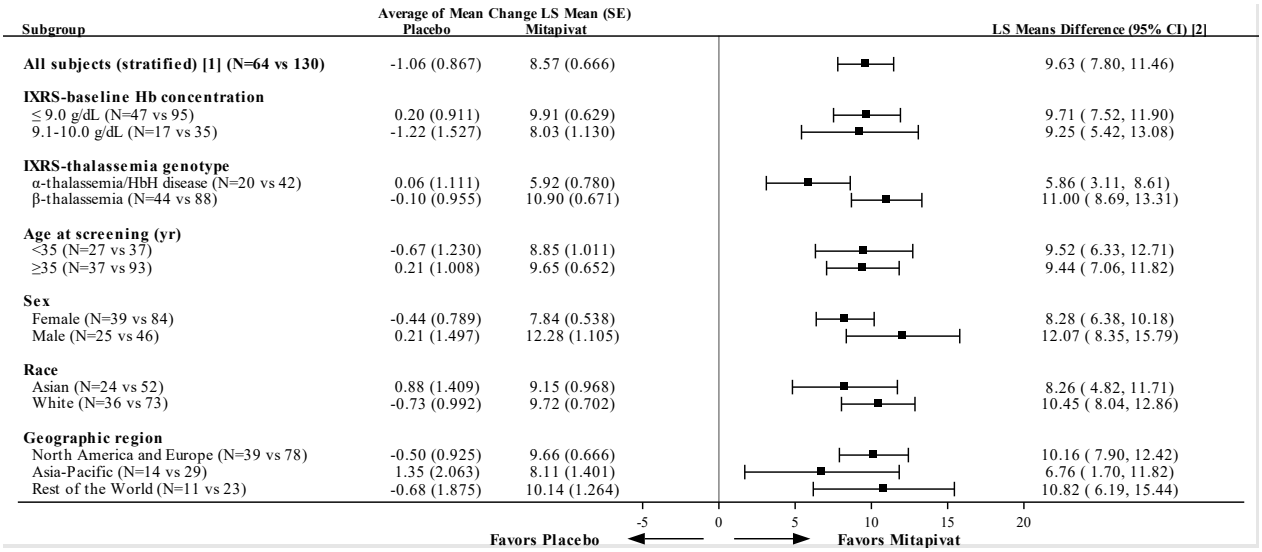


Figure 44: Forest Plot of Change from Baseline in Average Hemoglobin Concentration from Week 12 through Week 24 (Full Analysis Set)



Efficacy data from the ongoing OLE period of pivotal study AG348-C-017

Cumulative efficacy data from 55 subjects in Study 017 who were randomized to mitapivat and achieved Hb response during the Double-blind Period are included in this efficacy update. This represents cumulative efficacy and safety data from the Double-blind Periods and OLE Periods through 90 days after the last patient's first dose in the OLE Period (this cutoff date includes an additional minimum 241 days [34.4 weeks] follow-up for subjects ongoing in Study 017).

For subjects who achieved Hb response, duration of Hb response was calculated as the number of days from start of Hb response through the date of loss of Hb response, where:

Start of Hb response was the earliest date on or after the first dose of mitapivat with Hb change from baseline ≥1.0 g/dL; loss of Hb response occurred on the first date with Hb change from baseline <1.0 g/dL after the last Hb assessment in Double-blind Period with change from baseline ≥1.0 g/dL

For subjects who did not have loss of Hb response, the duration of Hb response was censored at the end of the 24-week Double-blind Period or the date of the last Hb assessment in the OLE Period with change from baseline ≥1.0 g/dL, whichever occurred later.

Hb concentrations assessed within 8 weeks after an RBC transfusion were excluded from the analysis.

Duration of exposure to mitapivat during the Double-blind Period and the cumulative duration of exposure to mitapivat through the cutoff date in the OLE Period are summarised in Table 69 for Hb responders.

Table 69: Summary of Exposure to Study Drug (Subjects in Full Analysis Set Who Were Randomized to Mitapivat and Achieved Hb Response)

	Mitapivat	
	Double-blind Period N=55	Efficacy Update N=55
Duration of exposure (Weeks)		
n	55	55
Mean (SD)	24.23 (0.592)	74.24 (19.738)
Median (Q1, Q3)	24.14 (24.14, 24.29)	69.86 (63.43, 88.14)
Min, Max	23.0, 27.6	23.0, 126.1
Person exposure-years	25.54	78.25

n is the number of subjects in the full analysis set who achieved Hb response.
Duration of exposure (weeks)=(date of last dose - date of first dose +1)/7.
Person exposure-years = sum of duration of exposure for each patient in days within each treatment group/365.25.
AG348 Thal Efficacy Update Data Cutoff: Study 017 (2024-07-10)

The duration of Hb response based on the data from the Double-blind Period and the updated duration of Hb response incorporating data from the OLE Period are summarised in Table 70.

Table 70: Summary of Duration of Hemoglobin Response (Subjects in Full Analysis Set Who Were Randomized to Mitapivat and Achieved Hb Response).

	Mitapivat	
	Double-blind Period N=55	Efficacy Update N=55
Duration of Hb response (weeks)		
n	55	55
Mean (SD)	17.90 (3.553)	43.60 (24.189)
Median (Q1, Q3)	19.57 (16.14, 20.29)	32.86 (20.14, 60.43)
Min, Max	4.0+, 23.4+	15.4, 93.3+

With continued mitapivat treatment during the OLE Period, the increase in Hb concentrations compared to baseline was sustained as shown in Figure 45 and the increase in FACIT-Fatigue scores compared to baseline was sustained as shown in Figure 46.

Figure 45: Mean (95% CI) of Change from Baseline in Hemoglobin Over Time (Study 017 Double-blind and OLE Periods, Subjects Randomized to Mitapivat)

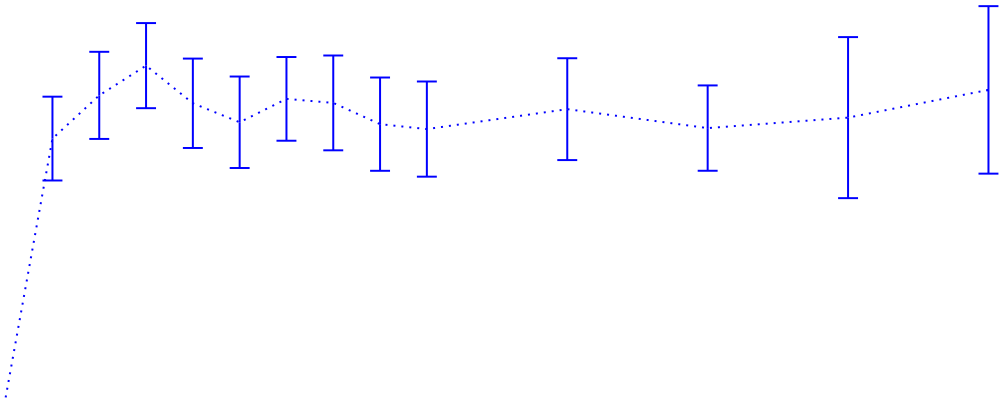
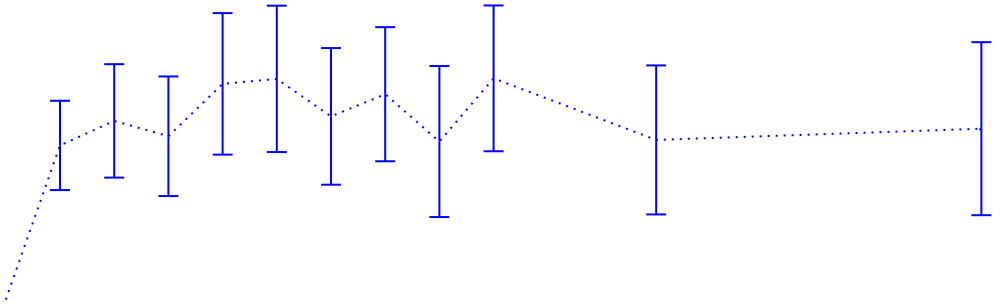


Figure 46: Mean (95% CI) of Change from Baseline in FACIT-Fatigue Subscale Score Over Time (Study 017 Double-blind and OLE Periods, Subjects Randomized to Mitapivat)



Additionally, the benefit seen on markers of hemolysis was sustained as shown in Figure 47 and Figure 48, respectively. Haptoglobin remained stable during the Double-blind and OLE Periods (Figure 49).

Figure 47: Mean (95% CI) of Change from Baseline in Indirect Bilirubin Over Time (Study 017 Double-blind and OLE Periods, Subjects Randomized to Mitapivat)

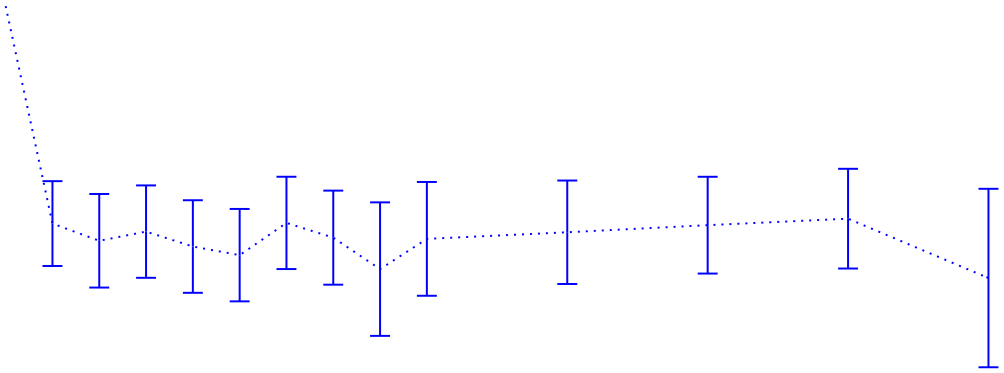


Figure 48: Mean (95% CI) of Change from Baseline in Lactate Dehydrogenase Over Time (Study 017 Double-blind and OLE Periods, Subjects Randomized to Mitapivat)

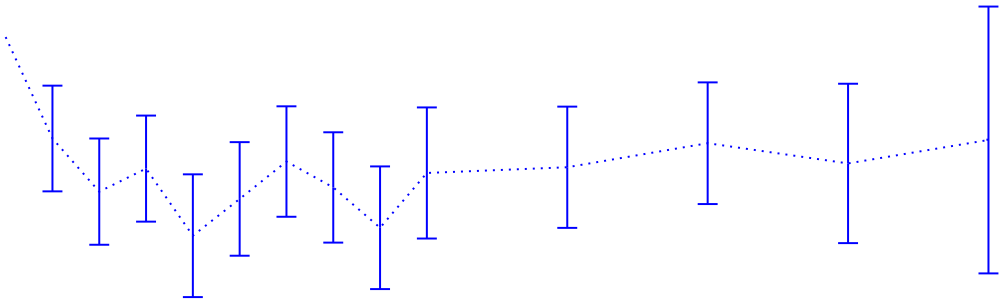
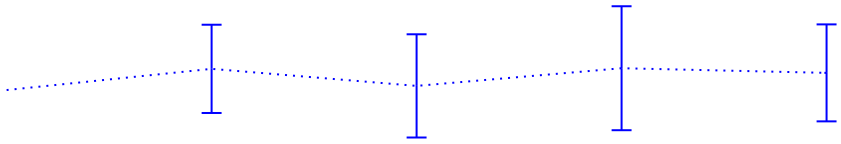
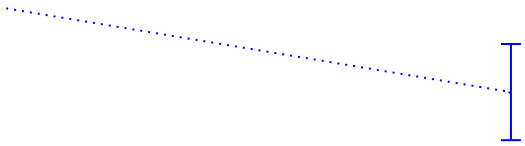


Figure 49: Mean (95% CI) of Change from Baseline in Haptoglobin Over Time (Study 017 Double-blind and OLE Periods, Subjects Randomized to Mitapivat)



The first assessment of hepatic iron concentration by MRI during treatment occurred, per protocol, at Week 48 during the OLE Period. As shown in Figure 6, hepatic iron concentration was substantially decreased after 48 weeks of treatment with mitapivat compared with baseline.

Figure 50: Mean (95% CI) of Change from Baseline in Hepatic Iron Concentration by MRI (Study 017 Double-blind and OLE Periods, Subjects Randomized to Mitapivat)



- **Summary of main efficacy results**

The following tables summarise the efficacy results from the main studies supporting the present application. The following tables (Table 71 and Table 72) summarise the efficacy results from the main studies supporting the present application.

Table 71: Primary and Key Secondary Efficacy Results – All Randomised Subjects (Study 018)

	Mitapivat N=171	**Placebo N=87**	**Difference[1]**	
Primary Endpoint	**n (%)**	**n (%)**	**Adjusted Difference (%) (95% CI)**	**p-value**
TRR, ≥50% reduction from baseline in RBC units transfused in any consecutive 12 weeks, with a reduction of at least 2 units	52 (30.4)	11 (12.6)	17.6 (8.0, 27.2)	0.0003
Key Secondary Endpoints	**n (%)**	**n (%)**	**Adjusted Difference (%) (95% CI)**	**p-value**
TRR2, ≥50% reduction from baseline in RBC units transfused in any consecutive 24 weeks	23 (13.5)	2 (2.3)	11.1 (5.1, 17.0)	0.0003
TRR3, ≥33% reduction from baseline in RBC units from Week 13 through Week 48	25 (14.6)	1 (1.1)	13.4 (7.7, 19.1)	<0.0001
TRR4, ≥50% reduction from baseline in RBC units from Week 13 through Week 48	13 (7.6)	1 (1.1)	6.4 (1.9, 10.9)	0.0056

Table 72: Primary and Key Secondary Efficacy Results – All Randomised Subjects (Study 017)

	Mitapivat N=130	**Placebo N=64**	**Difference**	
Primary Endpoint	**n (%)**	**n (%)**	**Adjusted Difference (%) (95% CI)**	**p-value**
Hb Response[1, 2]	55 (42.3)	1 (1.6)	40.9 (32.0, 49.8)	<0.0001
Key Secondary Endpoints	**LS Mean (95% CI)**	**LS Mean (95% CI)**	**LS Mean Difference (95% CI)**	**p-value**
Hemoglobin[3] (g/L)	8.57 (7.26, 9.88)	−1.06 (−2.77, 0.65)	9.63 (7.80, 11.46)	<0.0001
FACIT-Fatigue[4]	4.85 (3.41, 6.30)	1.46 (−0.43, 3.34)	3.40 (1.21, 5.59)	0.0026

2.6.5.3. In vitro biomarker test for patient selection for efficacy

Not Applicable.

2.6.5.4. Analysis performed across trials (pooled analyses and meta-analysis)

Not applicable.

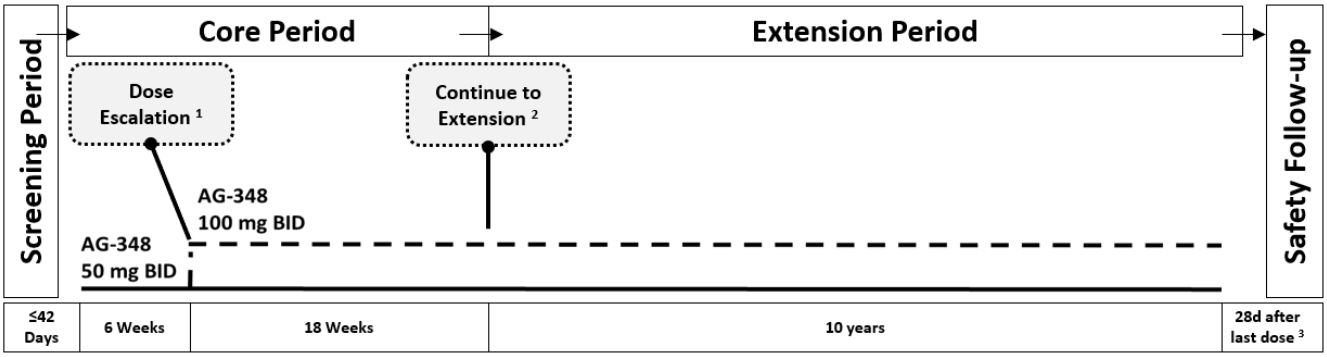
2.6.5.5. Supportive study(ies)

Study AG348-C-010

This is a Phase 2, open-label, multicenter study evaluating the efficacy, safety, pharmacokinetics, and PD of treatment with mitapivat in adult subjects with NTDT. This study consisted of a Core Period (up to 24 weeks) followed by an Extension Period (up to 10 years). There were 20 subjects enrolled in the Core Period. The Core Period has been completed and the OLE is ongoing. The study design is depicted in Figure 51.

Adult subjects with NTDT who tolerated mitapivat at 50 mg BID and achieved an Hb concentration increase from baseline that was <12 g/L for female subjects and <13 g/L for male subjects were eligible to have their dose escalated to 100 mg BID. Based on clinical studies conducted in healthy adults and in adult subjects with pyruvate kinase deficiency, 100 mg BID of mitapivat was expected to be safe and tolerable.

Figure 51: AG348-C-010 Study Design



Abbreviations: BID=twice daily; d=days; Hb=hemoglobin.

[1] Subjects could increase mitapivat dose from 50 to 100 mg at the Week 6 Visit based on an evaluation of safety and Hb concentration by the treating Investigator and after confirmation by the Medical Monitor (or designee). Criteria for dose escalation are described in AG348-C-010 CSR, Appendix 16.1.1, Study Protocol Section 7.3.

[2] Subjects who completed the 24-week Core Period and achieved an Hb response or a delayed Hb response with an acceptable safety profile could continue mitapivat for up to an additional 10 years in the Extension Period, after confirmation by the Medical Monitor (or designee).

[3] The Safety Follow-up Visit was to occur 28 days (±4 days) after the subject's last dose of mitapivat (including taper doses).

Subject population

The study was conducted in a population of adult subjects with NTDT, defined as having no more than 5 units of RBCs transfused during the 24-week period up to the first day of study drug and no RBC transfusions in the 8 weeks before the first day of study drug.

Additional key criteria included documented thalassemia, including β-thalassemia intermedia, HbE β-thalassemia, α-thalassemia (HbH disease), or β-thalassemia with mutations of 1 or more α genes and an Hb concentration ≤10.0 g/dL (100.0 g/L).

The study did not include subjects with a known history of HbS or HbC. The following prior treatments were excluded: sotatercept (ACE-011), luspatercept (ACE-536), ruxolitinib, or gene therapy; or prior bone marrow or stem cell transplant. Treatment with hematopoietic stimulating agents also was excluded (a washout period was allowed).

Endpoints

- Primary endpoint

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

- Secondary endpoints

-The mean change from baseline in Hb concentrations over a continuous 12-week interval from Week 12 to Week 24

-The sustained Hb response, defined as a subject who has achieved an Hb response and has achieved a ≥1.0 g/dL increase in Hb concentration at 2 or more evaluable Hb assessments out of the 4 scheduled assessments between the Week 12 Visit and Week 24 Visit (ie, Weeks 12, 16, 20, and 24)

-The delayed Hb response, defined as a subject who has not achieved an Hb response, but has achieved a ≥1.0 g/dL increase in Hb concentration at 1 or more Hb assessments after Week 12 (ie, Weeks 16, 20, and 24).

-Change from baseline in Hb concentration over the duration of the Extension Period • Time to first ≥1.0 g/dL increase in Hb concentration

-Change from baseline in markers of hemolysis: reticulocyte count, bilirubin, lactate dehydrogenase (LDH), and haptoglobin

-Change from baseline in markers of erythropoietic activity: nucleated RBC (NRBC), erythropoietin (EPO), and soluble transferrin receptor

Statistical method

The following statistical hypothesis was tested to address the primary objective:

H_0: λ_t = 0.3 vs H_1: λ_t > 0.3

where λ_t is the Hb response rate in the mitapivat arm. With a total of 17 subjects, the study would have 80% power to reject H_0 at a 1-sided α=0.05 when the true response rate is 0.6.

Disposition, Demographics, and Baseline Characteristics

There were 20 subjects enrolled; 15 subjects had β-thalassemia and 5 had α-thalassemia. Nineteen (95%) subjects completed the Core Period, while 1 (5%) subject discontinued after the Week 4 visit because of a TEAE that was an unrelated case of renal impairment.

The mean age for subjects in Study 010 was 45.3 years. Most subjects were female, and most subjects were Asian or White. Demographic characteristics were generally balanced between the thalassemia subgroups, except that subjects with α-thalassemia were younger than subjects with β-thalassemia (mean age: 37.0 vs 48.1 years), and a higher percentage of subjects with α-thalassemia were Asian compared with subjects with β-thalassemia (100% vs 33%).

Subjects were enrolled in the United States (45%), Canada (40%), and the United Kingdom (15%).

Baseline Hb concentrations for subjects treated with mitapivat in Study 010 were within the range of Hb concentrations observed for patients with NTDT, and signs of hemolysis at baseline were present.

Six (30%) subjects had a prior medical history of cholecystectomy, and 2 (10%) subjects had a prior medical history of splenectomy.

The mean liver iron concentration for all subjects was 9.44 mg Fe/g dw. Nine (45%) subjects had liver iron concentrations >3 mg Fe/g dw, consistent with hepatic iron overload. Three (15%) subjects received iron chelating agents within the 52 weeks before receiving the first dose of study treatment. Nineteen (95%) subjects had received hydroxyurea before the first dose of study treatment.

Summary of efficacy results

Primary endpoint

The study met the primary endpoint with a statistically significant result for Hb response. All 5 (100%) subjects with α-thalassemia and 11/15 (73.3%) subjects with β-thalassemia had an Hb response.

Secondary efficacy endpoints

- Average Change in Hemoglobin Concentrations

The mean for the secondary endpoint of average change from baseline in Hb concentrations from Week 12 to Week 24 was ~13 g/L; this was consistent with the means for the average change from baseline in Hb concentrations over all other prespecified periods within the 24-week period.

- Sustained Hemoglobin Response

Of the 16 subjects who achieved the primary endpoint of Hb response (≥1.0 g/dL [10 g/L] increase in Hb concentration from baseline between Week 4 and Week 12), the response was sustained for 13 subjects, with Hb concentration increases at 2 or more of the 4 scheduled assessments between the Week 12 and Week 24 visits.

- Delayed Hemoglobin response

In addition to the 16 subjects who achieved the primary endpoint of Hb response (≥ 10 g/L increase in Hb concentration from baseline between Week 4 and Week 12), an additional 2 subjects had a ≥10 g/L increase in Hb concentrations from baseline after Week 12, between the Week 16 and Week 24 visits.

Among the 16 Hb responders, the mean time to the first ≥ 10 g/L increase in Hb concentration was 4.54 weeks (Table 11). Of these 16 subjects, 13 (81.3%) reached this increase while receiving 50 mg BID doses in the first 6 weeks after dosing, whereas 3 first reached this increase after 6 weeks while receiving 100 mg BID doses.

- Markers of Hemolysis and Erythropoietic activity

Markers of hemolysis, which included reticulocyte count, indirect bilirubin, LDH, and haptoglobin, and their changes from baseline were evaluated.

A marked decrease in indirect bilirubin levels was observed by Week 2 (mean decrease from baseline to Week 2 of ~13 µmol/L) and sustained through Week 24 (mean decrease from baseline to Week 24 of ~11 µmol/L).

A marked decrease in LDH concentrations was observed by Week 2 (mean decrease from baseline to Week 2 of ~13 U/L) and sustained through Week 24 (mean decrease from baseline to Week 24 of ~42 U/L).

A marked decrease in erythropoietin concentrations was observed by Week 6 (mean change from baseline to Week 6 of ~619 IU/L) and sustained through Week 24 (mean decrease from baseline to Week 24 of ~830 IU/L).

2.6.6. Discussion on clinical efficacy

Design and conduct of clinical studies

The initially sought indication was the "*Treatment of adult patients with non-transfusion-dependent and transfusion-dependent alpha- or beta-thalassaemia*".

The agreed indication is the following: *'Pyrukynd is indicated in adults for the treatment of anaemia associated with transfusion-dependent and non-transfusion-dependent alpha- or beta-thalassaemia (see section 5.1).'*

Mitapivat was studied in a global program consisting of 3 studies that enrolled adult patients with thalassemia across genotypes and transfusion needs.

- The 2 global pivotal Phase 3 studies were randomized, double-blind, and placebo controlled and aimed to provide the primary evidence of efficacy of mitapivat in adults with thalassemia, reflective of the real-world thalassemia patient population. Study 018 enrolled subjects with TD α- or β-thalassemia and Study 017 enrolled subjects with NTD α- or β-thalassemia.

- The single-arm Phase 2 study (Study 010) aimed to provide supportive evidence of efficacy in patients with NTD α- or β-thalassemia.

The pivotal study AG348-C-018 was designed to assess the effect of mitapivat on transfusion burden, as measured by the proportion of subjects achieving a transfusion reduction response (TRR) in any consecutive 12-week period through Week 48 compared with the baseline in subjects with α- or β-TDT.

The pivotal study AG348-C-017 was designed to assess the effect of mitapivat on Hb concentration, as measured by the proportion of subjects achieving a ≥10 g/L (1.0 g/dL) increase in average Hb concentration from Week 12 through Week 24 compared with baseline in subjects with α- or β-NTDT.

Thalassemia is characterised by chronic hemolytic anemia, caused by toxic and unstable accumulation and aggregation of α-globin or β-globin chains, depending on where the mutation occurs, imposing metabolic stress on the RBCs. This hemolysis is accompanied by anemia of varying degrees depending on the magnitude of the globin mismatch and imbalance classifying patients as transfusion-dependent thalassemia or non-transfusion dependent thalassemia. The recommended treatment for TDT involves regular blood transfusions for life, usually administered every 2-4 weeks, to maintain a pre-transfusion hemoglobin level above 9.5-10.5 g/dl in β-TDT patients and above 8-9 g/dl in α-TDT. NTDT is characterised by a hemoglobin level between 7 and 10 g/dl.

Study AG348-C-018

The pivotal study AG348-C-018 was a Phase 3, Double-Blind, Randomized, Placebo-Controlled, Multicenter Study evaluating the efficacy and safety of mitapivat in adult subjects with Transfusion-Dependent Alpha- or Beta-Thalassemia. Subjects were randomly assigned in a 2:1 ratio to receive mitapivat 100 mg BID, or matching placebo BID for up to 48 weeks.

The design of the study was adequate. Randomisation according to thalassemia genotype and geographic region is endorsed, as they represent recognised covariates in the target population.

The choice of the 100 mg BID dose regimen was based on the results of the phase 2 study AG348-C-010, in which participants started with a 50 mg BID dose, followed by a dose increase to 100 mg BID at week 6 based on tolerability and a hemoglobin increase.

Inclusion and exclusion criteria were in correspondence with the proposed indication and the sought patient population.

Transfusion dependence was defined in this study as receiving 6 to 20 RBC units, with a transfusion-free period lower or equal to 6 weeks during the 24 weeks prior to randomization. At this time, the minimum of 6 RBC was defined based on potential transfusion practices, including a minimum of 1 unit every 4 weeks (or 6 RBC units/24 weeks), which may be observed in countries with limited blood supply. The maximum of 20 RBC units in the 24 weeks prior to randomisation was determined based on the clinical data from Phase 2 studies with luspatercept in β-thalassemia.

The primary endpoint of the study was the reduction in transfusion burden (TRR) defined as ≥50% reduction in transfused red blood cells (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 endpoints assessed the durability of the effect though different time intervals (rolling periods and fixed periods). Depth of transfusion reduction rate TRR2 was defined as a ≥33% reduction in transfused RBC units from Week 13 through Week 48 compared with baseline. TRR3 was defined as ≥50% reduction in transfused RBC units in any consecutive 24-week period through Week 48. TRR4 was defined as ≥50% reduction in transfused RBC units from Week 13 through Week 48 compared with baseline. A 33% reduction in transfusion burden from baseline was previously established as clinically meaningful and accepted as a primary endpoint in transfusion-dependent beta-thalassemia.

Only two major amendments to the protocol were made. The first amendment was submitted on Jul 21, 2021 and brought substantial changes in the study population allowing subjects who had received anabolic steroid and extending QTc to 470 milliseconds for eligibility criteria. The second amendment was submitted on Sep 28, 2022 and brought substantial changes on contraception, removing the requirement for male contraception.

Efficacy data and additional analyses

Overall, 305 subjects were screened. Forty-seven (47) subjects were excluded with 41 screen failure and 6 withdrawals by subject. Screen failures were linked to various unmet inclusion and exclusion criteria, with no single criteria acting as a major barrier to enrolment indicating a broad and relatively even distribution across multiple eligibility parameters.

The study enrolled a total of 258 subjects who were randomized in a 2:1 ratio to the mitapivat treatment group (N = 171) or placebo treatment group (N = 87). Most subjects (90.6% in the mitapivat treatment group and 95.4% in the placebo treatment group) completed the planned 48 weeks of treatment.

Patients enrolled were overall representative of patients with TD α- and β-thalassemia. However, subjects with TD-α-thalassemia were primarily captured under the non-β0/β0 TD population and accounted only for about 5% (12/258).

Baseline characteristics of included patients in study AG348-C-018 showed no major asymmetry in characteristics across treatment arms. It should be noted that most subjects enrolled were white and black or African Americans were underrepresented. Nevertheless, population pharmacokinetic modeling supports that race does not affect mitapivat exposure.

Major protocol deviations were reported in 51 subjects (19.8%). In the mitapivat arm, 18 subjects did not meet the protocol-specified eligibility criteria of transfusion dependent (inclusion criterion 3). This included 15 subjects who received >20 RBC units (11 in the mitapivat arm and 4 in the placebo arm) and 3 subjects had >6-week transfusion-free period in the 24 weeks before randomization (all in the mitapivat arm). These participants had comparable baseline characteristic to the overall population enrolled and were distinguished only by the splenectomy status (16.7% had undergo splenectomy compared to 57.5% in subjects who met

IC3). Specific efficacy analysis excluding the subjects who did not meet IC3 did not reveal inconsistent results with those for all subjects randomised. This deviation did not have a major impact on the efficacy data.

In study AG348-C-018, the benefit of mitapivat was demonstrated versus placebo on the reduction of transfusion burden, with a higher rate of subjects reaching at least a 50% reduction in transfusion volume with a reduction of ≥2 units of transfused RBCs in any consecutive 12-week period through Week 48. Among mitapivat-treated subjects, 30.4% were responders versus 12.6% in the placebo group, resulting in an adjusted difference of 17.6% (95% CI: 8.0 to 27.2; 2-sided p value 0.0003). These results are encouraging despite the small treatment's effect.

Durability of transfusion reduction response assessed by the 3 key secondary endpoints was also superior in the mitapivat arm. The proportion of patients with ≥50% Reduction in Transfused RBC Units in any consecutive 24-week period compared with baseline was significantly higher in the mitapivat arm, with a rate of 13.5% versus 2.30% in the placebo arm, leading to an adjusted difference between arms of 11.1% (95% CI 5.1,17.0) p-value 0.0003).

The proportion of subjects with ≥33% Reduction in Transfused RBC Units from Week 13 through Week 48 Compared with Baseline was significantly higher in the mitapivat treatment arm, with a rate of 14.6% versus 1.1% in the placebo group, leading to an adjusted difference between arms of 13.4% (95% CI 7.7,19.1) p-value <0.0001).

The proportion of subjects with ≥50% Reduction in Transfused RBC Units from Week 13 through Week 48 Compared with Baseline was significantly higher in the mitapivat treatment arm, with a rate of 7.60% in the mitapivat arm versus 1.10% in the placebo arm, with an adjusted difference between arms of 6.4% (95% CI 1.9,10.9) p-value 0.0056).

Specific efficacy analyses of the primary TRR endpoint and key secondary endpoints TRR2, TRR3 and TRR4 were provided on the 12 α-thalassemia subject and showed that the difference in TRR remained statistically significant compared to placebo, however the rate of responders was lower compared to the overall population (77.8% versus 30.4%, respectively). The very small sample of α-thalassemia subjects and wide CIs however challenge the reliability of the results. Moreover, the difference compared to placebo for key secondary endpoints TRR3 and TRR4 was not statistically significant (adjusted difference between arms of 66.7% (95% CI -10.8, 92.5) for both).

Heterogeneity test were performed to assess the treatment-by- α- or β-thalassemia genotype interaction. Large p-values considering the primary and key secondary endpoint TRR, TRR2, TRR3 and TRR4 (0.1021, 0.2090, 0.4947 and 0.2929 respectively) suggest non-significant evidence of heterogeneity supporting the interpretation that the true treatment effect of mitapivat on transfusion burden reduction endpoints is consistent for patients with α- and β-TDT.

It should still be emphasized that α-thalassemia patients have not been included into clinical studies so far. Although the globin imbalance differs, α-and β-thalassemia share the same pathophysiology with similar consequences (i.e hemolytic anemia). A mechanistic rationale supports the assumption that efficacy could be considered similar between α-and β genotypes, also given the consistency of the efficacy data on TRR and TRR2.

Overall, despite the statistically significant difference between the placebo and mitapivat arms for key secondary endpoints, the responder rates remain low, particularly over longer assessment periods. The impact is however considered substantial for responders. Further subsequent investigations would be valuable to better identify the population that could best benefit from the treatment and establish clinical and/or biological

characteristics that could predict optimal response with mitapivat. In the meantime, as per the SmPC, treatment with mitapivat should be discontinued in non-responders as stated in section 4.2 of the SmPC.

An overall 10.96% reduction of RBC units from Week 13 through Week 48 was observed in the mitapivat arm, leading to a difference of 7.63% [95% CI (1.06, 14.20)] with the placebo arm. With continued exposure to mitapivat in the OLE Period, the mean percent reduction from baseline in RBC transfusion burden was 13.86%, increasing from 10.96% observed during the double-blind period which translates in a reduction of approximately 4 transfusion visits per year.

Transfusion independence (TI), defined as transfusion-free for at least 8 consecutive weeks through Week 48 in the double-blind Period, was reported in 17 subjects (9.9%) in the mitapivat arm, versus 1 (1.1%) in the placebo arm, leading to a difference of 8.8% in favor of mitapivat treatment [95% CI (3.8, 13.8)]. At the end of the double-blind Period, 5 subjects had their transfusion-free period ongoing, 4 of whom had remained transfusion free for >40 weeks. The remaining subject had a more delayed response and had been transfusion-free since week 40.

Updated analysis incorporating data from the OLE Period shows a mean duration of TI increased from 21.69 weeks to 30.49 weeks with duration of TI up to 84.3 weeks. The 3 patients who were transfusion-free from the beginning (week 0) remained transfusion free through the OLE Period. The patient that became transfusion independent at week 8 achieved a transfusion-free period of 84.3 weeks and the remaining patient that became transfusion independent late at week 40 achieved a longer transfusion-free period of 20.9 weeks.

In the mitapivat arm, the translated benefit of transfusion reduction into lower iron indices was mainly observed with serum iron levels with a reduction from baseline of -4.76 umol/L at week 24 (95% CI (-6.59, -2.94)] and -4.57 umol/L at week 48 [95% IC (-6.44, -2.69)], leading to a statistical difference with placebo of -3.43 [95% CI (-6.22,-0.64)] at week 24 and -6.63 [95% CI (-9.53,-3.72)] at week 48 and TIBC. However, these markers can be influenced by other events such as inflammation, infection, autoimmune diseases or reactions, non-iron associated liver diseases and so on. Notably, no statistical difference from baseline in ferritin levels, could be evidenced, neither at week 24 (LS mean -104.6 ug/L, [95% CI (-248.5, 39.4), nor at week 48 (LS mean 45.3 [95% CI (-129.9, 220.5)]. Similarly, no notable effect of mitapivat on transferrin saturation could be demonstrated (LS mean 0.108 [95%CI (0.049, 0.166)] at week 24 and 0.079 [95% CI (0.027, 0.130)] at week 48).

Exploratory analyses on changes in hepatic iron concentration did not objectivise a reduction in LIC. At Week 48, mean changes from baseline in hepatic concentration were 0.576 mg/g (2.9720) in the mitapivat arm and −0.662 mg/g (2.0469) in the placebo arm (Baseline 5.731 mg/g in the mitapivat arm and 5.712 mg/g in the placebo arm). Data from the OLE period indicated that liver iron concentrations remained stable with continued mitapivat treatment. However, it is acknowledged that potential benefits on iron parameters may be less discernible in the TDT population, in whom transfusions continue, albeit with a reduced burden, potentially masking treatment-related improvements in iron metabolism.

Four quality of life (QoL) questionnaires were used in the protocol: the Transfusion-Dependent Quality of Life Questionnaire, PGIS-Thalassemia Symptoms, PGIC-Thalassemia Symptoms, the EuroQoL Group 5-Level EQ-5D-5L, and the PROMIS Physical Function 8-Item Short Form. These assessments were exploratory in nature, and no statistical testing was performed. Across all instruments, the differences observed between the treatment and placebo groups appeared minimal. Transfusion-Dependent Quality of Life Questionnaires (TranQoL) were improved in the same way in the mitapivat arm as placebo by an about 3 points in both treatment arms (3.14 mitapivat and 2.98 placebo) with mean baselines scores of 63.55 in the mitapivat arm and 63.41 in the placebo arm. The same trend is observed for EQ-VAS questionnaire (Week 48 mean change from baseline for the EQ-VAS score was 3.0 in the mitapivat arm and 2.0 in the placebo arm) and Promis

Physical Function T-scores (the Week 48 mean (SD) change from baseline for the PROMIS Physical Function T-Score was 0.10 (5.948) in the mitapivat arm and −1.10 (6.394) in the placebo arm). Notably, only the PGIC-Thalassemia Symptoms questionnaire, which captures patients' perceived change in symptoms compared to baseline, showed a more favorable trend. A higher proportion of participants in the treatment arm reported feeling "much better" compared to those in the placebo arm (13.5% vs 4.6%) at Week 48 compared with baseline. While this finding may suggest a perceived benefit, the overall impact on quality of life remains unclear in the absence of statistical confirmation and consistent trends across all measures.

Subgroup analyses raised some initial concerns, as several 95% CI crossed the null axis, indicating a lack of statistical significance within individual subgroups (β0/β0 genotype, Asian Race, Asia-Pacific region, Rest of the world region and transfusion burden of ≤12 RBC). However, considering the greater susceptibility to bias in subgroup analyses, the overall trend consistently favored mitapivat treatment. Indeed, the Breslow test did not detect significant heterogeneity between subgroups, supporting consistency of the treatment effect. Nevertheless, these findings should be interpreted with caution as not fully robust. However, these data reinforce the previous remark on the need to better identify and target the patient population most likely to benefit from mitapivat to improve the therapeutic impact.

Overall, the data primarily support an improvement in anaemia, as evidenced by the reduction in transfusion burden observed in both the primary and key secondary endpoints, although the magnitude of this effect is limited and appears heterogeneous with a potential poorer response for the β0/β0 genotype subgroup along with other sub-groups populations. Even so, these data are encouraging considering the very few therapeutic options for TDT subjects and the substantial impact for responders.

Study AG348-C-017

The pivotal study AG348-C-017 is a Phase 3, Double-Blind, Randomized, Placebo-Controlled, Multicenter Study evaluating the efficacy and safety of mitapivat in adult subjects with non-Transfusion-Dependent Alpha- or Beta-Thalassemia. Patients were randomly assigned in a 2:1 ratio to receive mitapivat 100 mg BID, or matching placebo BID for up to 24 weeks. NTDT encompasses three clinically distinct forms: β-thalassaemia intermedia, haemoglobin E/β-thalassaemia (mild and moderate forms), and α-thalassaemia intermedia (haemoglobin H disease).

The primary endpoint of the study was Hb response defined as a ≥1.0-g/dL increase in average Hb concentration from Week 12 through Week 24 compared with baseline. The use of surrogate endpoints is acceptable if they reliably predict a positive effect on clinical outcomes such as mortality or morbidity. The MAH relies on recent evidence linking hemoglobin levels ≥1 g/dL with improved survival in NTDT patients. Literature data from observational studies also support a decrease in development of multiple morbidities for each 1 g/dL Hb increase, with greater benefits seen in patients with lower Hb levels. Key secondary endpoints are generally endorsed and included the change in average FACIT-fatigue subscale scores, according to a pre-defined meaningful within-person change threshold, and change from baseline in average Hb concentration. The severity bands for FACIT-Fatigue score were derived as minimal (>46 to 52), mild (>37 to 46), moderate (>20 to 37), and severe (0 to 20).

Two major amendments to the protocol were reported. The first amendment was submitted on July 21, 2021 and brought substantial changes in the study population allowing subjects who had received anabolic steroid and extending QTc to 470 milliseconds for eligibility criteria. The second amendment was submitted on September 28, 2022 and brought substantial changes on contraception, removing the requirement for male contraception.

Efficacy data and additional analyses

A total of 235 subjects were screened with 33 screen failure recorded, linked to various unmet inclusion and exclusion criteria, without any one in particular being more concerning.

This study enrolled a total of 194 subjects who were randomized in a 2:1 ratio to the mitapivat treatment group (N = 130) or placebo treatment group (N = 64). Most subjects (93.8% in the mitapivat treatment group and 96.9% in the placebo treatment group) completed the planned 24 weeks of treatment.

Patients included were overall representative of patients with NTD α- and β-thalassemia. The targeted population had to be anemic but not transfusion-dependent and this was defined as administration of ≤5 RBC units during the 24-week period before randomization and no RBC transfusions ≤8 weeks before providing informed consent and during the Screening Period. Some patients required occasional or intermittent transfusions but the majority (86.6%) had no RBC unit transfused in the 24-week period prior to randomization.

Overall, around 32% (62/194) of subjects enrolled in Study 017 had α-thalassemia and 73.2% had baseline Hb ≤ 9.0 g/dL.

Baseline characteristics of enrolled patients reflect the population without major asymmetry in characteristics across treatment arms, except that a slight imbalance is observed between mitapivat and placebo arm for patients under 35 years-old with 28.5% in the mitapivat arm versus 42.2% in the placebo arm. There were also a higher proportion of women than men enrolled (63.4% vs 36.6%), rather attributed to behavioral trends than to an epidemiological rationale as prevalence of thalassemia is generally similar across genders. It should also be noted that most subjects enrolled were white and black or African Americans were underrepresented, explained by the greater rarity of the disease in populations of African descent.

The most frequently reported concomitant medication in both treatment arms was Folic acid (>75% subjects), followed by Colecalciferol, Paracetamol, and Acetylsalicylic acid, Deferasirox, and Deferiprone (>10% subjects). Concomitant iron chelators were reported in 49 (38.0%) subjects in the mitapivat arm and in 24 (38.1%) subjects in the placebo arm.

Regarding the primary endpoint, a ≥1.0 g/dL increase in average Hb concentration from Week 12 through Week 24 was observed in 42.3% participant (55/130) in the mitapivat arm versus 1.6% (1/64) in the placebo arm demonstrating a statistically significant difference of 40.9% with the placebo arm [95% CI (32.0, 49.8); 2 sided p-value <0.0001].

Transfusions were permitted during the study, as clinically indicated. In the analysis of the primary endpoint, Hb concentration assessed within 8 weeks after an RBC transfusion (relative to the mean half-life of RBC quoted as 50-60 days) were considered as missing and excluded for evaluating Hb response, and subjects who did not have at least 2 on-treatment Hb concentration assessments from Week 12 to Week 24 were considered non-responders. The exclusion of these values did not had major impact on efficacy results.

The Hb response was sustained until week 24 for all responders with the exception of 9 patients who experienced loss of response, mostly related to transient drops in Hb, rapidly offset by a further increase in Hb to reach again a >1 g/dL increase compared to baseline.

Among responders in the mitapivat arm, the average increase from baseline in Hb concentration from Week 12 through Week 24 was 1.56 g/dl. There was 1 Hb responder in the placebo arm with a baseline Hb of 7.3 g/dL with an Hb increase of 1.2 g/dl from Week 12 through Week 24.

Data from the OLE period provides an additional minimum follow-up of 34.4 weeks (241 days) beyond the initial 24-week double-blind phase, allowing assessment of the durability of response.

The MAH provided graphical representation showing the mean change from baseline in Haemoglobin in FACIT-Fatigue Subscale Score, Indirect Bilirubin, Lactate Dehydrogenase, Haptoglobin and Hepatic Iron concentration over time, including the available data from the Double-blind and OLE Period. The data, as presented graphically, indicates a sustained increase in haemoglobin compared to baseline, with continued mitapivat treatment during the OLE Period.

The impact of the Hb change on the clinical symptoms of anemia was supported by participant self-reported fatigue outcomes (FACIT-Fatigue questionnaire). A meaningful within-person change threshold for FACIT-Fatigue was however observed with over 4.5-point improvement from baseline in average score from Week 12 through 24 in the protocol. According to this criterion, a significant improvement was observed from Week 12 through Week 24 in the mitapivat arm with a LS mean change of 4.85 points (95% CI (3.41, 6.30)]. A higher proportion of patients in the mitapivat arm compared to the placebo arm (36.2 % versus 21.9%) experienced a meaningful within-person change threshold, with an adjusted difference in response rate statistically significant [14.2% (95% CI: 1.1, 27.2)]. The impact of mitapivat treatment on patient's quality of life is however still only assessed in terms of fatigue, which has its limitations and it would have been more informative to assess specific NTDT-PRO. Updated analysis incorporating data from the OLE Period show sustained treatment effect of mitapivat in increasing FACIT-fatigue In addition to the statistically significant improvement observed in the FACIT-Fatigue questionnaire, several other QoL instruments were assessed exploratorily to evaluate broader patient-reported outcomes. These included the PROMIS® SF-8 Physical Function, FACT-G, PGIS/PGIC-Thalassemia Symptoms, PGIS/PGIC-Walking Capacity, and the EQ-5D-5L. Although no formal statistical testing was conducted for these exploratory measures, numerical trends favored the treatment arm. For example, the FACT-G total score increased by 3.25 (11.343) versus 0.84 (13.190), and EQ-5D-5L VAS scores rose by 4.2 (15.78) in the treatment arm compared to 1.6 (14.70) in placebo. Patient-perceived symptom improvement was also more frequent in the treatment group: 28.5% of subjects reported feeling "much better" for thalassemia symptoms (vs. 12.5% in placebo) and 24.6% reported "much better" walking capacity (vs. 12.5%). While exploratory and not statistically conclusive, these findings support the direction of benefit observed in the FACIT-Fatigue results, suggesting a consistent trend toward improved patient experience with treatment.

The proportion of patients who achieved an Hb 1.5+ response was significantly higher in the mitapivat arm with 24.6% responders (32/130) versus 0% (0/65) in the placebo arm. The adjusted difference was 24.7% (95% CI: 17.3, 32.1).

Moreover, a greater decrease in haemolysis markers was observed in the mitapivat arm compared to placebo.

Considering iron metabolism, the benefit of mitapivat on iron overload was not demonstrated, with small and similar reduction in ferritin concentrations observed in both treatment arms leading to no significant difference (LS mean (SE) difference was −2.26 (49.529) (95% CI: −100.04, 95.52)).

Baseline mean (SD) transferrin saturation (fraction of 1) was 0.519 (0.2101) in the mitapivat arm and 0.518 (0.2164) in the placebo arm. LS mean (SE) change from baseline to Week 24 was −0.029 (0.0212) in the mitapivat arm and −0.047 (0.0262) in the placebo arm; the LS mean (SE) difference was 0.017 (0.0322) (95% CI: −0.047, 0.081). However, subjects were not heavily iron loaded at baseline and the limited evaluation period may compromise the discernment of changes in markers of iron metabolism. However, the updated analysis incorporating data from the OLE period, which includes the assessment of hepatic iron concentration by MRI at Week 48, shows an apparent decrease from baseline, with a reduction approaching, however not reaching, 1 mg/g. However, the exact numerical values are not provided, and the data are presented only graphically, with two time points, baseline and Week 48. Considering the baseline mean liver concentration of

5.357 mg/g in the mitapivat arm, which represents a moderate iron overload, a reduction approaching 1 mg/g may be regarded as clinically meaningful, particularly when it reflects a sustained downward trend.

As a result, the trend suggests a potential treatment effect on liver iron levels, as Liver iron concentration (LIC) measured by MRI is endorsed by international guidelines as a gold-standard, non-invasive biomarker for assessing iron overload.

As a reminder, the number of patients receiving iron chelators during the study was balanced between the two arms (38% and 38.1% in the mitapivat and placebo arm, respectively).

From the sub-group analyses, it emerges that the effect of mitapivat on haemoglobin response was particularly more pronounced in patients with baseline Hb concentration ≤ 9.0 g/dL, β-thalassemia genotype and male patients however taking necessary interpretation precautions due to the known biases of subgroup analyses.

Regarding subgroup analyses with respect to FACIT-fatigue subscale score, the LS means difference was not significant among the ≥ 35 years age group (LS mean difference 2.84 CI -0.12;5.81), male (LS mean difference 3.12 CI -0.16;6.40), white (LS mean difference 2.47 CI -0.44;5.38) and RoW (LS mean difference 2.45 CI -2.46;7.36) subgroups. A Breslow-Day test was performed to assess the treatment-by-subgroup interaction and did not detect significant heterogeneity between subgroups, supporting consistency of the treatment effect. Nevertheless, this finding should be interpreted with caution as not fully robust.

In summary, available data from study 017 supports a rather moderate (less than 50% responders), but robust effect of mitapivat on hemoglobin improvement versus placebo, and more markedly in patients with baseline Hb concentration ≤ 9.0 g/dL and β-thalassemia genotype. The increase in haemoglobin translated to significant improvement in fatigue alongside improvement in haemolysis markers and LIC, Long-term data provided during the OLE period further strengthen these results, supporting the durable effect of mitapivat in improving anaemia.

Results from a phase 2 supportive study, AG348-C-010, with 20 NTDT patients enrolled, were included in the dossier. This study consists of a Core Period (up to 24 weeks) followed by an Extension Period (up to 10 years). Mitapivat was administered orally at 50 mg BID for the first 6 weeks of the core period followed by an escalation to 100 mg BID for 18 weeks thereafter. The main objective of the study was to assess a haemoglobin response defined as a ≥1.0 g/dL increase in Hb concentration in adults NTD α-thalassemia (5 subjects) and β-thalassemia (15 subjects). Results are consistent with those observed in pivotal study 017, with a rapid hb responses within the first few weeks of mitapivat treatment. However, the sequential dose escalation reveals that most patients had an increase in haemoglobin of 1·0 g/dL at a dose of 50 mg BID. Dose increase to 100 mg BID was necessary in only 3 patients within the timeframe set to achieve the objective, which calls into question the initial dose of 100 mg BID applied in the two pivotal studies 017 and 018, however this uncertainty remains on the margins given the sequential dose escalation design of the study not allowing for clear attribution of efficacy observations to one or other of the dosages.

Overall, considering outcomes of study AG348-C-017 in adults with NTDT and study AG348-C-018 in adults with TDT, mitapivat can lead to a durable increase in haemoglobin levels and reduce transfusion burden in patients suffering from thalassemia.

Subjects with α-thalassemia were primarily captured under the non-β0/β0 population and were quite underrepresented, particularly TD- α-thalassemia. Although the globin imbalance differs, α-and β-thalassemia share the same pathophysiology. Therefore, considering mitapivat MoA, there is a mechanistic rationale to support the assumption that efficacy should not differ a priori between α- and β-genotypes. This supports the generalisability of the results from the ITT population to α- thalassemia genotype, especially given the consistency of the efficacy data. Given the limited treatment options, particularly for α-thalassaemia, these

findings are meaningful and support a positive benefit/risk for the new indication, irrespective of genotype and transfusion requirement.

Considering that the data in all 3 studies primarily supports a significant improvement in anaemia, the following indication wording was agreed by the CHMP to adequately reflect the treatment's objective, and has been agreed by the MAH: "Pyrukynd is indicated in adults for the treatment of anaemia associated with transfusion-dependent and non-transfusion-dependent alpha or beta-thalassaemia"

2.6.7. Conclusions on the clinical efficacy

The MAH initially applied for the following extension of indication for Mitapivat: "*Treatment of adult patients with non-transfusion-dependent and transfusion-dependent alpha- or beta-thalassaemia*".

Considering that the data primarily support a significant improvement in anaemia, the following indication wording is now proposed to adequately reflect the treatment's objective, and has been agreed by the MAH:

"*Pyrukynd is indicated in adults for the treatment of anaemia associated with transfusion-dependent and non-transfusion-dependent alpha or beta-thalassaemia*"

Efficacy data were mainly based on two pivotal studies, AG348-C-018 (TDT) and AG348-C-017(NTDT), each covering a different part of the sought indication.

The pivotal trial AG348-C-018 demonstrated significant reduction in transfusion burden with mitapivat compared to placebo in TD- α and β-thalassemia, however with a modest responder rate, particularly over longer assessment periods. In addition, data regarding iron overload parameters are insufficiently robust to reflect the expected effect on iron burden. Similarly, and in line with these expectations, no improvement in quality of life was observed, noting that this was an exploratory endpoint within the study.

The pivotal trial AG348-C-017 demonstrated significant and sustained improvement on Hb response with mitapivat compared to placebo resulting in a significant improvement in average FACIT-Fatigue subscale and in haemolysis markers in subjects with NTD- α and β-thalassemia Although blood biomarkers did not demonstrate a benefit on iron overload, hepatic iron concentration assessed by MRI at Week 48 showed an apparent decrease from baseline.

Furthermore, mitapivat is the first therapeutic agent to be studied for the treatment of α-thalassemia major or intermedia. Although α-thalassemia subjects were underrepresented, particularly in study 018, the shared pathophysiology, and the mechanistic rationale relative to mitapivat MoA, the generalisability of the results from the ITT population to α- thalassemia genotype is considered acceptable, especially given the consistency of the efficacy data. Given the limited treatment options, particularly for α-thalassaemia, these findings are meaningful and support a positive benefit/risk for the new indication, irrespective of genotype and transfusion requirement.

2.6.8. Clinical safety

The safety of mitapivat in the thalassemia indication is primarily supported by the 2 pivotal phase 3 clinical studies (study 017 and 018), and a phase 2 study (010) conducted in patients with thalassemia. In addition, supportive safety data from other indications, pyruvate kinase deficiency and sickle-cell disease (SCD), is presented.

The safety data for this extension of indication includes safety analyses from 2 pooled safety sets:

- The Comparative Thalassemia Safety Pool, which includes safety data from subjects with thalassemia who were administered mitapivat or matched placebo in the Double-blind Periods of either the pivotal Study 017 or the pivotal Study 018.
- The All Indications Safety Pool, which includes safety data from subjects treated in Studies 017, 018, 010, 003, 006, 007, 011, and the Phase 2 portion of Study 020.

The data from Study 010 are not included in the Comparative Thalassemia Safety Pool due to differences in study design (Study 010 was not randomized and Studies 017 and 018 were randomized) and in dosing schema (in Study 010 subjects received mitapivat 50 mg BID and at the end of Week 6, subjects could undergo an intrasubject dose escalation to mitapivat 100 mg BID; in Studies 017 and 018, all subjects received 100 mg BID of mitapivat or matched placebo). The data from Study 010 are included in the All Indications Safety Pool.

In the pivotal studies, adverse events and SAEs that occurred from the time of providing informed consent until 28 days after the administration of the last dose of study drug were recorded. Safety assessments were performed every 4 weeks during the 24-week double-blind Period in Study 017, every 4 weeks during the 48-week Double-blind Period in Study 018, and every 2-4 weeks during the 24-week double-blind Period in study 010. In study 010, long-term safety continues to be assessed in the ongoing 10-year Extension Period, with visits approximately every 12 to 24 weeks.

2.6.8.1. Patient exposure

The safety data for this extension of indication includes safety analyses from 2 pooled safety sets:

- The Comparative Thalassemia Safety Pool, which includes safety data from subjects with thalassemia who were administered mitapivat or matched placebo in the Double-blind Periods of either the pivotal Study 017 or the pivotal Study 018.
- The All Indications Safety Pool, which includes safety data from subjects treated in Studies 017, 018, 010, 003, 006, 007, 011, and the Phase 2 portion of Study 020.

Comparative thalassemia safety pool

The thalassemia studies included 301 patients who received mitapivat 100mg BID and 148 patients who received placebo in phase 3; For each pivotal study, the duration of exposure to study treatment was similar for both treatment arms (Table 73). The median duration of exposure to mitapivat was consistent with the duration of the Double-blind Period in each study: 24 weeks (study 017) and 48 weeks (study 018). In the supportive phase 2 study 20 subjects received 50mg BID for 6 weeks followed by 100mg BID; the total duration of exposure to mitapivat was 207 weeks.

The data from Study 010 are not included in the Comparative Thalassemia Safety Pool due to differences in study design (Study 010 was not randomized and Studies 017 and 018 were randomized) and in dosing schema

(in Study 010 subjects received mitapivat 50 mg BID and at the end of Week 6, subjects could undergo an intrasubject dose escalation to mitapivat 100 mg BID; in Studies 017 and 018, all subjects received 100 mg BID of mitapivat or matched placebo). The data from Study 010 are included in the All Indications Safety Pool.

Table 73: Summary of Exposure to Study Drug – Comparative Thalassemia Safety Pool (Safety Analysis Set)

	Study 017		Study 018		Pooled	
	Mitapivat N=129	Placebo N=63	Mitapivat N=172	Placebo N=85	Mitapivat N=301	Placebo N=148
Duration of exposure (weeks)						
n	129	63	172	85	301	148
Mean	23.60	24.15	45.81	47.43	36.29	37.52
SD	3.526	1.151	9.288	4.543	13.253	12.070
Median	24.14	24.14	48.14	48.00	46.86	46.93
Q1	24.14	24.00	47.29	47.14	24.14	24.14
Q3	24.29	24.43	48.86	48.71	48.29	48.14
Min	1.1	17.6	0.3	14.3	0.3	14.3
Max	28.1	28.0	59.9	53.1	59.9	53.1
Duration of exposure category, n (%)						
>0 - ≤4 weeks	2 (1.6)	0	3 (1.7)	0	5 (1.7)	0
>4 - ≤8 weeks	1 (0.8)	0	1 (0.6)	0	2 (0.7)	0
>8 - ≤12 weeks	1 (0.8)	0	0	0	1 (0.3)	0
>12 - ≤16 weeks	1 (0.8)	0	3 (1.7)	1 (1.2)	4 (1.3)	1 (0.7)
>16 - ≤20 weeks	0	1 (1.6)	2 (1.2)	0	2 (0.7)	1 (0.7)
>20 - ≤24 weeks	27 (20.9)	16 (25.4)	0	0	27 (9.0)	16 (10.8)
>24 - ≤36 weeks	97 (75.2)	46 (73.0)	4 (2.3)	2 (2.4)	101 (33.6)	48 (32.4)
>36 - ≤48 weeks	0	0	55 (32.0)	41 (48.2)	55 (18.3)	41 (27.7)
>48 weeks	0	0	104 (60.5)	41 (48.2)	104 (34.6)	41 (27.7)
Person exposure-years	58.35	29.16	151.01	77.26	209.36	106.42

Source: AG348 Thal ISS/RMP Table 18.3-1.1.1 (Agios internal use only).
The denominator used to calculate percentages is N, the number of subjects in the safety analysis set within each treatment group. Duration of exposure (weeks)=(date of last dose - date of first dose +1)/7.
Person exposure-years = sum of duration of exposure for each patient in days within each treatment group/365.25.

The demographics of patients enrolled in the pivotal studies were consistent with the inclusion criteria and reflective of the real-world thalassemia adult patient population with haemolytic anaemia.

Subjects ranged in age from 18 to 68 years of age, and as expected in this patient population, most subjects (97.7%) were less than 65 years of age, most (91.7%) non-Hispanic, 57.5% were white and 35.5% Asian.

Pivotal Study 018 enrolled subjects with TD α- or β-thalassemia and pivotal Study 017 enrolled subjects with NTD α- or β-thalassemia, as receiving transfusions has a confounding impact on the hematologic parameters evaluated as primary endpoints in each of the studies. The baseline characteristics across the pivotal studies were as expected and representative of the entire thalassemia population, irrespective of genotype or transfusion requirements, and representative of the real-world patient population with thalassemia.

All subjects had haemolytic anaemia with low Hb and/or varying baseline transfusion requirements and expected baseline sequela such as prior splenectomy, cholecystectomy, and low BMD. The presence of haemolytic anaemia, presence of iron overload, and coadministration of chelation therapy of subjects enrolled in the pivotal studies were typical of the global thalassemia population.

Other indications safety pool

Safety data from the following completed or ongoing studies of mitapivat in other diseases contribute to the pooled analyses of safety.

Studies in adult subjects with pyruvate kinase deficiency:

- Study AG348-C-003 (Study 003), a Phase 2, first-in-patient, open-label, 2-arm, multicenter, randomized, dose-ranging study in adult subjects with pyruvate kinase deficiency; the study includes a Core Period (24 weeks) and an Extension Period (90 months). The study included 52 patients, not regularly receiving transfusions, and treated with 50 mg BID (n=18) or 300 mg BID (n=34).

- Study AG348-C-006 (Study 006, ACTIVATE), a Phase 3, randomized, multicenter, double-blind, placebo-controlled study consisting of a Dose Optimization Period (Part 1) followed by a Fixed Dose Period (Part 2). This study evaluated the efficacy and safety of orally administered mitapivat as compared with placebo in subjects with pyruvate kinase deficiency who were not regularly receiving blood transfusions. Treatment duration was 26 weeks. The study included 80 patients (40 in mitapivat arm), not regularly receiving transfusions, and treated with doses ranging from 5 to 50 mg BID.

- Study AG348-C-007 (Study 007, ACTIVATE-T), a 2-part, multicenter, open-label, Phase 3 study consisting of a Dose Optimization Period (Part 1) followed by a Fixed-Dose Period (Part 2). The study evaluated the efficacy and safety of treatment with mitapivat in adult subjects with pyruvate kinase deficiency who were regularly receiving blood transfusions. Treatment duration was 42 weeks. The study included 27 subjects, regularly receiving transfusions, treated with doses ranging 5 to 50 mg BID.

- Study AG348-C-011 (Study 011), a multicenter, open-label, extension study to evaluate the long-term safety, tolerability, and efficacy of treatment with mitapivat in subjects who were previously enrolled in Studies 006 or 007. Treatment duration is 194 weeks. The study included 88 subjects, of which 71 (cohort 1 and 2) did not regularly receive transfusions and 17 (from study 007) regularly receiving transfusions.

Study in subjects with SCD:

- Phase 2 portion of **Study AG348-C-020 (Study 020),** a Phase 2/3, double-blind, randomized, placebo-controlled, multicenter study to evaluate the efficacy and safety of mitapivat in subjects with SCD. The Phase 2 Double-blind Period was 12 weeks and the Phase 2 OLE Period is up to 216 weeks.

In the other indications pooled safety analysis, 209 patients received mitapivat at different dose levels and 66 received placebo.

Table 74 Summary of Exposure to Study Drug – All indications Safety Pool (Safety Analysis Set)

	PK Deficiency		Sickle Cell Disease		Thalassemia		Pooled	
	Mitapivat N=157	Placebo N=39	Mitapivat N=52	Placebo N=27	Mitapivat N=321	Placebo N=148	Mitapivat N=530	Placebo N=214
Duration of exposure (weeks)								
n	157	39	52	27	321	148	530	214
Mean	141.18	24.36	11.95	12.45	43.14	37.52	69.12	31.96
SD	112.781	0.723	1.217	1.353	33.225	12.070	81.798	13.460
Median	139.57	24.14	12.07	12.14	47.00	46.93	47.14	24.43
Q1	38.00	24.14	11.86	12.00	24.14	24.14	24.14	24.00
Q3	191.43	24.29	12.29	12.43	48.43	48.14	49.86	47.71
Min	8.1	23.6	3.9	11.7	0.3	14.3	0.3	11.7
Max	430.4	27.4	14.1	19.0	207.4	53.1	430.4	53.1
Duration of exposure category, n (%)								
>0 - ≤12 weeks	1 (0.6)	0	26 (50.0)	12 (44.4)	9 (2.8)	0	36 (6.8)	12 (5.6)
>12 - ≤24 weeks	15 (9.6)	9 (23.1)	26 (50.0)	15 (55.6)	33 (10.3)	18 (12.2)	74 (14.0)	42 (19.6)
>24 - ≤48 weeks	30 (19.1)	30 (76.9)	0	0	158 (49.2)	89 (60.1)	188 (35.5)	119 (55.6)
>48 weeks - ≤2 years	23 (14.6)	0	0	0	105 (32.7)	41 (27.7)	128 (24.2)	41 (19.2)
>2 years - ≤3 years	20 (12.7)	0	0	0	2 (0.6)	0	22 (4.2)	0
>3 years - ≤5 years	50 (31.8)	0	0	0	14 (4.4)	0	64 (12.1)	0
>5 years	18 (11.5)	0	0	0	0	0	18 (3.4)	0
Person exposure-years	424.80	18.20	11.91	6.44	265.38	106.42	702.09	131.06

The denominator used to calculate percentages is N, the number of subjects in the safety analysis set within each treatment group.
Duration of exposure (weeks)=(date of last dose - date of first dose +1)/7.
Duration of exposure (years)=(date of last dose - date of first dose +1)/365.25.
Person exposure-years = sum of duration of exposure for each patient in days within each treatment group/365.25.
AG348 Thal ISS/RMP Data Cutoff: Study 006 (Final), Study 007 (Final), Study 003 (2023-11-10), Study 010 (2023-03-10), Study 011 (2023-03-09), Study 017 (Primary CSR), Study 018 (Primary CSR), Study 020 Phase 2 (Primary CSR)

Overall, across all indications, 530 patients were treated with mitapivat (across all dose levels) and 214 with placebo.

2.6.8.2. Adverse events

2.6.8.2.1. Comparative thalassemia safety pool

Across the pivotal studies, a similar frequency of subjects with TEAEs is reported for both treatment arms. In the mitapivat treatment arm, most subjects experienced TEAEs that were non-serious, low grade (Grade 1 or 2), and considered not treatment related by the Investigator.

The frequency of subjects with serious TEAEs, serious treatment-related TEAEs, or TEAEs leading to interruption of study drug was similar across the treatment arms. There were no serious treatment-related TEAEs in Study 017. A higher frequency of subjects who received mitapivat experienced treatment-related TEAEs, Grade ≥3

TEAEs, Grade ≥3 treatment-related TEAEs, and TEAEs leading to dose reduction compared with subjects who received placebo. TEAEs that led to discontinuation of study drug were infrequent in both treatment arms. There were no TEAEs that led to death.

Table 75: Overall summary of treatment-Emergent Adverse Events- Comparative Thalassemia Safety Pool (Safety Analysis Set)

	Study 017		Study 018		Pooled	
Number (%) of Subjects with	Mitapivat N=129 n (%)	Placebo N=63 n (%)	Mitapivat N=172 n (%)	Placebo N=85 n (%)	Mitapivat N=301 n (%)	Placebo N=148 n (%)
Any TEAEs	107 (82.9)	50 (79.4)	155 (90.1)	71 (83.5)	262 (87.0)	121 (81.8)
Grade≥3 TEAEs	18 (14.0)	2 (3.2)	32 (18.6)	12 (14.1)	50 (16.6)	14 (9.5)
Treatment-related TEAEs	56 (43.4)	13 (20.6)	65 (37.8)	16 (18.8)	121 (40.2)	29 (19.6)
Grade≥3 treatment-related TEAEs	5 (3.9)	0	13 (7.6)	1 (1.2)	18 (6.0)	1 (0.7)
Serious TEAEs	8 (6.2)	0	19 (11.0)	13 (15.3)	27 (9.0)	13 (8.8)
Serious treatment-related TEAEs	0	0	4 (2.3)	1 (1.2)	4 (1.3)	1 (0.7)
TEAEs leading to discontinuation of study drug	4 (3.1)	0	10 (5.8)	1 (1.2)	14 (4.7)	1 (0.7)
TEAEs leading to dose reduction of study drug	7 (5.4)	2 (3.2)	20 (11.6)	2 (2.4)	27 (9.0)	4 (2.7)
TEAEs leading to interruption of study drug	2 (1.6)	1 (1.6)	13 (7.6)	5 (5.9)	15 (5.0)	6 (4.1)
TEAEs leading to death	0	0	0	0	0	0
Treatment-related TEAEs leading to death	0	0	0	0	0	0

Source: AG348 Thal ISS/RMP Table 18.3.1-1.1.1 (Agios internal use only).
The denominator used to calculate percentages is N, the number of subjects in the safety analysis set within each treatment group.

The adverse events were considered related to study treatment if occurring or worsening during study drug treatment; events were counted once per patient, with the highest grade reported in the assessment of severity or causality.

Common TEAE

Table 76: Summary of Most Common (Any Grade in ≥ 10% Subjects in Any Treatment Group) Treatment-Emergent Adverse Events by Preferred Term-Comparative Thalassemia Safety Pool (Safety Analysis Set)

	Study 017		Study 018		Pooled	
Preferred Term (PT)	Mitapivat N=129 n (%)	Placebo N=63 n (%)	Mitapivat N=172 n (%)	Placebo N=85 n (%)	Mitapivat N=301 n (%)	Placebo N=148 n (%)
Subjects with events	107 (82.9)	50 (79.4)	155 (90.1)	71 (83.5)	262 (87.0)	121 (81.8)
Headache	29 (22.5)	6 (9.5)	46 (26.7)	10 (11.8)	75 (24.9)	16 (10.8)
Initial insomnia	18 (14.0)	3 (4.8)	24 (14.0)	4 (4.7)	42 (14.0)	7 (4.7)
Upper respiratory tract infection	14 (10.9)	4 (6.3)	27 (15.7)	14 (16.5)	41 (13.6)	18 (12.2)
Diarrhoea	11 (8.5)	6 (9.5)	19 (11.0)	7 (8.2)	30 (10.0)	13 (8.8)
Fatigue	12 (9.3)	4 (6.3)	18 (10.5)	2 (2.4)	30 (10.0)	6 (4.1)
Nausea	15 (11.6)	5 (7.9)	13 (7.6)	5 (5.9)	28 (9.3)	10 (6.8)

Source: AG348 Thal ISS/RMP Table 18.3.1-2.3.1 (Agios internal use only).
Summarized in order of decreasing frequency of subjects with events based on the frequencies observed in any grade for the pooled mitapivat arm.
The denominator used to calculate percentages is N, the number of subjects in the safety analysis set within each treatment group.
Subjects with multiple AEs within a PT are counted only once in that PT.
MedDRA version 27.0 is used.

The most common AE occurring in ≥10% of patients treated with mitapivat versus placebo were headache (24.9% vs 10.8%), initial insomnia (14.0% vs 4.7%), upper respiratory tract infection (13.6% vs 12.2%), diarrhoea (10.0% vs 8.8%), fatigue (10% vs 4.1%), and nausea (9.3% vs 6.8%).

Treatment-related TEAEs occurred with higher frequency in subjects who received mitapivat (40.2%) compared to placebo (19.6%). The most frequent treatment related TEAE occurred in the SOC of Psychiatric disorders, Nervous system disorders, GI disorders, and general disorders. The most common (occurring in ≥10% of subjects) treatment-related TEAEs in subjects who received mitapivat were insomnia (20.9%), Headache (15.0%); the frequency of subjects with these events was similar in both studies.

In the pooled thalassemia dataset (n=301), in mitapivat treated patients, the treatment related TEAE, occurring in >1% of patients irrespective of grade, were headache (15.0%), initial insomnia (10.3%), nausea (6.0%), middle insomnia (5.6%), insomnia (3.3%), fatigue (2.7%), diarrhoea, ALT increased (2.0%), terminal insomnia (1.9%), palpitations (1.7%), pruritus (1.3%), dizziness (1.3%), asthenia, abdominal distension, AST increased, pain in extremity, decreased appetite (1.0% each). Most subjects with a treatment-related TEAE had an event that was Grade 1 or 2 in severity.

Frequency of subjects with Grade ≥3 TEAEs was higher in subjects who received mitapivat compared to placebo (16.6% vs 9.5%). There were no PT of Grade ≥3 TEAEs occurring in ≥5% of subjects in the pivotal studies. Most Grade ≥3 TEAEs occurred in 1 subject each. The TEAEs of Anaemia, Alanine aminotransferase increased, Aspartate aminotransferase increased, and Initial insomnia were the only Grade ≥3 TEAEs (by PT) that occurred in ≥1% of subjects treated with mitapivat. Grade ≥3 TEAEs (by PT) Alanine aminotransferase increased, Aspartate aminotransferase increased, and Initial insomnia were the only treatment-related which occurred in ≥1% of subjects treated with mitapivat in both pivotal studies combined. No treatment-related Grade ≥3 TEAE (by PT) occurred in ≥2% of subjects. No serious treatment-related TEAEs were reported for Study 017 and no serious treatment-related TEAEs (by PT) were reported for >1 subject in Study 018.

In study 017, Treatment related Grade ≥ 3 TEAE occurred in 5 patients (3.9%) and were initial insomnia, joint swelling, drug-induced liver injury, hypertrygliceridaemia, thrombocytopenia, ALT increased, AST increased,

international normalized ratio increased, blood bilirubin increased, blood lactate dehydrogenase increased (1 event each). There were no Grade 4 or 5 treatment related AE.

In study 018, Treatment related Grade 3 events occurred in 12 patients (7%) and were: insomnia (2.4%), ALT increased (1.7%), AST increased (1.2%), abdominal distension, blood pressure increased, arrythmia supraventricular and supraventricular tachycardia, hypertransaminasaemia, and lower respiratory tract infection (0.6% each). One Grade 4 event was renal mass in 1 patient (0.6%); there were no Grade 5 events**.**

Table 77: Summary of Treatment-Emergent Grade ≥3 Adverse Events Related to Study Treatment, by System Organ Class and Preferred Term- Comparative Thalassemia Safety Pool (Safety Analysis Set)

System Organ Class (SOC) Preferred Term (PT)	Study 017		Study 018		Pooled	
	Mitapivat N=129 n (%)	Placebo N=63 n (%)	Mitapivat N=172 n (%)	Placebo N=85 n (%)	Mitapivat N=301 n (%)	Placebo N=148 n (%)
Subjects with events	5 (3.9)	0	13 (7.6)	1 (1.2)	18 (6.0)	1 (0.7)
Investigations	1 (0.8)	0	4 (2.3)	1 (1.2)	5 (1.7)	1 (0.7)
Alanine aminotransferase increased	1 (0.8)	0	3 (1.7)	0	4 (1.3)	0
Aspartate aminotransferase increased	1 (0.8)	0	2 (1.2)	0	3 (1.0)	0
Blood bilirubin increased	1 (0.8)	0	0	0	1 (0.3)	0
Blood lactate dehydrogenase increased	1 (0.8)	0	0	0	1 (0.3)	0
Blood pressure increased	0	0	1 (0.6)	0	1 (0.3)	0
International normalised ratio increased	1 (0.8)	0	0	0	1 (0.3)	0
Blood creatine phosphokinase increased	0	0	0	1 (1.2)	0	1 (0.7)
Psychiatric disorders	1 (0.8)	0	3 (1.7)	0	4 (1.3)	0
Initial insomnia	1 (0.8)	0	2 (1.2)	0	3 (1.0)	0
Insomnia	0	0	1 (0.6)	0	1 (0.3)	0
Middle insomnia	0	0	1 (0.6)	0	1 (0.3)	0
Cardiac disorders	0	0	2 (1.2)	0	2 (0.7)	0
Arrhythmia supraventricular	0	0	1 (0.6)	0	1 (0.3)	0
Supraventricular tachycardia	0	0	1 (0.6)	0	1 (0.3)	0
Hepatobiliary disorders	1 (0.8)	0	1 (0.6)	0	2 (0.7)	0
Drug-induced liver injury	1 (0.8)	0	0	0	1 (0.3)	0
Hypertransaminasaemia	0	0	1 (0.6)	0	1 (0.3)	0
Blood and lymphatic system disorders	1 (0.8)	0	0	0	1 (0.3)	0
Thrombocytopenia	1 (0.8)	0	0	0	1 (0.3)	0
Gastrointestinal disorders	0	0	1 (0.6)	0	1 (0.3)	0
Abdominal distension	0	0	1 (0.6)	0	1 (0.3)	0
Infections and infestations	0	0	1 (0.6)	0	1 (0.3)	0
Lower respiratory tract infection	0	0	1 (0.6)	0	1 (0.3)	0
Metabolism and nutrition disorders	1 (0.8)	0	0	0	1 (0.3)	0
Hypertriglyceridaemia	1 (0.8)	0	0	0	1 (0.3)	0
Musculoskeletal and connective tissue disorders	1 (0.8)	0	0	0	1 (0.3)	0
Joint swelling	1 (0.8)	0	0	0	1 (0.3)	0
Renal and urinary disorders	0	0	1 (0.6)	0	1 (0.3)	0
Renal mass	0	0	1 (0.6)	0	1 (0.3)	0

Source: AG348 Thal ISS/RMP Table 18.3.1-2.7.1.1 (Agios internal use only).
Summarized in order of decreasing frequency of subjects with events based on the frequencies observed in any grade for the pooled mitapivat arm.
The denominator used to calculate percentages is N, the number of subjects in the safety analysis set within each treatment group.
Subjects with multiple AEs within a PT are counted only once in that PT.
Subjects with multiple AEs within a SOC are counted only once in that SOC.
MedDRA version 27.0 and CTCAE version 4.03 are used.

2.6.8.2.2. All indications safety pool

Across all indications, most subjects treated with mitapivat experienced TEAEs that were nonserious and low grade (Grade 1 or 2), similar to what was observed for the pivotal thalassemia studies. One subject with pyruvate kinase deficiency experienced a serious TEAE of Accident that led to death (verbatim: death from a fire accident) while receiving mitapivat 50 mg BID in Study 011. This serious TEAE was considered not related to study treatment.

Table 78: Overall Summary of Treatment-Emergent Adverse Events-All Indications Safety Pool (Safety Analysis Set)

	PK Deficiency		Sickle Cell Disease		Thalassemia		Pooled	
Number (%) of Subjects with	Mitapivat N=157 n (%)	Placebo N=39 n (%)	Mitapivat N=52 n (%)	Placebo N=27 n (%)	Mitapivat N=321 n (%)	Placebo N=148 n (%)	Mitapivat N=530 n (%)	Placebo N=214 n (%)
Any TEAEs	156 (99.4)	35 (89.7)	42 (80.8)	22 (81.5)	282 (87.9)	121 (81.8)	480 (90.6)	178 (83.2)
Grade≥3 TEAEs	66 (42.0)	5 (12.8)	8 (15.4)	2 (7.4)	59 (18.4)	14 (9.5)	133 (25.1)	21 (9.8)
Treatment-related TEAEs	113 (72.0)	14 (35.9)	18 (34.6)	7 (25.9)	134 (41.7)	29 (19.6)	265 (50.0)	50 (23.4)
Grade≥3 treatment-related TEAEs	17 (10.8)	0	0	0	19 (5.9)	1 (0.7)	36 (6.8)	1 (0.5)
Serious TEAEs	46 (29.3)	2 (5.1)	6 (11.5)	3 (11.1)	30 (9.3)	13 (8.8)	82 (15.5)	18 (8.4)
Serious treatment-related TEAEs	11 (7.0)	0	0	0	4 (1.2)	1 (0.7)	15 (2.8)	1 (0.5)
TEAEs leading to discontinuation of study drug	8 (5.1)	0	0	0	15 (4.7)	1 (0.7)	23 (4.3)	1 (0.5)
TEAEs leading to dose reduction of study drug	16 (10.2)	0	0	0	32 (10.0)	4 (2.7)	48 (9.1)	4 (1.9)
TEAEs leading to interruption of study drug	15 (9.6)	2 (5.1)	0	0	16 (5.0)	6 (4.1)	31 (5.8)	8 (3.7)
TEAEs leading to death	1 (0.6)	0	0	0	0	0	1 (0.2)	0
Treatment-related TEAEs leading to death	0	0	0	0	0	0	0	0

Source: AG348 Thal ISS/RMP Table 18.3.1-1.1.2 (Agios internal use only).
The denominator used to calculate percentages is N, the number of subjects in the safety analysis set within each treatment group.

The most common adverse events (≥10%) in the all indications safety set in the mitapivat treated versus placebo were headache (31.7% vs 16.8%), fatigue (17.0% vs 7.0%), nausea (15.7% vs 10.7%), initial insomnia (13.8% vs 6.1%), upper respiratory tract infections (12.6 vs 10.7%), arthralgia (12.3% vs 9.3%), diarrhoea (11.9% vs 9.8%), Covid-19 (11.3% vs 6.1%), cough (10.9% vs 4.2%), nasopharyngitis (10.8% vs 5.6%), pyrexia (10.6% vs 7.0%), and back pain (10% vs 7%).

The most common treatment-related TEAEs (occurring in ≥10% of subjects) in subjects who received mitapivat for all indications combined were Initial insomnia and Headache. Most subjects with a treatment-related TEAE had an event that was Grade 1 or 2 in severity.

Table 79: Summary of Treatment-Emergent Grade ≥3 Adverse Events Related to Study Treatment, by System Organ Class and Preferred Term- All Indications Safety pool (Safety Analysis Set)

System Organ Class (SOC) Preferred Term (PT)	PK Deficiency		Sickle Cell Disease		Thalassemia		Pooled	
	Mitapivat N=157 n (%)	Placebo N=39 n (%)	Mitapivat N=52 n (%)	Placebo N=27 n (%)	Mitapivat N=321 n (%)	Placebo N=148 n (%)	Mitapivat N=530 n (%)	Placebo N=214 n (%)
Subjects with events	17 (10.8)	0	0	0	19 (5.9)	1 (0.7)	36 (6.8)	1 (0.5)
Investigations	2 (1.3)	0	0	0	5 (1.6)	1 (0.7)	7 (1.3)	1 (0.5)
Alanine aminotransferase increased	0	0	0	0	4 (1.2)	0	4 (0.8)	0
Aspartate aminotransferase increased	1 (0.6)	0	0	0	3 (0.9)	0	4 (0.8)	0
Blood bilirubin increased	1 (0.6)	0	0	0	1 (0.3)	0	2 (0.4)	0
Blood lactate dehydrogenase increased	0	0	0	0	1 (0.3)	0	1 (0.2)	0
Blood pressure increased	0	0	0	0	1 (0.3)	0	1 (0.2)	0
International normalised ratio increased	0	0	0	0	1 (0.3)	0	1 (0.2)	0

System Organ Class (SOC) Preferred Term (PT)	PK Deficiency		Sickle Cell Disease		Thalassemia		Pooled	
	Mitapivat N=157 n (%)	Placebo N=39 n (%)	Mitapivat N=52 n (%)	Placebo N=27 n (%)	Mitapivat N=321 n (%)	Placebo N=148 n (%)	Mitapivat N=530 n (%)	Placebo N=214 n (%)
Blood creatine phosphokinase increased	0	0	0	0	0	1 (0.7)	0	1 (0.5)
Metabolism and nutrition disorders	6 (3.8)	0	0	0	1 (0.3)	0	7 (1.3)	0
Hypertriglyceridaemia	6 (3.8)	0	0	0	1 (0.3)	0	7 (1.3)	0
Psychiatric disorders	1 (0.6)	0	0	0	5 (1.6)	0	6 (1.1)	0
Initial insomnia	1 (0.6)	0	0	0	4 (1.2)	0	5 (0.9)	0
Insomnia	1 (0.6)	0	0	0	1 (0.3)	0	2 (0.4)	0
Middle insomnia	0	0	0	0	1 (0.3)	0	1 (0.2)	0
Blood and lymphatic system disorders	3 (1.9)	0	0	0	1 (0.3)	0	4 (0.8)	0
Haemolytic anaemia	2 (1.3)	0	0	0	0	0	2 (0.4)	0
Anaemia	1 (0.6)	0	0	0	0	0	1 (0.2)	0
Haemolysis	1 (0.6)	0	0	0	0	0	1 (0.2)	0
Thrombocytopenia	0	0	0	0	1 (0.3)	0	1 (0.2)	0
Nervous system disorders	4 (2.5)	0	0	0	0	0	4 (0.8)	0
Headache	3 (1.9)	0	0	0	0	0	3 (0.6)	0
Dizziness	1 (0.6)	0	0	0	0	0	1 (0.2)	0
Musculoskeletal and connective tissue disorders	2 (1.3)	0	0	0	1 (0.3)	0	3 (0.6)	0
Joint swelling	1 (0.6)	0	0	0	1 (0.3)	0	2 (0.4)	0
Arthralgia	1 (0.6)	0	0	0	0	0	1 (0.2)	0
Musculoskeletal pain	1 (0.6)	0	0	0	0	0	1 (0.2)	0
Cardiac disorders	0	0	0	0	2 (0.6)	0	2 (0.4)	0
Arrhythmia supraventricular	0	0	0	0	1 (0.3)	0	1 (0.2)	0
Supraventricular tachycardia	0	0	0	0	1 (0.3)	0	1 (0.2)	0
Hepatobiliary disorders	0	0	0	0	2 (0.6)	0	2 (0.4)	0
Drug-induced liver injury	0	0	0	0	1 (0.3)	0	1 (0.2)	0
Hypertransaminasaemia	0	0	0	0	1 (0.3)	0	1 (0.2)	0
Infections and infestations	1 (0.6)	0	0	0	1 (0.3)	0	2 (0.4)	0
Gastroenteritis	1 (0.6)	0	0	0	0	0	1 (0.2)	0
Lower respiratory tract infection	0	0	0	0	1 (0.3)	0	1 (0.2)	0
Gastrointestinal disorders	0	0	0	0	1 (0.3)	0	1 (0.2)	0
Abdominal distension	0	0	0	0	1 (0.3)	0	1 (0.2)	0
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	1 (0.6)	0	0	0	0	0	1 (0.2)	0
Renal cell carcinoma	1 (0.6)	0	0	0	0	0	1 (0.2)	0
Renal and urinary disorders	0	0	0	0	1 (0.3)	0	1 (0.2)	0
Renal mass	0	0	0	0	1 (0.3)	0	1 (0.2)	0

Source: AG348 Thal ISS/RMP Table 18.3.1-2.7.1.2 (Agios internal use only).
Summarized in order of decreasing frequency of subjects with events based on the frequencies observed in any grade for the pooled mitapivat arm.
The denominator used to calculate percentages is N, the number of subjects in the safety analysis set within each treatment group.
Subjects with multiple AEs within a PT are counted only once in that PT.
Subjects with multiple AEs within a SOC are counted only once in that SOC.
MedDRA version 27.0 and CTCAE version 4.03 are used.

For all indications combined, there were no PT of Grade ≥3 TEAEs occurring in ≥5% of subjects treated with mitapivat. Most Grade ≥3 TEAEs occurred in 1 subject each. The TEAEs of Anaemia, Alanine aminotransferase increased, Aspartate aminotransferase increased, Hypertriglyceridaemia, and Initial insomnia were the only Grade ≥3 TEAEs (by PT) that occurred in ≥1% of subjects treated with mitapivat.

Treatment related Grade ≥ 3 TEAE occurred in 17 (10.8%) subjects with PK deficiency and 19 (5.9%) in subjects with thalassemia and were more frequent than in placebo (0% and 0.7% respectively). Hypertriglyceridemia and headache were the only treatment-related Grade ≥3 TEAEs (by PT) that occurred in ≥1% of subjects treated with mitapivat (all indications combined). No treatment-related Grade ≥3 TEAE (by PT) occurred in ≥2% of subjects.

Treatment related Grade 3 occurred in 16 subjects (10.2%) with PKD treated with mitapivat and were hypertriglyceridemia (3.2%), headache, anaemia (1.9% each), insomnia (1.2%), dizziness, arthralgia, joint swelling, musculoskeletal pain, ALT increased, bilirubin increased (0.6% each). The Grade 4 event in one subject (0.6%) was hypertriglyceridemia. In patients with thalassemia pooled, treated with mitapivat, treatment related Grade 3 occurred in 18 patients (5.6%), and were ALT increased (1.2%), insomnia (1.6%), AST increased (0.9%), abdominal distension, joint swelling, bilirubin, LDH increased, blood pressure increased supraventricular tachycardia, arrhythmia supraventricular, thrombocytopenia, DILI, and LRTI (0.3% each). The Grade 4 in one patient (0.3%) was renal mass.

2.6.8.3. Serious adverse event/deaths/other significant events

2.6.8.3.1. Comparative thalassemia safety pool

Serious TEAEs were reported in 9.0% of subjects who received mitapivat and in 8.8% of subjects who received placebo. Most serious TEAEs occurred in 1 subject. In subjects that received mitapivat, the serious TEAEs of Pneumonia, Gastroenteritis, and Lower respiratory tract infection occurred in ≥2 subjects. The frequency of subjects with serious treatment-related TEAEs was low across the 2 pivotal studies and similar across the treatment arms. No serious treatment-related TEAE (by PT) occurred in >1 subject.

Table 80: Summary of Serious Treatment-Emergent Adverse Events Related to Study Treatment, by System Organ Class and Preferred Term- Comparative Thalassemia Safety Pool (Safety Analysis Set)

	Study 017		Study 018		Pooled	
System Organ Class (SOC) Preferred Term (PT)	Mitapivat N=129 n (%)	Placebo N=63 n (%)	Mitapivat N=172 n (%)	Placebo N=85 n (%)	Mitapivat N=301 n (%)	Placebo N=148 n (%)
Subjects with events	0	0	4 (2.3)	1 (1.2)	4 (1.3)	1 (0.7)
Cardiac disorders	0	0	2 (1.2)	0	2 (0.7)	0
Arrhythmia supraventricular	0	0	1 (0.6)	0	1 (0.3)	0
Supraventricular tachycardia	0	0	1 (0.6)	0	1 (0.3)	0
Infections and infestations	0	0	1 (0.6)	0	1 (0.3)	0
Lower respiratory tract infection	0	0	1 (0.6)	0	1 (0.3)	0
Renal and urinary disorders	0	0	1 (0.6)	0	1 (0.3)	0
Renal mass	0	0	1 (0.6)	0	1 (0.3)	0
Investigations	0	0	0	1 (1.2)	0	1 (0.7)
Blood creatine phosphokinase increased	0	0	0	1 (1.2)	0	1 (0.7)

Source: AG348 Tha1 ISS/RMP Table 18.3.1-3.2.1 (Agios internal use only).
Summarized in order of decreasing frequency of subjects with events based on the frequencies observed for the pooled mitapivat arm.
The denominator used to calculate percentages is N, the number of subjects in the safety analysis set within each treatment group.
Subjects with multiple AEs within a PT are counted only once in that PT.
Subjects with multiple AEs within a SOC are counted only once in that SOC.
MedDRA version 27.0 is used.

AESI

There were no AESI specified for mitapivat.

Other Adverse events of interest

- Acute haemolysis upon abrupt withdrawal of mitapivat (not a risk in thalassemia)

The event has been observed in subjects with pyruvate kinase deficiency and is not expected to occur in subjects with thalassemia for the following reasons: residual activity of wild-type pyruvate kinase is retained in the setting of thalassemia compared with decreased activity from mutant pyruvate kinase in the setting pyruvate kinase deficiency. No events of acute hemolysis were observed in subjects with thalassemia who interrupted or discontinued mitapivat treatment, including subjects who discontinued or interrupted treatment abruptly.

- Hepatocellular injury in thalassemia

Hepatocellular injury is a risk for subjects with thalassemia that is based on events observed only in subjects with thalassemia in Studies 017 and 018 during the Double blind and OLE Periods. During the Double-blind Period for the pivotal studies, 2 subjects with non-serious events of hepatocellular injury leading to discontinuation of mitapivat treatment were reported. In the OLE Periods of Studies 017 and 018, there have been 3 subjects who had events of hepatocellular injury leading to discontinuation of mitapivat treatment with subsequent improvement of liver tests. Two events were reported as serious AEs and one was reported as a nonserious AE.

All 5 events were characterized by a time to onset within the first 6 months of treatment with subsequent peak elevations of aminotransferases of >5×ULN. Four of the 5 subjects had preexisting liver disease and/or other confounders. The fifth subject had no preexisting liver disease and no known confounders identified. Thus, an association with mitapivat treatment could not be ruled out. For 2 of the 5 subjects, the events were serious (requiring hospitalization). All observed cases of hepatocellular injury have been identified as part of routine protocol-defined monitoring, and liver tests improved following discontinuation of mitapivat. None of these events led to liver failure or death.

A comprehensive search of all cases of hepatocellular injury across all clinical program has been conducted: 58 subjects with relevant TEAE were identified, 36 in PKD, 2 in SCD, and 21 in thalassemia indication. Upon review of cases in PKD and SCD, there were no events suggestive of potential drug-induced hepatocellular injury.

The 21 adult subjects with thalassemia identified had one of the following PTs: ALT increased, AST increased, Blood bilirubin increased, Transaminases increased, Hypertransaminasaemia, Hepatic cytolysis, Hepatic pain, Drug-induced liver injury, Hepatic enzyme increased, and Hepatomegaly. Of the 21 subjects, 19 did not have an event suggestive of hepatocellular injury. The remaining 2 subjects with thalassemia had drug withdrawn and a relationship to mitapivat could not be ruled out.

Additionally, as described above, 3 subjects with thalassemia experienced AEs suggestive of hepatocellular injury during the OLE Periods after switching from placebo to mitapivat.

Overall, events hepatocellular injury in which mitapivat effect could not be ruled out have been observed only in subjects with thalassemia.

No death occurred during the double-blind period in thalassemia studies.

2.6.8.3.2. All indications safety pool

Serious TEAEs were reported in 15.5% of subjects who received mitapivat (all indications combined) versus 8.4% in placebo. There were 46 (29.3%) SAE in patients treated with mitapivat and 2 (5%) in placebo in the PK deficiency indication, 6 (11%) SAE in patients treated with mitapivat and 3 (11%) in placebo in SCD

indication, and 30 (9.3%) SAE in patients treated with mitapivat and 13 (8.8%) in placebo in the thalassemia indication. Most serious TEAEs (by PT) occurred in <=2 subjects. In subjects who received mitapivat, the serious TEAEs of Gastroenteritis, Pneumonia, Tonsillitis, Tibia fracture, Haemolysis, and Haemolytic anaemia occurred in >2 subjects.

Table 81: Summary of Serious Treatment-Emergent Adverse Events Related to Study Treatment, by System Organ Class and Preferred Term- All Indications Safety Pool (Safety Analysis Set)

	PK Deficiency		Sickle Cell Disease		Thalassemia		Pooled	
System Organ Class (SOC) Preferred Term (PT)	Mitapivat N=157 n (%)	Placebo N=39 n (%)	Mitapivat N=52 n (%)	Placebo N=27 n (%)	Mitapivat N=321 n (%)	Placebo N=148 n (%)	Mitapivat N=530 n (%)	Placebo N=214 n (%)
Subjects with events	11 (7.0)	0	0	0	4 (1.2)	1 (0.7)	15 (2.8)	1 (0.5)
Blood and lymphatic system disorders	3 (1.9)	0	0	0	0	0	3 (0.6)	0
Haemolytic anaemia	3 (1.9)	0	0	0	0	0	3 (0.6)	0
Haemolysis	1 (0.6)	0	0	0	0	0	1 (0.2)	0
Cardiac disorders	0	0	0	0	2 (0.6)	0	2 (0.4)	0
Arrhythmia supraventricular	0	0	0	0	1 (0.3)	0	1 (0.2)	0
Supraventricular tachycardia	0	0	0	0	1 (0.3)	0	1 (0.2)	0
Infections and infestations	1 (0.6)	0	0	0	1 (0.3)	0	2 (0.4)	0
Gastroenteritis	1 (0.6)	0	0	0	0	0	1 (0.2)	0
Lower respiratory tract infection	0	0	0	0	1 (0.3)	0	1 (0.2)	0
Musculoskeletal and connective tissue disorders	2 (1.3)	0	0	0	0	0	2 (0.4)	0
Musculoskeletal pain	1 (0.6)	0	0	0	0	0	1 (0.2)	0
Osteoporosis	1 (0.6)	0	0	0	0	0	1 (0.2)	0
Nervous system disorders	2 (1.3)	0	0	0	0	0	2 (0.4)	0
Headache	1 (0.6)	0	0	0	0	0	1 (0.2)	0
Seizure	1 (0.6)	0	0	0	0	0	1 (0.2)	0
Metabolism and nutrition disorders	1 (0.6)	0	0	0	0	0	1 (0.2)	0
Hypertriglyceridaemia	1 (0.6)	0	0	0	0	0	1 (0.2)	0
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	1 (0.6)	0	0	0	0	0	1 (0.2)	0
Renal cell carcinoma	1 (0.6)	0	0	0	0	0	1 (0.2)	0
Psychiatric disorders	1 (0.6)	0	0	0	0	0	1 (0.2)	0
Initial insomnia	1 (0.6)	0	0	0	0	0	1 (0.2)	0
Renal and urinary disorders	0	0	0	0	1 (0.3)	0	1 (0.2)	0
Renal mass	0	0	0	0	1 (0.3)	0	1 (0.2)	0
Investigations	0	0	0	0	0	1 (0.7)	0	1 (0.5)
Blood creatine phosphokinase increased	0	0	0	0	0	1 (0.7)	0	1 (0.5)

Source: AG348 Thal ISS/RMP Table 18.3.1-3.2.2 (Agios internal use only).
Summarized in order of decreasing frequency of subjects with events based on the frequencies observed for the pooled mitapivat arm.
The denominator used to calculate percentages is N, the number of subjects in the safety analysis set within each treatment group.
Subjects with multiple AEs within a PT are counted only once in that PT.
Subjects with multiple AEs within a SOC are counted only once in that SOC.
MedDRA version 27.0 is used.

Serious treatment-related TEAEs were reported infrequently across all the indications. In the all indications safety pool, there were 11 (7.0%) SAE related to mitapivat in the PK deficiency, and 4 SAE (1.2%) in the thalassemia. Most serious treatment-related TEAEs occurred in only 1 subject. The serious treatment-related TEAE of Haemolytic anaemia and haemolysis occurred in 3 subjects due to abrupt withdrawal of mitapivat treatment in subjects with pyruvate kinase deficiency.

AESI:

Acute haemolysis upon abrupt withdrawal of mitapivat is an important identified risk in PK deficiency. Acute haemolysis has occurred in subjects with pyruvate kinase deficiency who were Hb responders and underwent an abrupt withdrawal of mitapivat (without a gradual reduction in dosing) that led to rapid and marked returns to pre-treatment Hb levels, accompanied by signs and symptoms of haemolysis.

Deaths

One subject with pyruvate kinase deficiency experienced a serious TEAE of Accident that led to death (verbatim: death from a fire accident) while receiving mitapivat 50 mg BID in Study 011. This serious TEAE was considered not related to study treatment.

2.6.8.4. Laboratory findings

Clinical laboratory results were to be reported as an AE if they were considered clinically significant in the Investigator's judgement, were associated with clinical signs and symptoms, resulted in dose modification or study drug discontinuation, or required medical or surgical intervention or a change in concomitant therapy.

Newly occurring or worsening clinical chemistry laboratory abnormalities with onset during the on-treatment period were generally Grade 1 or 2 in severity for subjects with thalassemia. The most common (occurring in >20% of subjects) chemistry laboratory abnormalities reported in both treatment arms were high ALT and high AST. The most common chemistry laboratory abnormalities observed in a single treatment arm were low glucose (mitapivat arm only), high bilirubin (placebo arm only), and low calcium (placebo arm only). Differences between mitapivat and placebo arms in newly occurring or worsening any grade (=5% difference) chemistry parameters are:

• A lower frequency of subjects with high bilirubin and low calcium values in the mitapivat arm compared with the placebo arm.

• A higher frequency of subjects with low glucose, high potassium, and high urate values in the mitapivat arm compared with the placebo arm.

Liver tests

Subjects with hemoglobinopathies have ongoing hemolysis leading to increases in bilirubin and transaminases. Liver involvement in the forms of intrahepatic cholestasis, cholelithiasis, and hepatic iron overload are common findings in subjects with hemoglobinopathies due to the underlying hemolysis. Furthermore, concurrent use of iron chelators, a common concomitant medication in patients with hemoglobinopathies, has been associated with hepatotoxicity; and in subjects with a transfusion history, there is an increased risk of concomitant HCV infection and cirrhosis. Results from a recent, large long-term registry study in patients with hemoglobinopathies has shown that cirrhosis, hepatocellular carcinomas, and liver failure are common comorbidities or causes of mortality in these patients (Delicou et al, 2024).

During the on-treatment period, the most common (occurring in =10% of subjects) elevation in liver function tests in subjects who received mitapivat or placebo was total bilirubin >2×ULN; this was observed less

frequently in subjects who received mitapivat compared with placebo (35.5% vs 54.1%). ALT elevations above 3-fold the ULN were observed more frequently in subjects receiving mitapivat compared with placebo (8.0% vs 2.7%, respectively).

Triglycerides

A similar frequency of subjects experienced newly occurring or worsening triglyceride elevations between the mitapivat and placebo arms (10.0% vs 14.7%, respectively). Newly occurring or worsening elevated triglycerides with onset during the on-treatment period were generally Grade 1 or 2 in severity. One TEAE of Hypertriglyceridaemia was reported in 1 subject receiving mitapivat in Study 017. No clinically significant triglyceride laboratory abnormalities were reported as TEAEs in Study 018.

Haematology

Newly occurring or worsening haematology laboratory abnormalities with onset during the on treatment period were generally Grade 1 or 2 in severity for subjects with thalassemia. The most common (occurring in >20% of subjects) heamatology laboratory abnormalities in subjects who received mitapivat and subjects who received placebo were low Hb and high lymphocytes.

Differences between the mitapivat and placebo arms in newly occurring or worsening any grade (=5% difference) heamatology parameters are:

• A lower frequency of subjects with newly occurring or worsening any grade low Hb values in the mitapivat arm compared with the placebo arm.

• A higher frequency of subjects with newly occurring or worsening any grade low platelet values in the mitapivat arm compared with the placebo arm.

Aromatase inhibition

Based on *in vitro* data that show mitapivat is a weak aromatase inhibitor, sex hormone laboratory abnormalities and BMD were monitored in the mitapivat clinical trials. Collection of sex hormones included nonfasting estradiol, estrone, and testosterone (total and free). BMD was assessed by DXA scans of the hip and spine performed according to the instructions provided by the central-read vendor for some of the mitapivat clinical trials.

Male Subjects

In the thalassemia safety pool, for both pivotal studies combined, the frequency of male subjects with on-treatment estrone and estradiol <LLN and on-treatment testosterone and free testosterone >ULN, where baseline was >=LLN or <=ULN, respectively is: Estrone decreased: 51.2% mitapivat and 4.4% placebo, Estradiol decreased: 13.6% mitapivat and 10.3% placebo, Testosterone increased: 32.0% mitapivat and 11.8% placebo, and Free testosterone increased: 48.8% mitapivat and 19.1% placebo.

For the all indications pool in subjects who received mitapivat: Estrone decreased: 46.2%, Estradiol decreased: 14.0%, Testosterone increased: 24.0%, and Free testosterone, increased: 51.6%.

Female Subjects

Sex hormone analysis in female subjects was limited due to physiologic variations in hormone levels expected throughout the normal menstrual cycle and with the various types of hormonal contraceptives used, the range in subject age, and the potential of thalassemia to cause menstrual cycle changes.

Dual energy X-ray Absorptiometry (DXA) scans

Across both Study 017 and 018, subjects had low BMD at baseline, which is expected in the thalassemia population as severe anaemia and ineffective erythropoiesis lead to bone marrow expansion while iron overload directly disrupts bone formation, has toxic effects on osteoblasts, and induces a decrease in the recruitment of cells of the osteoblastic lineage (Yavropoulou *et al,* 2022).

Follow-up DXA scans were not scheduled during the Double-blind Period of Study 017.

In Study 018, no clinically meaningful changes were observed in BMD (as assessed by DXA scores for adjusted spine and femoral total) from baseline to Week 48 in either treatment arm. Observed changes in subject DXA T-score categories were infrequent and similar between subjects treated with mitapivat or placebo. Across Studies 017 and 018, a total of 9 subjects who received mitapivat treatment experienced bone abnormality TEAEs: 6 subjects experienced fractures and 3 subjects experienced events of osteopenia or osteoporosis. None of these events were considered related to mitapivat treatment.

2.6.8.5. In vitro biomarker test for patient selection for safety

Not applicable.

2.6.8.6. Safety in special populations

No meaningful differences in TEAEs by age, sex, race and ethnic origin, and geographic region were observed with mitapivat treatment; no dose modifications with mitapivat are recommended in these populations.

2.6.8.6.1. Comparative thalassemia safety pool

Most (97.7%) subjects treated with mitapivat were 18 to <65 years of age; there were no subjects 75 years or older. While the reported TEAE data in subjects >=65 years of age are limited, overall, the reported TEAEs in these subjects are consistent with the established safety profile of mitapivat or the clinical complications typically associated with thalassemia, and no clinically meaningful differences in TEAEs by age were observed with mitapivat treatment. No dose modifications with mitapivat are recommended in subjects >=65 years of age.

Of the total subjects enrolled in the pivotal thalassemia studies who received mitapivat, 58.5% were female. The overall frequency of subjects with TEAEs was similar in male and female subjects who received mitapivat (87.2% and 86.9%, respectively). The overall type and incidence of TEAEs were similar in male and female subjects with thalassemia who received mitapivat.

Adverse events by thalassemia genotype and Hb concentration

The randomisation stratification factors for the 2 pivotal studies included 2 intrinsic factors in Study 017 (baseline Hb concentration [≤9.0 g/dL; 9.1-10.0 g/dL] and thalassemia genotype [α -thalassemia/HbH disease; β -thalassemia]) and 1 intrinsic factor in Study 018 (thalassemia genotype [non-[β 0/ β 0], including subjects with HbE/ β -thalassemia and α -thalassemia/HbH disease; β 0/ β 0]).

Of the total subjects enrolled in Study 017 who received mitapivat, more (68.0%) had the β -thalassemia genotype compared with the α -thalassemia genotype. The overall frequency of subjects with TEAEs was similar between subjects with β -thalassemia and α -thalassemia who received mitapivat (80.5% and 84.1%, respectively). The overall type and incidence of TEAEs was generally similar in subjects with β -thalassemia and α -thalassemia who received mitapivat. For the SOC of Musculoskeletal and connective tissue disorders,

there was an observed imbalance (>10%) of reported TEAEs between subjects with β-thalassemia and α-thalassemia (23.9% and 12.2%, respectively). There were no individual TEAEs within this SOC that occurred more frequently (≥10% difference) in subjects with β-thalassemia that would be suggestive of a drug effect.

Of all subjects enrolled in Study 017 who received mitapivat, more (73.2%) subjects had a baseline Hb concentration of ≤9 g/dL compared with a baseline Hb concentration of >9 g/dL. The overall frequency of subjects with TEAEs was similar between subjects with a baseline Hb concentration of ≤9 g/dL and >9 g/dL who received mitapivat (84.0% and 80.0%, respectively). The overall type and incidence of TEAEs was generally similar in subjects with a baseline Hb concentration of ≤9 g/dL and >9 g/dL who received mitapivat. For the SOC of General disorders and administration site conditions, there was an observed imbalance (>10%) in the percent of subjects reporting TEAEs between subjects with a baseline Hb concentration ≤9 g/dL and >9 g/dL (18.1% and 28.6%, respectively).

Of the total subjects enrolled in Study 018 who received mitapivat, more (55.8%) had the non-β0/β0 genotype compared with the β0/β0 genotype. The overall frequency of subjects with TEAEs was similar between subjects with non-β0/β0 and β0/β0 thalassemia who received mitapivat (86.5% and 94.7%, respectively). The overall type and incidence of TEAEs was generally similar in these subjects.

For the SOC of Musculoskeletal and connective tissue disorders, there was an observed difference (>10%) in frequency of TEAEs between subjects with non-β0/β0 and β0/β0 thalassemia (21.9% and 35.5%, respectively). There were no individual TEAEs within this SOC that occurred more frequently (≥10% difference) in subjects with non-β0/β0 thalassemia that would be suggestive of a drug effect. Additionally, for the SOC of Gastrointestinal disorders, there was an observed imbalance (>10% in the percentage of subjects reporting TEAEs between subjects with non-β0/β0 and β0/β0 thalassemia (22.9% and 36.8%, respectively). There were no individual TEAEs within this SOC that occurred more frequently (≥10% difference) in subjects with non-β0/β0 thalassemia that would cause the imbalance that would be suggestive of a drug effect.

2.6.8.6.2. All indications safety pool

Most (97.4%) subjects treated with mitapivat were 18 to <65 years of age; there were no subjects 85 years or older. While the reported TEAE data in subjects >=65 years of age are limited, overall, the reported TEAEs in these subjects are consistent with the established safety profile of mitapivat or the clinical complications typically associated with thalassemia, SCD, and pyruvate kinase deficiency, and no clinically meaningful differences in TEAEs by age were observed with mitapivat treatment. No dose modifications with mitapivat are recommended in subjects >=65 years of age.

Of the total subjects enrolled across all indications who received mitapivat, 58.3% were female. The overall frequency of subjects with TEAEs was similar in male and female subjects who received mitapivat (91.9% and 89.6%, respectively). The overall type and incidence of TEAEs were similar in male and female subjects who received mitapivat.

Pregnancy and lactation

There are no or limited amount of data from the use of mitapivat in pregnant women. Studies in animals have shown reproductive toxicity. PYRUKYND is not recommended during pregnancy and in women of childbearing potential not using contraception.

As of 11 April 2024, 6 pregnancies in female subjects have been reported in clinical studies of mitapivat. In addition, 2 pregnancies had occurred at the time of the initial MA assessment. In total, 8 pregnancies were exposed to mitapivat, of which 3 resulted in termination, 1 spontaneous abortion, 1 outcome of pregnancy was

unknown and 3 were carried to term. Considering the 3 pregnancies that were carried to term: One pregnancy occurred in a subject's partner; Two pregnancies occurred in a female subject, with discontinuation of treatment during the first trimester of pregnancy; All three pregnancy resulted to the birth of a normal healthy newborn.

In addition, mitapivat is a mild inhibitor of human aromatase activity, based on *in vitro* data. Mitapivat may decrease the systemic exposure of hormonal contraceptives that are sensitive substrates of CYP3A4.

No information is available on the clinical use of mitapivat during breastfeeding, on the presence of mitapivat in human milk, on the effect on the breastfed infant, or effects on milk production. Because mitapivat is highly protein bound (97.7%) to human plasma proteins, the amount in milk is likely to be low. The elimination half-life is 16 to 79 hours and is indicative of potential accumulation in infant. A risk not to be excluded. In consequence, a decision must be made whether to discontinue breast-feeding or to abstain from PYRUKYND therapy taking into account the benefit of breast feeding for the child and the benefit of therapy for the woman.

Dosing errors

One subject in Study 011 Cohort 2 received a mitapivat dose that exceeded the protocol-specified dose. The subject was prescribed mitapivat 50 mg BID but incorrectly took 3 tablets at once (150 mg BID) over a period of 70 days (30 October 2019 to 07 January 2020). The subject was retrained by the Investigator, and efforts were made to determine the extent of overdose, but the actual number of extra doses could not be determined,

A dosing error with mitapivat was reported in a paediatric patient who was not a participant in a clinical study with mitapivat nor a patient in an access program with mitapivat. Due to a pharmacy error, mitapivat was inadvertently dispensed to the patient' s family and the patient received mitapivat for 5 days. The patient experienced nonserious AEs of Rash pustular, Vomiting, and Headache, all of which resolved within 3 days of the last dose of mitapivat.

Overdose

No events of overdose were reported in subjects who received mitapivat in clinical studies.

Drug abuse

Based on the cumulative data across the clinical development program, as of the data cutoff date, no potential for drug dependence or drug abuse has been observed for mitapivat during any of the studies with mitapivat.

Withdrawal and rebound

No events associated with withdrawal were reported for subjects with thalassemia during any of the studies with mitapivat. Events of acute haemolysis following abrupt withdrawal of mitapivat were observed in PK deficiency and dose tapering is recommended.

Effects on ability to drive or operate machinery or mental ability impairment

The effect of mitapivat on the ability to drive vehicles and operate machinery has not been evaluated. As of the data cutoff date for the marketing application, no effect of mitapivat on ability to drive or operate machinery has been reported during any of the studies with mitapivat.

2.6.8.7. Immunological events

Not applicable.

2.6.8.8. Safety related to drug-drug interactions and other interactions

Mitapivat is primarily metabolised by CYP3A4/5 in vitro. In addition, it is an inducer and a metabolism-based inhibitor of CYP3A. Therefore, coadministration of mitapivat with CYP3A inhibitors/inducers or CYP3A substrates has the potential for DDI. The DDI potential was observed in a clinical DDI study (AG348-C-012) and PBPK simulations. The DDIs that may have a safety impact are summarised below.

Coadministration of mitapivat with CYP3A inhibitors may increase mitapivat plasma concentrations, which may increase the risk of adverse reactions. Therefore, coadministration of strong CYP3A inhibitors with mitapivat should be avoided, and alternative therapies should be considered in place of moderate CYP3A inhibitors during treatment with mitapivat. If co-administered with a moderate CYP3A inhibitor, the dose should be 100 mg once daily. Do not exceed 100 mg once daily.

Coadministration of mitapivat with CYP3A inducers may decrease mitapivat plasma concentrations and may reduce the efficacy of mitapivat. Therefore, coadministration of strong CYP3A inducers with mitapivat should be avoided, and alternative therapies should be considered in place of moderate CYP3A inducers during treatment with mitapivat. If coadministration of a moderate CYP3A inducer is unavoidable, do not exceed a maximum recommended dose of 100 mg BID, patients should be monitored for reduced activity of mitapivat.

Coadministration of mitapivat with sensitive CYP3A substrates may decrease systemic exposure of these medicinal products. Alternative therapies that are not sensitive substrates of CYP3A should be considered while on treatment with mitapivat. If concomitant use of mitapivat with sensitive CYP3A substrates is unavoidable, then patients should be monitored for loss of therapeutic effect of drugs that fall under this category.

Mitapivat may decrease the systemic concentrations of hormonal contraceptives that are sensitive substrates of CYP3A (eg, ethinyl estradiol). Alternative contraceptive methods such as barrier contraceptives, intrauterine devices, implants, or depot contraceptives should be considered.

Based on *in vitro* data, mitapivat may induce UGT1A1 and may decrease systemic exposure to substrates of this enzyme. Patients should be monitored for loss of therapeutic effect of UGT1A1 substrates with a narrow therapeutic index when coadministered with mitapivat.

2.6.8.9. Discontinuation due to adverse events

2.6.8.9.1. Comparative thalassemia safety pool

Discontinuation of study drug

Table 82: Summary of Treatment-Emergent Adverse Events Leading to discontinuation of Study Treatment, by System Organ Class and Preferred Term- Comparative Thalassemia Safety Pool (Safety Analysis Set)

System Organ Class (SOC) Preferred Term (PT)	Study 017		Study 018		Pooled	
	Mitapivat N=129 n (%)	Placebo N=63 n (%)	Mitapivat N=172 n (%)	Placebo N=85 n (%)	Mitapivat N=301 n (%)	Placebo N=148 n (%)
Subjects with events	4 (3.1)	0	10 (5.8)	1 (1.2)	14 (4.7)	1 (0.7)
Gastrointestinal disorders	1 (0.8)	0	2 (1.2)	0	3 (1.0)	0
Abdominal distension	1 (0.8)	0	0	0	1 (0.3)	0
Diarrhoea	0	0	1 (0.6)	0	1 (0.3)	0
Paraesthesia oral	0	0	1 (0.6)	0	1 (0.3)	0
Psychiatric disorders	0	0	2 (1.2)	0	2 (0.7)	0
Anxiety	0	0	1 (0.6)	0	1 (0.3)	0
Initial insomnia	0	0	1 (0.6)	0	1 (0.3)	0
Insomnia	0	0	1 (0.6)	0	1 (0.3)	0
Investigations	1 (0.8)	0	0	1 (1.2)	1 (0.3)	1 (0.7)
Alanine aminotransferase increased	1 (0.8)	0	0	0	1 (0.3)	0
Aspartate aminotransferase increased	1 (0.8)	0	0	0	1 (0.3)	0
Blood bilirubin increased	1 (0.8)	0	0	0	1 (0.3)	0
Blood lactate dehydrogenase increased	1 (0.8)	0	0	0	1 (0.3)	0
International normalised ratio increased	1 (0.8)	0	0	0	1 (0.3)	0
Blood creatine phosphokinase increased	0	0	0	1 (1.2)	0	1 (0.7)
Blood and lymphatic system disorders	1 (0.8)	0	0	0	1 (0.3)	0
Thrombocytopenia	1 (0.8)	0	0	0	1 (0.3)	0
Cardiac disorders	0	0	1 (0.6)	0	1 (0.3)	0
Supraventricular tachycardia	0	0	1 (0.6)	0	1 (0.3)	0
General disorders and administration site conditions	0	0	1 (0.6)	0	1 (0.3)	0
Fatigue	0	0	1 (0.6)	0	1 (0.3)	0
Hepatobiliary disorders	0	0	1 (0.6)	0	1 (0.3)	0
Hypertransaminasaemia	0	0	1 (0.6)	0	1 (0.3)	0
Infections and infestations	0	0	1 (0.6)	0	1 (0.3)	0
Hepatitis C	0	0	1 (0.6)	0	1 (0.3)	0
Musculoskeletal and connective tissue disorders	1 (0.8)	0	0	0	1 (0.3)	0
Arthralgia	1 (0.8)	0	0	0	1 (0.3)	0
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	0	0	1 (0.6)	0	1 (0.3)	0
Hepatic cancer	0	0	1 (0.6)	0	1 (0.3)	0
Renal and urinary disorders	0	0	1 (0.6)	0	1 (0.3)	0
Renal mass	0	0	1 (0.6)	0	1 (0.3)	0

Summarized in order of decreasing frequency of subjects with events based on the frequencies observed for the pooled mitapivat arm.
The denominator used to calculate percentages is N, the number of subjects in the safety analysis set within each treatment group.
Subjects with multiple AEs within a PT are counted only once in that PT.
Subjects with multiple AEs within a SOC are counted only once in that SOC.
MedDRA version 27.0 is used.

Dose interruption

Across the pivotal studies, TEAEs leading to dose interruption of study treatment were reported in 5.0% of subjects who received mitapivat and in 4.1% of subjects who received placebo. For subjects receiving mitapivat

treatment, most TEAEs (by PT) leading to dose interruption occurred in 1 subject each; the TEAE of Influenza led to interruption of mitapivat treatment in 2 subjects.

Dose reduction

Across the pivotal studies, TEAEs leading to dose reduction of study treatment were reported in 9.0% of subjects who received mitapivat and in 2.7% of subjects who received placebo. For subjects receiving mitapivat, most TEAEs (by PT) leading to dose reduction occurred in <1% of subjects; the TEAEs leading to dose reduction of study drug reported for >=1% of subjects were Middle insomnia, Alanine aminotransferase increased, and Dizziness.

2.6.8.9.2. All indications safety set

TEAEs leading to discontinuation of study drug were infrequent in for all indications combined (Table 73), similar to what was observed for the pivotal thalassemia studies.

Table 83: Summary of Treatment-Emergent Adverse Events Leading to discontinuation of Study Treatment, by System Organ Class and Preferred Term- All Indications Safety Pool (Safety Analysis Set)

	PK Deficiency		Sickle Cell Disease		Thalassemia		Pooled	
System Organ Class (SOC) Preferred Term (PT)	Mitapivat N=157 n (%)	Placebo N=39 n (%)	Mitapivat N=52 n (%)	Placebo N=27 n (%)	Mitapivat N=321 n (%)	Placebo N=148 n (%)	Mitapivat N=530 n (%)	Placebo N=214 n (%)
Subjects with events	8 (5.1)	0	0	0	15 (4.7)	1 (0.7)	23 (4.3)	1 (0.5)
Gastrointestinal disorders	1 (0.6)	0	0	0	3 (0.9)	0	4 (0.8)	0
Abdominal distension	0	0	0	0	1 (0.3)	0	1 (0.2)	0
Diarrhoea	0	0	0	0	1 (0.3)	0	1 (0.2)	0
Nausea	1 (0.6)	0	0	0	0	0	1 (0.2)	0
Paraesthesia oral	0	0	0	0	1 (0.3)	0	1 (0.2)	0
Psychiatric disorders	1 (0.6)	0	0	0	2 (0.6)	0	3 (0.6)	0
Anxiety	0	0	0	0	1 (0.3)	0	1 (0.2)	0
Initial insomnia	0	0	0	0	1 (0.3)	0	1 (0.2)	0
Insomnia	0	0	0	0	1 (0.3)	0	1 (0.2)	0
Middle insomnia	1 (0.6)	0	0	0	0	0	1 (0.2)	0
Investigations	1 (0.6)	0	0	0	1 (0.3)	1 (0.7)	2 (0.4)	1 (0.5)
Alanine aminotransferase increased	1 (0.6)	0	0	0	1 (0.3)	0	2 (0.4)	0
Aspartate aminotransferase increased	0	0	0	0	1 (0.3)	0	1 (0.2)	0
Blood bilirubin increased	0	0	0	0	1 (0.3)	0	1 (0.2)	0
Blood lactate dehydrogenase increased	0	0	0	0	1 (0.3)	0	1 (0.2)	0
International normalised ratio increased	0	0	0	0	1 (0.3)	0	1 (0.2)	0
Blood creatine phosphokinase increased	0	0	0	0	0	1 (0.7)	0	1 (0.5)

System Organ Class (SOC) Preferred Term (PT)	PK Deficiency		Sickle Cell Disease		Thalassemia		Pooled	
	Mitapivat N=157 n (%)	Placebo N=39 n (%)	Mitapivat N=52 n (%)	Placebo N=27 n (%)	Mitapivat N=321 n (%)	Placebo N=148 n (%)	Mitapivat N=530 n (%)	Placebo N=214 n (%)
Blood and lymphatic system disorders	1 (0.6)	0	0	0	1 (0.3)	0	2 (0.4)	0
Haemolytic anaemia	1 (0.6)	0	0	0	0	0	1 (0.2)	0
Thrombocytopenia	0	0	0	0	1 (0.3)	0	1 (0.2)	0
Hepatobiliary disorders	1 (0.6)	0	0	0	1 (0.3)	0	2 (0.4)	0
Hypertransaminasaemia	0	0	0	0	1 (0.3)	0	1 (0.2)	0
Non-alcoholic steatohepatitis	1 (0.6)	0	0	0	0	0	1 (0.2)	0
Infections and infestations	1 (0.6)	0	0	0	1 (0.3)	0	2 (0.4)	0
Hepatitis C	0	0	0	0	1 (0.3)	0	1 (0.2)	0
Pharyngitis	1 (0.6)	0	0	0	0	0	1 (0.2)	0
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	1 (0.6)	0	0	0	1 (0.3)	0	2 (0.4)	0
Hepatic cancer	0	0	0	0	1 (0.3)	0	1 (0.2)	0
Renal cell carcinoma	1 (0.6)	0	0	0	0	0	1 (0.2)	0
Renal and urinary disorders	0	0	0	0	2 (0.6)	0	2 (0.4)	0
Renal impairment	0	0	0	0	1 (0.3)	0	1 (0.2)	0
Renal mass	0	0	0	0	1 (0.3)	0	1 (0.2)	0
Cardiac disorders	0	0	0	0	1 (0.3)	0	1 (0.2)	0
Supraventricular tachycardia	0	0	0	0	1 (0.3)	0	1 (0.2)	0
General disorders and administration site conditions	0	0	0	0	1 (0.3)	0	1 (0.2)	0
Fatigue	0	0	0	0	1 (0.3)	0	1 (0.2)	0
Metabolism and nutrition disorders	1 (0.6)	0	0	0	0	0	1 (0.2)	0
Hypertriglyceridaemia	1 (0.6)	0	0	0	0	0	1 (0.2)	0
Musculoskeletal and connective tissue disorders	0	0	0	0	1 (0.3)	0	1 (0.2)	0
Arthralgia	0	0	0	0	1 (0.3)	0	1 (0.2)	0
Nervous system disorders	1 (0.6)	0	0	0	0	0	1 (0.2)	0
Seizure	1 (0.6)	0	0	0	0	0	1 (0.2)	0
Respiratory, thoracic and mediastinal disorders	1 (0.6)	0	0	0	0	0	1 (0.2)	0
Pleural effusion	1 (0.6)	0	0	0	0	0	1 (0.2)	0

Source: AG348 Thal ISS/RMP Table 18.3.1-4.1.2 (Agios internal use only).
Summarized in order of decreasing frequency of subjects with events based on the frequencies observed for the pooled mitapivat arm.
The denominator used to calculate percentages is N, the number of subjects in the safety analysis set within each treatment group.
Subjects with multiple AEs within a PT are counted only once in that PT.

Treatment discontinuation across all indications occurred in 23 (4.3%) patients treated with mitapivat versus 1 (0.5%) receiving placebo. Rate of discontinuations were comparable in the PK deficiency (5.1%) and thalassemia (4.7%) indications.

Dose interruption

TEAEs leading to dose interruption were reported in 5.8% of subjects who received mitapivat (all indications combined), similar to what was observed for the pivotal thalassemia studies. Most TEAEs (by PT) leading to dose interruption occurred in 1 subject each, the TEAEs leading to dose interruption of study drug for >1 subject who received mitapivat were Influenza, Gastroenteritis, Haemolytic anaemia, Cholelithiasis, and Insomnia.

Dose reduction

TEAEs leading to dose reduction were reported in 9.1% of subjects who received mitapivat (all indications combined), similar to what was observed for the pivotal thalassemia studies. Most TEAEs (by PT) leading to dose reduction occurred in <1% of subjects; the TEAE leading to dose reduction of study drug reported for =1% of subjects was Insomnia.

2.6.8.10. Post marketing experience

Mitapivat is approved in the US for the treatment of haemolytic anaemia in adults with pyruvate kinase deficiency and in the EU (as well as Norway, Iceland, and Liechtenstein as part of the centralized authorization procedure) and Great Britain for the treatment of pyruvate kinase deficiency in adult patients. Commercial distribution of PYRUKYND is currently limited to the US. The MAH uses a single pharmacy for commercial mitapivat distribution; therefore, the methodology for capturing patient exposure from marketing experience is expected to represent a 100% capture rate. Currently, there is no need for extrapolation or estimation. A patient is counted in the summary if they receive a shipment of PYRUKYND within a requested time period, and the patient exposure date is the ship date of the medicine to the patient.

Cumulatively, as of 16 August 2024, approximately 197 patients were exposed to mitapivat in the post-marketing setting. Exposure data include all patients who have received mitapivat in the post-marketing setting as of 16 August 2024. In addition, cumulatively, as of 16 August 2024, approximately 161 adult patients with pyruvate kinase deficiency or an Agios approved haemolytic anaemia indication received mitapivat outside of the US through an expanded access program, known as Global Managed Access Program.

Additional safety data from the OLE Periods of Study 017 and Study 018

The updated summaries include data for subjects in Study 017 and Study 018 with up to 127.3 and 127.1 weeks of exposure to mitapivat, respectively, compared with the ISS for thalassemia which included data for subjects in study 017 and Study 018 with up to 28.1 and 59.9 weeks of exposure to mitapivat, respectively.

This update includes 444 subjects with thalassemia who received mitapivat across the pivotal thalassemia clinical studies (Studies 017 and 018) during the Double-blind and OLE Periods compared with the ISS for thalassemia that included 301 subjects with thalassemia who received mitapivat across these pivotal clinical studies during the Double-blind Period only. The additional subjects who received mitapivat are subjects with thalassemia who received placebo during the Double-blind Period and first received mitapivat during the OLE Period. This update also includes the safety data from the Double-blind Period for the 148 subjects who received placebo in the pivotal clinical studies as had been included in the ISS for thalassemia.

Disposition

For the mitapivat and placebo arms, the frequency of subjects who did not enter the OLE Periods was low (6.1% and 3.4%, respectively). As of the data cutoff date for the safety update, most (84.5%) subjects who received mitapivat are ongoing in the studies.

A summary of subject disposition is provided in Table 84 and Table 85 for the safety update and the ISS for thalassemia, respectively.

Table 84: [ISS 90-day Update] Subject Disposition

	Study 017		Study 018		Pooled	
	Mitapivat N=190 n (%)	Placebo N=63 n (%)	Mitapivat N=254 n (%)	Placebo N=85 n (%)	Mitapivat N=444 n (%)	Placebo N=148 n (%)
Disposition: Treatment open-label extension period						
Not Entered	8 (4.2)	2 (3.2)	19 (7.5)	3 (3.5)	27 (6.1)	5 (3.4)
Discontinued	21 (11.1)	0	21 (8.3)	0	42 (9.5)	0
Reason						
Adverse event	5 (2.6)	0	3 (1.2)	0	8 (1.8)	0
Pregnancy	2 (1.1)	0	0	0	2 (0.5)	0
Physician decision	3 (1.6)	0	1 (0.4)	0	4 (0.9)	0
Withdrawal by subject	10 (5.3)	0	17 (6.7)	0	27 (6.1)	0
Lost to follow-up	1 (0.5)	0	0	0	1 (0.2)	0
Completed	0	0	0	0	0	0
Ongoing	161 (84.7)	0	214 (84.3)	0	375 (84.5)	0
Disposition: End of study						
Discontinued	29 (15.3)	2 (3.2)	40 (15.7)	3 (3.5)	69 (15.5)	5 (3.4)
Reason						
Adverse event	7 (3.7)	0	12 (4.7)	1 (1.2)	19 (4.3)	1 (0.7)
Pregnancy	3 (1.6)	0	0	1 (1.2)	3 (0.7)	1 (0.7)
Physician decision	3 (1.6)	0	1 (0.4)	0	4 (0.9)	0
Did not enter extension	1 (0.5)	1 (1.6)	3 (1.2)	0	4 (0.9)	1 (0.7)
Withdrawal by subject	13 (6.8)	1 (1.6)	24 (9.4)	1 (1.2)	37 (8.3)	2 (1.4)
Lost to follow-up	1 (0.5)	0	0	0	1 (0.2)	0
Other	1 (0.5)	0	0	0	1 (0.2)	0
Completed	0	0	0	0	0	0
Ongoing	161 (84.7)	0	214 (84.3)	0	375 (84.5)	0

Table 85: Subject Disposition – Comparative Thalassemia Safety Pool (Safety Analysis Set)

	Study 017		Study 018		Pooled	
	Mitapivat N=129 n (%)	Placebo N=63 n (%)	Mitapivat N=172 n (%)	Placebo N=85 n (%)	Mitapivat N=301 n (%)	Placebo N=148 n (%)
Disposition: Treatment double-blind period						
Discontinued	7 (5.4)	1 (1.6)	16 (9.3)	3 (3.5)	23 (7.6)	4 (2.7)
Reason						
Adverse event	2 (1.6)	0	9 (5.2)	1 (1.2)	11 (3.7)	1 (0.7)
Pregnancy	1 (0.8)	0	0	1 (1.2)	1 (0.3)	1 (0.7)
Withdrawal by subject	3 (2.3)	1 (1.6)	7 (4.1)	1 (1.2)	10 (3.3)	2 (1.4)
Other	1 (0.8)	0	0	0	1 (0.3)	0
Completed	122 (94.6)	62 (98.4)	156 (90.7)	82 (96.5)	278 (92.4)	144 (97.3)
Ongoing	0	0	0	0	0	0

Overall Extent of Exposure

As of the data cutoff date for the safety update, the median (maximum) duration of exposure to mitapivat was 64.79 weeks (127.3 weeks) and the total exposure to mitapivat was 515.22 patient-years. As of the data cutoff date for the ISS for thalassemia, the median (maximum) exposure to mitapivat was 46.86 weeks (59.9 weeks) and the total exposure to mitapivat was 209.36 patient-years (Table 86 and Table 87).

Table 86: [ISS 90-day Update] Summary of Exposure to Study Drug

	Study 017		Study 018		Pooled	
	Mitapivat N=190	Placebo N=63	Mitapivat N=254	Placebo N=85	Mitapivat N=444	Placebo N=148
Duration of exposure (weeks)						
n	190	63	254	85	444	148
Mean	63.13	24.15	58.61	47.43	60.55	37.52
SD	23.372	1.151	27.628	4.543	25.961	12.070
Median	64.36	24.14	65.79	48.00	64.79	46.93
Q1	47.29	24.00	33.43	47.14	39.29	24.14
Q3	78.43	24.43	78.43	48.71	78.43	48.14
Min	1.1	17.6	0.3	14.3	0.3	14.3
Max	127.3	28.0	127.1	53.1	127.3	53.1
Duration of exposure category, n (%)						
>0 - ≤4 weeks	2 (1.1)	0	3 (1.2)	0	5 (1.1)	0
>4 - ≤8 weeks	2 (1.1)	0	2 (0.8)	0	4 (0.9)	0
>8 - ≤12 weeks	3 (1.6)	0	0	0	3 (0.7)	0
>12 - ≤16 weeks	1 (0.5)	0	8 (3.1)	1 (1.2)	9 (2.0)	1 (0.7)
>16 - ≤20 weeks	1 (0.5)	1 (1.6)	16 (6.3)	0	17 (3.8)	1 (0.7)
>20 - ≤24 weeks	2 (1.1)	16 (25.4)	7 (2.8)	0	9 (2.0)	16 (10.8)
>24 - ≤36 weeks	9 (4.7)	46 (73.0)	40 (15.7)	2 (2.4)	49 (11.0)	48 (32.4)
>36 - ≤48 weeks	28 (14.7)	0	15 (5.9)	41 (48.2)	43 (9.7)	41 (27.7)
>48 weeks - ≤2 years	137 (72.1)	0	155 (61.0)	41 (48.2)	292 (65.8)	41 (27.7)
>2 years - ≤3 years	5 (2.6)	0	8 (3.1)	0	13 (2.9)	0
>3 years	0	0	0	0	0	0
Person exposure-years	229.89	29.16	285.33	77.26	515.22	106.42

Table 87: Summary of Exposure to Study Drug – Comparative Thalassemia Safety Pool (Safety Analysis Set)

	Study 017		Study 018		Pooled	
	Mitapivat N=129	Placebo N=63	Mitapivat N=172	Placebo N=85	Mitapivat N=301	Placebo N=148
Duration of exposure (weeks)						
n	129	63	172	85	301	148
Mean	23.60	24.15	45.81	47.43	36.29	37.52
SD	3.526	1.151	9.288	4.543	13.253	12.070
Median	24.14	24.14	48.14	48.00	46.86	46.93
Q1	24.14	24.00	47.29	47.14	24.14	24.14
Q3	24.29	24.43	48.86	48.71	48.29	48.14
Min	1.1	17.6	0.3	14.3	0.3	14.3
Max	28.1	28.0	59.9	53.1	59.9	53.1
Duration of exposure category, n (%)						

	Study 017		Study 018		Pooled	
	Mitapivat N=129	Placebo N=63	Mitapivat N=172	Placebo N=85	Mitapivat N=301	Placebo N=148
>0 - ≤4 weeks	2 (1.6)	0	3 (1.7)	0	5 (1.7)	0
>4 - ≤8 weeks	1 (0.8)	0	1 (0.6)	0	2 (0.7)	0
>8 - ≤12 weeks	1 (0.8)	0	0	0	1 (0.3)	0
>12 - ≤16 weeks	1 (0.8)	0	3 (1.7)	1 (1.2)	4 (1.3)	1 (0.7)
>16 - ≤20 weeks	0	1 (1.6)	2 (1.2)	0	2 (0.7)	1 (0.7)
>20 - ≤24 weeks	27 (20.9)	16 (25.4)	0	0	27 (9.0)	16 (10.8)
>24 - ≤36 weeks	97 (75.2)	46 (73.0)	4 (2.3)	2 (2.4)	101 (33.6)	48 (32.4)
>36 - ≤48 weeks	0	0	55 (32.0)	41 (48.2)	55 (18.3)	41 (27.7)
>48 weeks	0	0	104 (60.5)	41 (48.2)	104 (34.6)	41 (27.7)
Person exposure-years	58.35	29.16	151.01	77.26	209.36	106.42

Dose Modification

In the safety update, AEs were the most common reason for subjects to undergo a dose modification, as had been observed in the ISS for thalassemia.

The overall frequency of subjects who received mitapivat and had at least 1 dose modification was 92 (20.7%) subjects in the safety update (Table 88) and 48 (15.9%) subjects in the ISS for thalassemia (Table 89).

Table 88: [ISS 90-day Update] Summary of Dose Modifications

	Study 017		Study 018		Pooled	
	Mitapivat N=190 n (%)	Placebo N=63 n (%)	Mitapivat N=254 n (%)	Placebo N=85 n (%)	Mitapivat N=444 n (%)	Placebo N=148 n (%)
Subjects with at least 1 dose modification	32 (16.8)	5 (7.9)	60 (23.6)	7 (8.2)	92 (20.7)	12 (8.1)
Reason						
Adverse event	19 (10.0)	3 (4.8)	39 (15.4)	7 (8.2)	58 (13.1)	10 (6.8)
Dose taper due to treatment discontinuation	8 (4.2)	2 (3.2)	22 (8.7)	0	30 (6.8)	2 (1.4)
Other	5 (2.6)	0	5 (2.0)	1 (1.2)	10 (2.3)	1 (0.7)
Subjects with at least 1 dose reduction	26 (13.7)	4 (6.3)	51 (20.1)	2 (2.4)	77 (17.3)	6 (4.1)
Reason						
Adverse event	16 (8.4)	2 (3.2)	30 (11.8)	2 (2.4)	46 (10.4)	4 (2.7)
Dose taper due to treatment discontinuation	8 (4.2)	2 (3.2)	22 (8.7)	0	30 (6.8)	2 (1.4)
Other	2 (1.1)	0	4 (1.6)	1 (1.2)	6 (1.4)	1 (0.7)
Subjects with at least 1 interruption of study drug	8 (4.2)	1 (1.6)	20 (7.9)	5 (5.9)	28 (6.3)	6 (4.1)
Reason						
Adverse event	5 (2.6)	1 (1.6)	16 (6.3)	5 (5.9)	21 (4.7)	6 (4.1)
Other	3 (1.6)	0	4 (1.6)	0	7 (1.6)	0

Table 89: Summary of Dose Modifications – Comparative Thalassemia Safety Pool (Safety Analysis Set)

	Study 017		Study 018		Pooled	
	Mitapivat N=129 n (%)	Placebo N=63 n (%)	Mitapivat N=172 n (%)	Placebo N=85 n (%)	Mitapivat N=301 n (%)	Placebo N=148 n (%)
Subjects with at least 1 dose modification	13 (10.1)	5 (7.9)	35 (20.3)	7 (8.2)	48 (15.9)	12 (8.1)
Reason						
Adverse event	9 (7.0)	3 (4.8)	28 (16.3)	7 (8.2)	37 (12.3)	10 (6.8)
Dose taper due to treatment discontinuation	3 (2.3)	2 (3.2)	8 (4.7)	0	11 (3.7)	2 (1.4)
Other	1 (0.8)	0	1 (0.6)	1 (1.2)	2 (0.7)	1 (0.7)
Subjects with at least 1 dose reduction	10 (7.8)	4 (6.3)	29 (16.9)	2 (2.4)	39 (13.0)	6 (4.1)
Reason						
Adverse event	7 (5.4)	2 (3.2)	22 (12.8)	2 (2.4)	29 (9.6)	4 (2.7)
Dose taper due to treatment discontinuation	3 (2.3)	2 (3.2)	8 (4.7)	0	11 (3.7)	2 (1.4)
Other	0	0	1 (0.6)	1 (1.2)	1 (0.3)	1 (0.7)
Subjects with at least 1 interruption of study drug	3 (2.3)	1 (1.6)	11 (6.4)	5 (5.9)	14 (4.7)	6 (4.1)
Reason						
Adverse event	2 (1.6)	1 (1.6)	11 (6.4)	5 (5.9)	13 (4.3)	6 (4.1)
Other	1 (0.8)	0	0	0	1 (0.3)	0

Analysis of Adverse Events

Given the design of the pivotal clinical studies, where OLE Periods were included to allow continued use of mitapivat beyond a Double-blind Period and initiation of mitapivat for subjects who previously received placebo, the results of the safety update are interpreted in the context of the longer exposure to mitapivat than to placebo. Since all subjects who entered the OLE Period received mitapivat, comparisons between the mitapivat and placebo arms for the safety update tabulations should be interpreted with caution due to the difference in the duration of exposure to mitapivat (which includes data from the Double-blind and OLE Periods) and of exposure to placebo (which includes only data from the Double-blind Period).

The presentation of treatment-related TEAEs is based on Investigator assessment of causality.

Overall Summary of Adverse Events

As of the safety update and consistent with what had been reported in the ISS, in the mitapivat treatment arm, most subjects experienced TEAEs that were nonserious, low grade (Grade 1 or 2), and considered not treatment related by the Investigator. The additional exposure data in the safety update showed that mitapivat continued to be well tolerated.

In the safety update (Table 90) and the ISS for thalassemia (Table 91), 391 (88.1%) and 262 (87.0%) subjects, respectively, had any TEAE. The frequency of subjects who received mitapivat with a TEAE that led to discontinuation of mitapivat was 5.4% in the safety update and 4.7% in the ISS for thalassemia. As of the data cutoff date for the safety update, one subject in Study 017 experienced a TEAE of Pneumonia that led to death and was considered unrelated to study treatment by the Investigator (Deaths).

The overall frequency of subjects experiencing TEAEs in the Infections and infestations SOC was 52.5% in the safety update and 41.2% in the ISS for thalassemia. This is to be expected as there is a high background rate for infections and disease-related factors that predispose patients with thalassemia to infection risk factors

such as anemia, iron overload, and splenectomy or functional asplenia resulting in immunological abnormalities (Ricerca *et al,* 2009). No treatment-related Infections and infestations TEAEs were reported in the OLE Period of the thalassemia studies. The frequency of Infections and infestations Grade ⩾3 TEAEs was 2.6% in the safety update and 1.6% in the ISS for thalassemia. Mitapivat is not an immunosuppressant and is not expected to increase the risk of a subject developing an infection.

Table 90: [ISS 90-day Update] Overall Summary of Treatment-Emergent Adverse Events

	Study 017		Study 018		Pooled	
Number (%) of Subjects with	**Mitapivat N=190 n (%)**	**Placebo N=63 n (%)**	**Mitapivat N=254 n (%)**	**Placebo N=85 n (%)**	**Mitapivat N=444 n (%)**	**Placebo N=148 n (%)**
Any TEAEs	169 (88.9)	50 (79.4)	222 (87.4)	71 (83.5)	391 (88.1)	121 (81.8)
Grade≥3 TEAEs	32 (16.8)	2 (3.2)	49 (19.3)	12 (14.1)	81 (18.2)	14 (9.5)
Treatment-related TEAEs	77 (40.5)	13 (20.6)	84 (33.1)	17 (20.0)	161 (36.3)	30 (20.3)
Grade≥3 treatment-related TEAEs	10 (5.3)	0	16 (6.3)	1 (1.2)	26 (5.9)	1 (0.7)
Serious TEAEs	22 (11.6)	0	38 (15.0)	13 (15.3)	60 (13.5)	13 (8.8)
Serious treatment-related TEAEs	2 (1.1)	0	4 (1.6)	1 (1.2)	6 (1.4)	1 (0.7)
TEAEs leading to discontinuation of study drug	9 (4.7)	0	15 (5.9)	2 (2.4)	24 (5.4)	2 (1.4)
TEAEs leading to dose reduction of study drug	14 (7.4)	2 (3.2)	26 (10.2)	2 (2.4)	40 (9.0)	4 (2.7)
TEAEs leading to interruption of study drug	8 (4.2)	1 (1.6)	19 (7.5)	5 (5.9)	27 (6.1)	6 (4.1)
TEAEs leading to death	1 (0.5)	0	0	0	1 (0.2)	0
Treatment-related TEAEs leading to death	0	0	0	0	0	0

Table 91: Overall Summary of Treatment-Emergent Adverse Events – Comparative Thalassemia Safety Pool (Safety Analysis Set)

	Study 017		Study 018		Pooled	
Number (%) of Subjects with	**Mitapivat N=129 n (%)**	**Placebo N=63 n (%)**	**Mitapivat N=172 n (%)**	**Placebo N=85 n (%)**	**Mitapivat N=301 n (%)**	**Placebo N=148 n (%)**
Any TEAEs	107 (82.9)	50 (79.4)	155 (90.1)	71 (83.5)	262 (87.0)	121 (81.8)
Grade≥3 TEAEs	18 (14.0)	2 (3.2)	32 (18.6)	12 (14.1)	50 (16.6)	14 (9.5)
Treatment-related TEAEs	56 (43.4)	13 (20.6)	65 (37.8)	16 (18.8)	121 (40.2)	29 (19.6)
Grade≥3 treatment-related TEAEs	5 (3.9)	0	13 (7.6)	1 (1.2)	18 (6.0)	1 (0.7)
Serious TEAEs	8 (6.2)	0	19 (11.0)	13 (15.3)	27 (9.0)	13 (8.8)
Serious treatment-related TEAEs	0	0	4 (2.3)	1 (1.2)	4 (1.3)	1 (0.7)
TEAEs leading to discontinuation of study drug	4 (3.1)	0	10 (5.8)	1 (1.2)	14 (4.7)	1 (0.7)
TEAEs leading to dose reduction of study drug	7 (5.4)	2 (3.2)	20 (11.6)	2 (2.4)	27 (9.0)	4 (2.7)
TEAEs leading to interruption of study drug	2 (1.6)	1 (1.6)	13 (7.6)	5 (5.9)	15 (5.0)	6 (4.1)

Number (%) of Subjects with	Study 017		Study 018		Pooled	
	Mitapivat N=129 n (%)	Placebo N=63 n (%)	Mitapivat N=172 n (%)	Placebo N=85 n (%)	Mitapivat N=301 n (%)	Placebo N=148 n (%)
TEAEs leading to death	0	0	0	0	0	0
Treatment-related TEAEs leading to death	0	0	0	0	0	0

Summary of Common Adverse Events, Grade ⩾3 Adverse Events, and Treatment-Related Adverse Events

Summary of Common Adverse Events:

Any-grade TEAEs (by PT) that occurred in ≥10% of all subjects in any treatment group in the safety update (Table 82) and the ISS for thalassemia (Table 83) included Headache, Upper respiratory tract infections, Initial insomnia, Fatigue, and Nausea. The TEAEs of COVID-19 and Arthralgia met this criterion for the safety update only and the TEAE of Diarrhoea met this criterion for the ISS for thalassemia only.

The frequency of subjects who received mitapivat who reported a TEAE by PT of Upper respiratory tract infection was 18.9% in the safety update and 13.6% in the ISS for thalassemia. As noted in the section Overall Summary of Adverse Events this is because patients with thalassemia have a high background rate for infections and have disease-related risk factors that predispose them to infections such as anemia, iron overload, and splenectomy or functional asplenia resulting in immunological abnormalities; mitapivat is not expected to increase the risk of a subject developing an infection.

Table 92: [ISS 90-day Update] Summary of Most Common (Any Grade in ≥10% Subjects in Any Treatment Group) Treatment-Emergent Adverse Events by Preferred Term

Preferred Term (PT)	Study 017		Study 018		Pooled	
	Mitapivat N=190 n (%)	Placebo N=63 n (%)	Mitapivat N=254 n (%)	Placebo N=85 n (%)	Mitapivat N=444 n (%)	Placebo N=148 n (%)
Subjects with events	169 (88.9)	50 (79.4)	222 (87.4)	71 (83.5)	391 (88.1)	121 (81.8)
Headache	40 (21.1)	6 (9.5)	57 (22.4)	10 (11.8)	97 (21.8)	16 (10.8)
Upper respiratory tract infection	33 (17.4)	4 (6.3)	51 (20.1)	13 (15.3)	84 (18.9)	17 (11.5)
Initial insomnia	25 (13.2)	3 (4.8)	26 (10.2)	4 (4.7)	51 (11.5)	7 (4.7)
Fatigue	19 (10.0)	4 (6.3)	24 (9.4)	2 (2.4)	43 (9.7)	6 (4.1)
COVID-19	28 (14.7)	5 (7.9)	13 (5.1)	8 (9.4)	41 (9.2)	13 (8.8)
Arthralgia	15 (7.9)	4 (6.3)	26 (10.2)	5 (5.9)	41 (9.2)	9 (6.1)
Nausea	22 (11.6)	5 (7.9)	18 (7.1)	5 (5.9)	40 (9.0)	10 (6.8)

Table 93: Summary of Most Common (Any Grade in ≥10% Subjects in Any Treatment Group) Treatment-Emergent Adverse Events by Preferred Term – Comparative Thalassemia Safety Pool (Safety Analysis Set)

	Study 017		Study 018		Pooled	
Preferred Term (PT)	Mitapivat N=129 n (%)	Placebo N=63 n (%)	Mitapivat N=172 n (%)	Placebo N=85 n (%)	Mitapivat N=301 n (%)	Placebo N=148 n (%)
Subjects with events	107 (82.9)	50 (79.4)	155 (90.1)	71 (83.5)	262 (87.0)	121 (81.8)
Headache	29 (22.5)	6 (9.5)	46 (26.7)	10 (11.8)	75 (24.9)	16 (10.8)
Initial insomnia	18 (14.0)	3 (4.8)	24 (14.0)	4 (4.7)	42 (14.0)	7 (4.7)
Upper respiratory tract infection	14 (10.9)	4 (6.3)	27 (15.7)	14 (16.5)	41 (13.6)	18 (12.2)
Diarrhoea	11 (8.5)	6 (9.5)	19 (11.0)	7 (8.2)	30 (10.0)	13 (8.8)
Fatigue	12 (9.3)	4 (6.3)	18 (10.5)	2 (2.4)	30 (10.0)	6 (4.1)
Nausea	15 (11.6)	5 (7.9)	13 (7.6)	5 (5.9)	28 (9.3)	10 (6.8)

Summary of Grade ⩾3 Adverse Events:

The frequency of subjects who received mitapivat with Grade ⩾3 TEAEs was 18.2% for the safety update and 16.6% for the ISS for thalassemia. As of the data cutoff date for the safety update, there were no Grade ⩾3 TEAEs occurring in ⩾5% of subjects in the pivotal clinical studies and most Grade ⩾3 TEAEs occurred in 1 subject each, similar to what had been reported in the ISS. The TEAEs (by PT) of Anaemia, Alanine aminotransferase increased, Initial insomnia, and Pneumonia were the only Grade ⩾3 TEAEs that occurred in ⩾1% of subjects treated with mitapivat.

Summary of Treatment-Related Treatment-Emergent Adverse Events:

The frequencies of subjects who received mitapivat with a treatment-related TEAE were 36.3% (any grade) and 5.9% (Grade ⩾3, Table 84) in the safety update and 40.2% (any grade) and 6.0% (Grade ⩾3, Table 85) in the ISS for thalassemia, respectively. As of the data cutoff date for the safety update, there were no treatment-related Grade ⩾3 TEAEs (by PT) that occurred in ⩾1% of subjects. By SOC, treatment-related Grade ⩾3 TEAEs that occurred in ⩾1% of subjects included Investigations, Hepatobiliary disorders, and Psychiatric disorders. The frequency of treatment-related Grade ⩾3 TEAEs within the SOC of Hepatobiliary disorders was higher in Study 017 compared with Study 018 (2.1% vs 0.4%, respectively).

Table 94: [ISS 90-day Update] Summary of Treatment-Emergent Grade ≥3 Adverse Events Related to Study Treatment, by System Organ Class and Preferred Term

	Study 017		Study 018		Pooled	
System Organ Class (SOC) Preferred Term (PT)	Mitapivat N=190 n (%)	Placebo N=63 n (%)	Mitapivat N=254 n (%)	Placebo N=85 n (%)	Mitapivat N=444 n (%)	Placebo N=148 n (%)
Subjects with events	10 (5.3)	0	16 (6.3)	1 (1.2)	26 (5.9)	1 (0.7)
Investigations	2 (1.1)	0	4 (1.6)	1 (1.2)	6 (1.4)	1 (0.7)
Alanine aminotransferase increased	1 (0.5)	0	3 (1.2)	0	4 (0.9)	0
Aspartate aminotransferase increased	1 (0.5)	0	2 (0.8)	0	3 (0.7)	0
Blood bilirubin increased	2 (1.1)	0	0	0	2 (0.5)	0

System Organ Class (SOC) Preferred Term (PT)	Study 017		Study 018		Pooled	
	Mitapivat N=190 n (%)	Placebo N=63 n (%)	Mitapivat N=254 n (%)	Placebo N=85 n (%)	Mitapivat N=444 n (%)	Placebo N=148 n (%)
Blood lactate dehydrogenase increased	1 (0.5)	0	0	0	1 (0.2)	0
Blood pressure increased	0	0	1 (0.4)	0	1 (0.2)	0
International normalised ratio increased	1 (0.5)	0	0	0	1 (0.2)	0
Blood creatine phosphokinase increased	0	0	0	1 (1.2)	0	1 (0.7)
Hepatobiliary disorders	4 (2.1)	0	1 (0.4)	0	5 (1.1)	0
Hypertransaminasaemia	2 (1.1)	0	1 (0.4)	0	3 (0.7)	0
Drug-induced liver injury	1 (0.5)	0	0	0	1 (0.2)	0
Hepatitis acute	1 (0.5)	0	0	0	1 (0.2)	0
Psychiatric disorders	2 (1.1)	0	3 (1.2)	0	5 (1.1)	0
Initial insomnia	2 (1.1)	0	2 (0.8)	0	4 (0.9)	0
Insomnia	0	0	1 (0.4)	0	1 (0.2)	0
Middle insomnia	0	0	1 (0.4)	0	1 (0.2)	0
Musculoskeletal and connective tissue disorders	1 (0.5)	0	2 (0.8)	0	3 (0.7)	0
Arthralgia	0	0	1 (0.4)	0	1 (0.2)	0
Back pain	0	0	1 (0.4)	0	1 (0.2)	0
Joint swelling	1 (0.5)	0	0	0	1 (0.2)	0
Blood and lymphatic system disorders	2 (1.1)	0	0	0	2 (0.5)	0
Anaemia	1 (0.5)	0	0	0	1 (0.2)	0
Thrombocytopenia	1 (0.5)	0	0	0	1 (0.2)	0
Cardiac disorders	0	0	2 (0.8)	0	2 (0.5)	0
Arrhythmia supraventricular	0	0	1 (0.4)	0	1 (0.2)	0
Supraventricular tachycardia	0	0	1 (0.4)	0	1 (0.2)	0
Metabolism and nutrition disorders	2 (1.1)	0	0	0	2 (0.5)	0
Hypertriglyceridaemia	1 (0.5)	0	0	0	1 (0.2)	0
Hypoglycaemia	1 (0.5)	0	0	0	1 (0.2)	0
Gastrointestinal disorders	0	0	1 (0.4)	0	1 (0.2)	0
Abdominal distension	0	0	1 (0.4)	0	1 (0.2)	0
Infections and infestations	0	0	1 (0.4)	0	1 (0.2)	0
Lower respiratory tract infection	0	0	1 (0.4)	0	1 (0.2)	0
Nervous system disorders	0	0	1 (0.4)	0	1 (0.2)	0
Peripheral sensorimotor neuropathy	0	0	1 (0.4)	0	1 (0.2)	0
Renal and urinary disorders	0	0	1 (0.4)	0	1 (0.2)	0
Renal mass	0	0	1 (0.4)	0	1 (0.2)	0
Skin and subcutaneous tissue disorders	0	0	1 (0.4)	0	1 (0.2)	0
Rash	0	0	1 (0.4)	0	1 (0.2)	0

Table 95: Summary of Treatment-Emergent Grade ≥3 Adverse Events Related to Study Treatment, by System Organ Class and Preferred Term – Comparative Thalassemia Safety Pool (Safety Analysis Set)

System Organ Class (SOC) Preferred Term (PT)	Study 017		Study 018		Pooled	
	Mitapivat N=129 n (%)	Placebo N=63 n (%)	Mitapivat N=172 n (%)	Placebo N=85 n (%)	Mitapivat N=301 n (%)	Placebo N=148 n (%)
Subjects with events	5 (3.9)	0	13 (7.6)	1 (1.2)	18 (6.0)	1 (0.7)
Investigations	1 (0.8)	0	4 (2.3)	1 (1.2)	5 (1.7)	1 (0.7)
Alanine aminotransferase increased	1 (0.8)	0	3 (1.7)	0	4 (1.3)	0
Aspartate aminotransferase increased	1 (0.8)	0	2 (1.2)	0	3 (1.0)	0
Blood bilirubin increased	1 (0.8)	0	0	0	1 (0.3)	0
Blood lactate dehydrogenase increased	1 (0.8)	0	0	0	1 (0.3)	0
Blood pressure increased	0	0	1 (0.6)	0	1 (0.3)	0
International normalised ratio increased	1 (0.8)	0	0	0	1 (0.3)	0
Blood creatine phosphokinase increased	0	0	0	1 (1.2)	0	1 (0.7)
Psychiatric disorders	1 (0.8)	0	3 (1.7)	0	4 (1.3)	0
Initial insomnia	1 (0.8)	0	2 (1.2)	0	3 (1.0)	0
Insomnia	0	0	1 (0.6)	0	1 (0.3)	0
Middle insomnia	0	0	1 (0.6)	0	1 (0.3)	0
Cardiac disorders	0	0	2 (1.2)	0	2 (0.7)	0
Arrhythmia supraventricular	0	0	1 (0.6)	0	1 (0.3)	0
Supraventricular tachycardia	0	0	1 (0.6)	0	1 (0.3)	0
Hepatobiliary disorders	1 (0.8)	0	1 (0.6)	0	2 (0.7)	0
Drug-induced liver injury	1 (0.8)	0	0	0	1 (0.3)	0
Hypertransaminasaemia	0	0	1 (0.6)	0	1 (0.3)	0
Blood and lymphatic system disorders	1 (0.8)	0	0	0	1 (0.3)	0
Thrombocytopenia	1 (0.8)	0	0	0	1 (0.3)	0
Gastrointestinal disorders	0	0	1 (0.6)	0	1 (0.3)	0
Abdominal distension	0	0	1 (0.6)	0	1 (0.3)	0
Infections and infestations	0	0	1 (0.6)	0	1 (0.3)	0
Lower respiratory tract infection	0	0	1 (0.6)	0	1 (0.3)	0
Metabolism and nutrition disorders	1 (0.8)	0	0	0	1 (0.3)	0
Hypertriglyceridaemia	1 (0.8)	0	0	0	1 (0.3)	0
Musculoskeletal and connective tissue disorders	1 (0.8)	0	0	0	1 (0.3)	0
Joint swelling	1 (0.8)	0	0	0	1 (0.3)	0
Renal and urinary disorders	0	0	1 (0.6)	0	1 (0.3)	0
Renal mass	0	0	1 (0.6)	0	1 (0.3)	0

Deaths

As of the data cutoff date for the safety update, 1 subject who received mitapivat in Study 017 experienced a TEAE of Pneumonia that led to death during the OLE Period after receiving mitapivat for 19 weeks. The TEAE of Pneumonia was considered unrelated to study treatment.

Other Serious Adverse Events

As of the data cutoff date for the safety update, most serious TEAEs occurred in 1 subject and the frequency of subjects with serious treatment-related TEAEs was low (Table 86), similar to what had been reported in the ISS.

The frequency of subjects who received mitapivat and experienced a serious TEAEs was 13.5% in the safety update and 9.0% in the ISS for thalassemia. As of the data cutoff date for the safety update, the serious TEAEs (by PT) of Pneumonia, Gastroenteritis, Cellulitis, Lower respiratory tract infection, Parvovirus infection, Cholecystitis acute, Cholecystitis, Anaemia, Hypersplenism, Abdominal pain, and Gastritis occurred in $\geqslant$2 subjects. The frequency of subjects who received mitapivat and experienced treatment-related serious TEAEs was 1.4% in the safety update and 1.3% in the ISS for thalassemia. Two subjects experienced new treatment-related serious TEAEs as of the data cutoff date for the safety update: Hepatitis acute and Hypertransaminasemia in 1 subject each. Safety narratives for these subjects were provided in the ISS for thalassemia as these events were 2 of the 5 events of hepatocellular injury that led to the important potential risk of hepatocellular injury in thalassemia.

Table 96: [ISS 90-day Update] Summary of Serious Treatment-Emergent Adverse Events Related to Study Treatment, by System Organ Class and Preferred Term

System Organ Class (SOC) Preferred Term (PT)	Study 017		Study 018		Pooled	
	Mitapivat N=190 n (%)	Placebo N=63 n (%)	Mitapivat N=254 n (%)	Placebo N=85 n (%)	Mitapivat N=444 n (%)	Placebo N=148 n (%)
Subjects with events	2 (1.1)	0	4 (1.6)	1 (1.2)	6 (1.4)	1 (0.7)
Cardiac disorders	0	0	2 (0.8)	0	2 (0.5)	0
Arrhythmia supraventricular	0	0	1 (0.4)	0	1 (0.2)	0
Supraventricular tachycardia	0	0	1 (0.4)	0	1 (0.2)	0
Hepatobiliary disorders	2 (1.1)	0	0	0	2 (0.5)	0
Hepatitis acute	1 (0.5)	0	0	0	1 (0.2)	0
Hypertransaminasaemia	1 (0.5)	0	0	0	1 (0.2)	0
Infections and infestations	0	0	1 (0.4)	0	1 (0.2)	0
Lower respiratory tract infection	0	0	1 (0.4)	0	1 (0.2)	0
Renal and urinary disorders	0	0	1 (0.4)	0	1 (0.2)	0
Renal mass	0	0	1 (0.4)	0	1 (0.2)	0
Investigations	0	0	0	1 (1.2)	0	1 (0.7)
Blood creatine phosphokinase increased	0	0	0	1 (1.2)	0	1 (0.7)

Table 97: Summary of Serious Treatment-Emergent Adverse Events Related to Study Treatment, by System Organ Class and Preferred Term – Comparative Thalassemia Safety Pool (Safety Analysis Set)

System Organ Class (SOC) Preferred Term (PT)	Study 017		Study 018		Pooled	
	Mitapivat N=129 n (%)	Placebo N=63 n (%)	Mitapivat N=172 n (%)	Placebo N=85 n (%)	Mitapivat N=301 n (%)	Placebo N=148 n (%)
Subjects with events	0	0	4 (2.3)	1 (1.2)	4 (1.3)	1 (0.7)
Cardiac disorders	0	0	2 (1.2)	0	2 (0.7)	0
Arrhythmia supraventricular	0	0	1 (0.6)	0	1 (0.3)	0
Supraventricular tachycardia	0	0	1 (0.6)	0	1 (0.3)	0
Infections and infestations	0	0	1 (0.6)	0	1 (0.3)	0
Lower respiratory tract infection	0	0	1 (0.6)	0	1 (0.3)	0
Renal and urinary disorders	0	0	1 (0.6)	0	1 (0.3)	0
Renal mass	0	0	1 (0.6)	0	1 (0.3)	0
Investigations	0	0	0	1 (1.2)	0	1 (0.7)
Blood creatine phosphokinase increased	0	0	0	1 (1.2)	0	1 (0.7)

Other Adverse Events of Interest

Hepatocellular Injury in Thalassemia

The ISS for thalassemia described 5 subjects with thalassemia, including 3 subjects in the OLE Period, who received mitapivat 100 mg BID and had events of hepatocellular injury in Studies 017 and 018 that led to discontinuation of mitapivat with subsequent improvement of liver tests. Although 3 subjects experienced events during the OLE Period, they were included in the ISS for thalassemia as part of the evaluation of this new important potential risk. No additional events of hepatocellular injury in thalassemia related to mitapivat have been reported as of the data cutoff date for the safety update.

Adverse Events Leading to Discontinuation of Study Treatment

As of the data cutoff date for the safety update, TEAEs leading to discontinuation of study drug were infrequent (Table 88), similar to what had been reported in the ISS for thalassemia (Table 89).

The frequency of subjects who received mitapivat with TEAEs that led to discontinuation of mitapivat was 5.4% in the safety update and 4.7% in the ISS for thalassemia. Most TEAEs leading to discontinuation of mitapivat occurred in 1 subject each; the TEAEs (by PT) of Abdominal distension, Hypertransaminasaemia, and Blood bilirubin increased led to discontinuation of mitapivat in $\geqslant$2 subjects. For the placebo group, there is 1 event of Initial insomnia that started during the Double-blind Period but did not lead to discontinuation of study treatment until after the subject initiated mitapivat in the OLE Period (and, therefore, was not included as a TEAE leading to discontinuation of study treatment in the ISS for thalassemia).

Table 98: [ISS 90-day Update] Summary of Treatment-Emergent Adverse Events Leading to Discontinuation of Study Treatment, by System Organ Class and Preferred Term

System Organ Class (SOC) Preferred Term (PT)	Study 017		Study 018		Pooled	
	Mitapivat N=190 n (%)	Placebo N=63 n (%)	Mitapivat N=254 n (%)	Placebo N=85 n (%)	Mitapivat N=444 n (%)	Placebo N=148 n (%)
Subjects with events	9 (4.7)	0	15 (5.9)	2 (2.4)	24 (5.4)	2 (1.4)
Gastrointestinal disorders	1 (0.5)	0	3 (1.2)	0	4 (0.9)	0
Abdominal distension	1 (0.5)	0	1 (0.4)	0	2 (0.5)	0
Diarrhoea	0	0	1 (0.4)	0	1 (0.2)	0
Dyspepsia	0	0	1 (0.4)	0	1 (0.2)	0
Paraesthesia oral	0	0	1 (0.4)	0	1 (0.2)	0
Hepatobiliary disorders	3 (1.6)	0	1 (0.4)	0	4 (0.9)	0
Hypertransaminasaemia	2 (1.1)	0	1 (0.4)	0	3 (0.7)	0
Hepatitis acute	1 (0.5)	0	0	0	1 (0.2)	0
Psychiatric disorders	0	0	3 (1.2)	1 (1.2)	3 (0.7)	1 (0.7)
Initial insomnia	0	0	1 (0.4)	1 (1.2)	1 (0.2)	1 (0.7)
Anxiety	0	0	1 (0.4)	0	1 (0.2)	0
Depressed mood	0	0	1 (0.4)	0	1 (0.2)	0
Insomnia	0	0	1 (0.4)	0	1 (0.2)	0
Investigations	2 (1.1)	0	0	1 (1.2)	2 (0.5)	1 (0.7)
Blood bilirubin increased	2 (1.1)	0	0	0	2 (0.5)	0
Alanine aminotransferase increased	1 (0.5)	0	0	0	1 (0.2)	0
Aspartate aminotransferase increased	1 (0.5)	0	0	0	1 (0.2)	0
Blood lactate dehydrogenase increased	1 (0.5)	0	0	0	1 (0.2)	0
International normalised ratio increased	1 (0.5)	0	0	0	1 (0.2)	0
Blood creatine phosphokinase increased	0	0	0	1 (1.2)	0	1 (0.7)
Blood and lymphatic system disorders	2 (1.1)	0	0	0	2 (0.5)	0
Anaemia	1 (0.5)	0	0	0	1 (0.2)	0
Thrombocytopenia	1 (0.5)	0	0	0	1 (0.2)	0
Musculoskeletal and connective tissue disorders	1 (0.5)	0	1 (0.4)	0	2 (0.5)	0
Arthralgia	1 (0.5)	0	0	0	1 (0.2)	0
Back pain	0	0	1 (0.4)	0	1 (0.2)	0
Nervous system disorders	0	0	2 (0.8)	0	2 (0.5)	0
Headache	0	0	1 (0.4)	0	1 (0.2)	0
Peripheral sensorimotor neuropathy	0	0	1 (0.4)	0	1 (0.2)	0
Cardiac disorders	0	0	1 (0.4)	0	1 (0.2)	0
Supraventricular tachycardia	0	0	1 (0.4)	0	1 (0.2)	0
General disorders and administration site conditions	0	0	1 (0.4)	0	1 (0.2)	0

System Organ Class (SOC) Preferred Term (PT)	Study 017		Study 018		Pooled	
	Mitapivat N=190 n (%)	Placebo N=63 n (%)	Mitapivat N=254 n (%)	Placebo N=85 n (%)	Mitapivat N=444 n (%)	Placebo N=148 n (%)
Fatigue	0	0	1 (0.4)	0	1 (0.2)	0
Infections and infestations	0	0	1 (0.4)	0	1 (0.2)	0
Hepatitis C	0	0	1 (0.4)	0	1 (0.2)	0
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	0	0	1 (0.4)	0	1 (0.2)	0
Hepatic cancer	0	0	1 (0.4)	0	1 (0.2)	0
Renal and urinary disorders	0	0	1 (0.4)	0	1 (0.2)	0
Renal mass	0	0	1 (0.4)	0	1 (0.2)	0
Vascular disorders	1 (0.5)	0	0	0	1 (0.2)	0
Superficial vein thrombosis	1 (0.5)	0	0	0	1 (0.2)	0

Table 99: Summary of Treatment-Emergent Adverse Events Leading to Discontinuation of Study Treatment, by System Organ Class and Preferred Term – Comparative Thalassemia Safety Pool (Safety Analysis Set)

System Organ Class (SOC) Preferred Term (PT)	Study 017		Study 018		Pooled	
	Mitapivat N=129 n (%)	Placebo N=63 n (%)	Mitapivat N=172 n (%)	Placebo N=85 n (%)	Mitapivat N=301 n (%)	Placebo N=148 n (%)
Subjects with events	4 (3.1)	0	10 (5.8)	1 (1.2)	14 (4.7)	1 (0.7)
Gastrointestinal disorders	1 (0.8)	0	2 (1.2)	0	3 (1.0)	0
Abdominal distension	1 (0.8)	0	0	0	1 (0.3)	0
Diarrhoea	0	0	1 (0.6)	0	1 (0.3)	0
Paraesthesia oral	0	0	1 (0.6)	0	1 (0.3)	0
Psychiatric disorders	0	0	2 (1.2)	0	2 (0.7)	0
Anxiety	0	0	1 (0.6)	0	1 (0.3)	0
Initial insomnia	0	0	1 (0.6)	0	1 (0.3)	0
Insomnia	0	0	1 (0.6)	0	1 (0.3)	0
Investigations	1 (0.8)	0	0	1 (1.2)	1 (0.3)	1 (0.7)
Alanine aminotransferase increased	1 (0.8)	0	0	0	1 (0.3)	0
Aspartate aminotransferase increased	1 (0.8)	0	0	0	1 (0.3)	0
Blood bilirubin increased	1 (0.8)	0	0	0	1 (0.3)	0
Blood lactate dehydrogenase increased	1 (0.8)	0	0	0	1 (0.3)	0
International normalised ratio increased	1 (0.8)	0	0	0	1 (0.3)	0
Blood creatine phosphokinase increased	0	0	0	1 (1.2)	0	1 (0.7)
Blood and lymphatic system disorders	1 (0.8)	0	0	0	1 (0.3)	0

System Organ Class (SOC) Preferred Term (PT)	Study 017		Study 018		Pooled	
	Mitapivat N=129 n (%)	Placebo N=63 n (%)	Mitapivat N=172 n (%)	Placebo N=85 n (%)	Mitapivat N=301 n (%)	Placebo N=148 n (%)
Thrombocytopenia	1 (0.8)	0	0	0	1 (0.3)	0
Cardiac disorders	0	0	1 (0.6)	0	1 (0.3)	0
Supraventricular tachycardia	0	0	1 (0.6)	0	1 (0.3)	0
General disorders and administration site conditions	0	0	1 (0.6)	0	1 (0.3)	0
Fatigue	0	0	1 (0.6)	0	1 (0.3)	0
Hepatobiliary disorders	0	0	1 (0.6)	0	1 (0.3)	0
Hypertransaminasaemia	0	0	1 (0.6)	0	1 (0.3)	0
Infections and infestations	0	0	1 (0.6)	0	1 (0.3)	0
Hepatitis C	0	0	1 (0.6)	0	1 (0.3)	0
Musculoskeletal and connective tissue disorders	1 (0.8)	0	0	0	1 (0.3)	0
Arthralgia	1 (0.8)	0	0	0	1 (0.3)	0
Neoplasms benign, malignant and unspecified (incl cysts and polyps)	0	0	1 (0.6)	0	1 (0.3)	0
Hepatic cancer	0	0	1 (0.6)	0	1 (0.3)	0
Renal and urinary disorders	0	0	1 (0.6)	0	1 (0.3)	0
Renal mass	0	0	1 (0.6)	0	1 (0.3)	0

Treatment-Emergent Adverse Events Leading to Dose Interruption of Study Treatment

The frequency of mitapivat-treated subjects with TEAEs that led to interruption of mitapivat was similar as of the data cutoff dates for the safety update and the ISS for thalassemia: 6.1% and 5.0%, respectively. Most TEAEs leading to interruption of mitapivat occurred in 1 subject each; the TEAEs (by PT) of Gastroenteritis, COVID-19, Influenza, Hypersplenism, and Cholecystitis led to interruption of mitapivat in ≥2 subjects.

Treatment-Emergent Adverse Events Leading to Dose Reduction of Study Treatment

The frequency of mitapivat-treated subjects with TEAEs that led to dose reduction of mitapivat was 9.0% in both the safety update and the ISS for thalassemia. Further, as of the data cutoff date for the safety update, most TEAEs leading to dose reduction occurred in <1% of subjects, and there were no TEAEs (by PT) leading to dose reduction of study drug reported for ≥1% of subjects, similar to what had been reported in the ISS.

2.6.9. Discussion on clinical safety

The main safety analysis supporting the thalassemia indication includes safety data from 2 pivotal phase 3 individual studies (017 and 018) and a pooled analysis also including a phase 2 study (010). In addition, supportive pooled analysis from several studies in different indications (pyruvate kinase deficiency and SCD), by treatment arm, by indication, and pooled, is presented allowing for an overall analysis to the safety profile across all indications.

In the thalassemia pool, in the 2 pivotal studies, 301 patients were treated with mitapivat 100mg BID and 148 with placebo and the mean duration of exposure was 36 weeks (min 0.3, max 59.9 weeks). In an updated safety analysis with a more recent data cutoff (DCO), including the open label period (OLE), 464 subjects with thalassemia received mitapivat, including 309 for more 12 months, and 292 (65.8%) subjects for >48 weeks to 2 years. The extent of exposure for the 464 subjects was 14.8 months (0.1-47.7 months). For the 309 subjects with >12 months exposure was 18.7 months (12.1-47.7 months). In study 010, 20 patients received 50mg BID for 6 weeks and, from week 6, 19 patients received 100mg BID and were followed for up to 207 weeks. The pivotal studies are ongoing for a duration of up to 5 years and study 010 includes an extension period of up to 10 years. The size of the safety database is considered sufficient to address the safety profile in the thalassemia indication, however the duration of follow-up in the pivotal studies is limited and long-term safety for more than 2 years is available for very few patients.

In the all indications safety pool, 530 patients were treated with mitapivat (at different dose levels) and 214 with placebo. Across all indications, the mean duration of safety exposure was 69.1 weeks (min 0.3, max 430.4 weeks), with 64 patients with > 3 years of follow-up and 18 patients with > 5 years follow-up.

Adverse events

The adverse events were considered related to study treatment if occurring or worsening during study drug treatment; events were counted once per patient, with the highest grade reported in the assessment of severity or causality.

In the pivotal thalassemia safety pool, the frequency of any AE was 87.0% in mitapivat treated and 81.8% in placebo treated subjects. In both studies the frequency of any TEAE was comparable in mitapivat and placebo arms.

In study 017 (NTDT), the frequency was substantially higher in the mitapivat versus placebo arm for Grade $\geqslant$ 3 treatment related TEAE (3.9% vs 0%), for Grade $\geqslant$ 3 TEAE (14% vs 3.2%), treatment related TEAEs (43.4 vs 20.6%), serious TEAE (6.2% vs 0%) and TEAE leading to discontinuation of study drug (3.1% vs 0%).

In study 018 (TDT), the frequency was substantially higher in the mitapivat versus placebo arm for Grade $\geqslant$ 3 treatment related TEAE (7.6% vs 1.2%), treatment related TEAE (37.8% vs 18.8%), TEAE leading to dose reduction of study drug (11.6% vs 2.4%).

In studies 017 and 018 there were no TEAE or treatment related TEAE leading to death.

The most common AE in the pooled pivotal thalassemia set occurred in the SOC of Infections and infestations (41.2% mitapivat and 41.9% placebo), Nervous system disorders (30.9% mitapivat and 18.2% placebo), GI disorders (29.9% mitapivat and 27.0% placebo), Psychiatric disorders (25.9% mitapivat and 11.5% placebo), Musculoskeletal and connective tissue disorders (24.6% mitapivat and 21.6% placebo), General disorders and administration site conditions (24.6% mitapivat and 18.9% placebo).

By PT, the most common AE occurring in the pooled thalassemia set included headache (24.9%), initial

insomnia (14.0%), upper respiratory tract infections (13.6%), diarrhoea, fatigue (10% each), and nausea (9.3%). All AE, except diarrhoea, occurred more frequently in mitapivat arm versus placebo. The frequency was slightly higher in study 018 than 017.

In the updated analysis including patients exposed for more than 12 months, the overall frequency of adverse events was comparable to the initial analysis with no new safety signals and the safety profile for exposure up to 2 years appears manageable.

Treatment related AE

In patients treated with mitapivat, treatment related TEAE occurred in 56 (43.4%) patients in study 017 and 65 (37.8%) patients in study 018, with higher frequency than in placebo (20.6% and 18.8% respectively). The most frequent treatment related events by SOC were psychiatric disorders e.g. (19.6% vs 6.8%), and nervous system disorders (16.3% vs 6.8%) and by PT insomnia (19.2% vs 6.1%) and respectively headache (15.0% vs 6.1%). Insomnia and headache are considered ADR.

In study 017, treatment related TEAE were Grade 1 in 29 patients (22.5%), Grade 2 in 22 patients (17.1%), Grade 3 in 5 patients (3.9%) and none were Grade 4 or 5.

In study 018, treatment related TEAE were Grade 1 in 26 patients (15.1%), Grade 2 in 26 patients (15.1%), Grade 3 in 12 patients (7%), Grade 4 in 1 patient (0.6%) and none of Grade 5.

Grade ⩾ 3 TEAE

Grade ⩾ 3 TEAE in mitapivat vs placebo occurred in 18 (14.0%) vs 2 (3.2%) patients in study 017 and 32 (18.6%) vs 12 (14.1%) patients in study 018. According to the MAH, PT of Grade ⩾ 3 TEAE with frequency ⩾5% did not occur in any subject. In study 017, the treatment related Grade ⩾ 3 TEAE were initial insomnia, joint swelling, drug-induced liver injury, hypertrygliceridaemia, thrombocytopenia, ALT/AST increased, international normalised ratio increased, bilirubin increased, LDH increased. In study 018, The Grade ⩾ 3 events were: insomnia (2.4%), ALT increased (1.7%), AST increased (n=2, 1.2%), abdominal distension, blood pressure increased, arrhythmia supraventricular and supraventricular tachycardia, hypertransaminasaemia, and lower respiratory tract infection (0.6% each). The Grade 4 event was renal mass (0.6%).

In the pooled all indications set, the most common AE (>10%) were slightly higher in the mitapivat treated (90.6%) than in placebo (83.2%), similarly in the PK deficiency and thalassemia safety sets. In patients treated with mitapivat, the frequency of TEAE was higher (almost twice) in the PK deficiency pooled set than the Thalassemia pooled set except for TEAE leading to discontinuation of study drug and dose reduction.

The most common AE in the PK deficiency safety set in subjects treated with mitapivat occurred in the SOC of Infections and infestations (75.2%), GI disorders (61.1%), Nervous system disorders (59.2%), General disorders and administration site conditions (55.4%), Musculoskeletal and connective tissue disorders (52.2%), and Psychiatric disorders (44.6%), consistent with the known safety profile in this indication. Overall the most common AE occurred in the same SOC for the thalassemia and PK deficiency indication.

In patients treated with mitapivat, treatment related TEAE occurred in 113 patients (72.0%) vs 14 (35.9%) placebo in PK deficiency and respectively 134 (41.7%) vs 29 (19.6%) placebo in thalassemia.

Serious adverse events

In the thalassemia indication pool, SAE occurred in 9% of patients treated with mitapivat and 8.8% patients treated with placebo. In the DBP, no SAE leading to death was reported.

Serious TEAE occurred in 8 (6.2%) patients treated with mitapivat versus 0 in placebo in study 017 and 19

(11%) patients versus 13 (15.3%) in placebo in study 018; the imbalance between the frequency of SAE in the placebo arm of the 2 studies is explained by the baseline characteristics (burden of disease).

In the pooled pivotal thalassemia set, serious AE occurring in more than one patient were in the SOC of Infections and infestations, Blood and lymphatic system disorders, Cardiac disorders, Injury, Hepatobiliary disorders, GI disorders.

In study 017, in mitapivat treated patients, there was no SAE occurring in more than 1 patient and no SAE was considered related to study treatment. No SAE occurred in Placebo. Two SAE of hypertransaminasaemia related to mitapivat of hypertransaminasaemia formed the basis for proposing hepatocellular injury as 'important potential risk' in thalassemia in the safety specifications in the RMP

In study 018, SAE were reported in 19 subjects (11%) treated with mitapivat and 13 subjects (15.3%) with placebo. The SAE related to study treatment were Grade 3 Arrhythmia supraventricular, supraventricular tachycardia, lower respiratory tract infection, and Grade 4 renal mass.

No deaths occurred in the DBP period in thalassemia studies); there was one fatal TEAE of Pneumonia in study 017 OLE, which was considered unrelated to study treatment.

In the all indications safety pool, SAE occurred in 15.5% patients who received mitapivat versus 8.4% Placebo. The frequency of SAE in the thalassemia indication (9.3% mitapivat and 8.8% placebo) was lower than in the PK deficiency (29.3% mitapivat and 5% placebo) indication and occurred globally in the same SOC as in PK deficiency (a higher frequency of SAE in the SOC 'Cardiac disorders'. Is reported in thalassemia).

AESI

No AESI were identified for the thalassemia indication clinical studies.

The AESI acute haemolysis upon abrupt treatment withdrawal identified for the PK deficiency indication was not considered applicable to thalassemia and no events were observed in thalassemia clinical studies upon dose modification (reduction or treatment abrupt discontinuation).

Hepatocellular injury is identified as a risk for patients treated for thalassemia. In studies 017 and 018, discontinuation of mitapivat treatment due to events of hepatocellular injury occurred in 5 subjects: 2 in the double-blind period and 3 in the open label extension (OLE).

The MAH was requested to discuss causality between mitapivat and the adverse event of hepatocellular injury that is identified in the thalassemia setting. The MAH considered that hypertransaminasaemia is the most appropriate term to characterise this event which indeed is considered related to mitapivat for the thalassemia indication. This is acknowledged. Because of this risk, liver tests should be obtained prior to the initiation of mitapivat and monthly thereafter for the first 6 months and as clinically indicated. Mitapivat should be interrupted if clinically significant increases in liver tests are observed or alanine aminotransferase is > 5 times the ULN. Mitapivat should be discontinued if hepatic injury due to mitapivat treatment is suspected. Hypertransaminasaemia and the related precautions are described in the SmPC section 4.4 and 4.8.

Discontinuations due to AEs

In the thalassemia safety set, TEAE leading to treatment modification were rather infrequent and overall occurred more in the mitapivat than placebo treated patients. TEAE leading to dose discontinuation were reported in 4.7% of patients on mitapivat vs 0.7% in placebo; dose interruptions in 5.0% in mitapivat vs 4.1% in placebo, and dose reduction in 9.0% in mitapivat vs 2.7% in placebo. In patients with exposure for

more than 12 months, the rate of discontinuation was 6.8%.

In study 017: 4 patients (3.1%) treated with mitapivat and none in placebo discontinued treatment. The events were thrombocytopenia, arthralgia (1 patient each) and abdominal distention, ALT, AST, blood bilirubin, blood LDH, and international normalised rate increased (1 patient). The events were considered related to study drug; thrombocytopenia and arthralgia were not resolved after discontinuation. Seven patients (5.4%) experienced dose reduction, with the following AE: hypertrigliceridaemia, joint swelling, atrial fibrillation, LRTI, fatigue, thrombocytopenia and abdominal distension, and DILI. These events were considered related to study drug. Though the DILI event resolved after dose reduction to QD and did not reoccur after BID treatment was resumed, the relationship to study drug could not be ruled out. Thrombocytopenia and abdominal distension (led to discontinuation), dizziness, and nausea (led to patient withdrawal) and joint swelling did not resolve.

In study 018, 10 patients (5.8%) treated with mitapivat and 1 patient treated with placebo had TEAE leading to treatment discontinuation. A serious event (SAE) of blood creatine phosphokinase increased occurred in a patient treated with placebo and required hospitalization. In patients treated with mitapivat, the TEAE were (by PT): diarrhoea, paraesthesia oral, initial insomnia, supraventricular tachycardia, fatigue, hypertransaminasaemia, hepatitis C, hepatic cancer, and renal mass (1 patient each), insomnia and anxiety (1 patient). Except renal mass which was Grade 4, all events were Grade 3. The events of hypertransaminasaemia, diarrhoea, insomnia and anxiety, renal mass, and supraventricular tachycardia were considered related to study drug. The events hepatitis C, hepatic cancer, renal mass, and supraventricular tachycardia were SAE. The events of hepatic cancer, hepatitis C, hypertransaminasaemia, and supraventricular tachycardia were not recovered/resolved after treatment discontinuation. The patient who experienced supraventricular tachycardia withdrew from the study.

13 patients (7.6%) in mitapivat vs 5 patients (5.9%) in placebo experienced dose interruption. For subjects receiving mitapivat treatment, most TEAEs (by PT) leading to dose interruption occurred in 1 subject each; the TEAE of Influenza led to interruption of mitapivat treatment in 2 subjects. A few events were considered SAE, however, with the exception of SAE supraventricular tachycardia and renal mass, all were considered not related and treatment was resumed after resolution of SAEs and patients terminated treatment in the DBP of the study.

Twenty subjects (11.6%) in mitapivat vs 2 subjects (2.4%) in placebo experienced dose reduction. SAE leading to dose reduction included asthenia and dizziness (1 patient), pneumonia and tonsillitis (1 patient), lower respiratory tract infection (1 patient, event occurred twice), arrhythmia supraventricular, pancytopenia, and influenza (1 patient each). With the exception of LRTI 2nd occurrence, none are considered related to study drug. In addition to the events described above, the non-serious TEAE of ALT/AST increased, migraine, hepatic pain, maculopapular rash, dermatitis, blood pressure increased, anxiety, vomiting, rash, flank pain, nausea led to dose modifications (reduction, interruption) were not related to mitapivat,

In the all indications safety set, treatment modifications due to TEAE were comparable in the PK deficiency versus thalassemia: except treatment interruption which was twice higher (9.6% vs 5.0%).

Laboratory and other findings

In the comparative thalassemia safety pool, the laboratory values with higher frequency in the mitapivat treated patients compared to placebo were Grade 3-4 ALT increased (2.0% vs 0), glucose hypo (23.8% vs 13.5%), potassium hyper (14.3% vs 8.8%), and urate hyper (19.0% vs 11.5%). The most frequent TEAE were ALT increased (24.9% vs 25.0%) and AST increased (27.6% vs 29.1%).

Frequency of Hb hypo at any time during treatment was, as expected, lower in mitapivat treated (52.5%)

than placebo (64.2%), and higher for platelets hypo (18.8% vs 10.1%). Leukocytes, lymphocytes, and neutrophils were comparable. Same trends are seen in the all indications pool.

Shifts of Haemoglobin values are observed during treatment with mitapivat in study 018 with an increase of worst on-treatment Grade 2 (50.6%) and Grade 3 (47.1%) events from baseline (32.0% and 5.8% respectively). It is mentioned in the SmPC that treatment should be discontinued if worsening of anaemia, and the intervals for patients monitoring to advise treatment discontinuation are recommended in the SmPC.

The majority of heamatology parameters were Grade 0, 1 and 2 throughout the study, with few events of Grade 3 in patients treated with mitapivat: decrease of neutrophils 3.1%, leukocytes 1.3%, lymphocytes 2.7%, and platelets 2.0%. Grade 4 lymphocytes and platelets decreased were reported each in 0.7% of patients in study 018.

The clinically significant events considered related to mitapivat treatment were thrombocytopenia in study 017, leading to discontinuation and reticulocytopenia in study 018 not requiring any treatment action. Aromatase activity is inhibited by mitapivat. As this enzyme is responsible for the transformation of androgens to oestrogens, a decrease of oestrogens and an increase in androgens were observed in male subjects: The frequency of sex hormone events in mitapivat versus placebo was Estrone decreased 51.2% vs 4.4%, Estradiol decreased 13.6% vs 10.3%, Testosterone increased 32.0% vs 11.8% and Free testosterone increased 48.8% vs 19.1%. This is consistent with the trend PK deficiency indication. Sex hormone analysis in females was limited due to physiologic variations in hormone levels throughout the normal menstrual cycle, use of hormonal contraceptives, subjects age, and the potential of thalassemia to cause menstrual cycle changes.

No clinically meaningful changes were observed in BMD (as assessed by DXA scores) from baseline to Week 48 in either treatment arm (study 018).

No formal analysis was performed on vital signs. It is stated that any clinically significant abnormalities in vital signs measurements were reported and summarised as AEs.

In the all indications safety pool, the most frequent laboratory abnormalities were ALT and AST increases and triglycerides increased. In the PK deficiency, triglycerides, cholesterol increased, elevation of liver tests (AST, bilirubinaemia) were higher than in thalassemia, while frequency of haematological parameters were higher in the thalassemia indication.

Safety in special populations

In the thalassemia safety pool, the safety profile is comparable within each of the following subgroups: age, sex, race, ethnic origin, geographic region.

In addition, the MAH has presented separately in the integrated safety summary the TEAE for the following subgroups: thalassemia genotype (α- and β thalassemia in study 017 and respectively β0/β0 and non-β0/β0 genotype in study 018) and by Hb concentration at baseline (study 017 only). In total safety profile appears comparable within each subgroup, however higher frequency in the SOC musculoskeletal and connective tissue disorders and GI disorders is observed in β-thalassemia in study 017 and respectively in β0/β0 genotype in study 018. The MAH considers that the imbalance does not exceed 10% difference between subgroups and is not suggestive of a drug effect. Though there are some differences in the frequency of AE between subgroups in terms of thalassemia genotypes and Hb concentration at baseline, no trend could be evidenced. According to the MAH's assessment none would justify dose modifications or any action based on subgroups. From a safety perspective this is acknowledged.

Pregnant and breast-feeding patients were excluded from clinical studies and treatment with mitapivat is not

recommended as stated in section 4.6 of the SmPC. As of 11 April 2024, 6 pregnancies in female subjects have been reported in clinical studies of mitapivat. In addition, 2 pregnancies had occurred at the time of the initial MA assessment. In total, 8 pregnancies were exposed to mitapivat, of which 3 resulted in termination, 1 spontaneous abortion, 1 outcome of pregnancy was unknown and 3 were carried to term. Considering the 3 pregnancies that were carried to term: One pregnancy occurred in a subject's partner; Two pregnancies occurred in a female subject, with discontinuation of treatment during the first trimester of pregnancy; All three pregnancy resulted to the birth of a normal healthy newborn.

In addition, no information is available on the clinical use of mitapivat during breastfeeding, on the presence of mitapivat in human milk, on the effect on the breastfed infant, or effects on milk production. Because mitapivat is highly protein bound (97.7%) to human plasma proteins, the amount in milk is likely to be low. The elimination half-life is 16 to 79 hours and is indicative of potential accumulation in infant. A risk not to be excluded. In consequence, a decision must be made whether to discontinue breast-feeding or to abstain from Pyrukynd therapy taking into account the benefit of breast feeding for the child and the benefit of therapy for the woman as reflected in Section 4.6.

Study AG348-C-024 in patients with mild and moderate impairment results and PBPK simulations showed no clinically meaningful differences in mitapivat exposure compared to healthy subjects. Patients with severe hepatic impairment have not been studied, and would not benefit from chronic treatment with mitapivat, due to short life expectancy. Based on PKPB data, no different safety profile is expected in patients with mild and moderate hepatic impairment. In addition, the levels of ALT are recommended to be monitored at baseline and during treatment as reflected in section 4.4.

Patients with severe renal impairment have not been studied, however ADME data suggest no impact of renal impairment on mitapivat exposure. Data available for few patients with renal impairment show a slightly higher frequency of AE compared to normal renal function, however no action in the SmPC is warranted.

Subjects with active and/or uncontrolled cardiac or pulmonary disease were excluded from both studies and this is reported in the SmPC in section 4.4.

Safety related to DDI

The potential for DDI is described in section 4.5 of the SmPC, consistent with the current approved indication.

ADRs of special interest, serious ADR and death causally related

There were no deaths in the DBP of pivotal studies in thalassemia. During the safety follow up, there was one death due to Pneumonia in Study 017 OLE. The TEAE of Pneumonia was considered unrelated to study treatment.

SAE from all studies were evaluated as possible ADRs. The criteria for determining adverse drug reactions (ADR) were the frequency of subjects, for Studies 017 and 018 combined, with the AE (all grades) in the mitapivat arm $\geqslant$5% higher than in the placebo arm and the AE considered clinically significant based on medical review, irrespective of frequency. However, in the D120 response, the MAH clarified that the methodology for assessing ADR integrated clinical judgment with consideration of AE severity, seriousness, frequency, pharmacologic plausibility, temporal association with drug exposure, and the background incidence of AEs in the target population. The 5% difference threshold was only one component of this approach.

All SAE were considered in the assessment of causality; the MAH clarified that the cardiac events (arrythmia, supraventricular tachycardia) were considered not treatment related.

The MAH stated that the 'medical concepts' headache, insomnia, and fatigue were medically reviewed. Eventually fatigue was not considered causally related to mitapivat since it is likely mediated by other aetiologies and also a symptom of haemolytic anaemia. Upon request, the TR-AE hypertransaminasemia, palpitations, pain in extremity, rash, arthralgia, dizziness, proteinuria, pruritus which occurred with higher frequency in the mitapivat arm were listed in section 4.8 of the SmPC.

For laboratory abnormalities, it is stated that many were not reported as TEAE, and, within TEAEs, if they were transient, incidental, or borderline at baseline, they were not considered ADRs. The laboratory abnormal values meeting the criteria for ADRs review were glucose decreased, platelets decreased, potassium increased, and urate increased. These abnormal values were not clinically relevant and not considered ADRs. In addition, sex hormone laboratory changes were medically reviewed. Ultimately, only sex hormone laboratory changes are retained as ADRs for the SmPC (see investigations).

Several laboratory abnormalities occurred with higher frequency in mitapivat arm than placebo (ALT/AST increased, LHD increased).

ADRs in the SmPC

Insomnia, headache, and changes in sex hormone levels are considered ADR (very common) in the tabulated list in section 4.8 of the SmPC. In addition, upon request, the following ADR have been added to the SmPC section 4.8: hypertransaminasemia, palpitations, pain in extremity, rash, arthralgia, dizziness, proteinuria, and pruritus, as well as changes in sex hormone levels (due to aromatase inhibition), hypertransaminasemia, and insomnia.

It was acknowledged that per the MAH's feedback decrease in hormone sex levels cannot be monitored in female subjects due physiological variability and absence of directional change in sex hormones and potential physiologically compensatory mechanisms which offset the aromatase inhibitory activity of mitapivat.

It is also endorsed that no action in the SmPC is needed regarding the aromatase activity in female subjects based on the experience with mitapivat in PK deficiency. The following warning is already stated in section 4.4 of the SmPC and was replicated for the new strength (100 mg): 'Mitapivat may decrease the systemic exposure of hormonal contraceptives that are sensitive substrates of cytochrome P450 3A4 (CYP3A4) (e.g. ethinylestradiol) (see section 4.5). Women of childbearing potential should be counselled regarding the use of additional or alternative contraception methods (see section 4.6).'

Post-marketing experience

Pyrukynd is approved in the USA (indication: haemolytic anaemia in adults with PK deficiency) and in the EU (indication: treatment of PK deficiency in adults) but is currently commercialised only in the USA. Up to 16 August 2024, 197 patients were exposed to mitapivat (161 patients with PK or haemolytic anaemia indication). A cumulative list of SAE was provided; a total of 196 SAE are reported post-marketing consistent with the safety profile during clinical development. In the latest PSUSA report, the B/R balance remains unchanged.

From the safety database all the adverse reactions reported in clinical trials and post-marketing have been included in the Summary of Product Characteristics.

2.6.10. Conclusions on the clinical safety

The overall safety profile of mitapivat in thalassemia is consistent with the safety profile of mitapivat for the approved indication of pyruvate kinase deficiency, with predominantly psychiatric disorders (insomnia),

nervous system disorders (headache), and aromatase inhibition (change in sex hormones levels) adverse events. Long-term safety (up to 2 years of exposure) is available for a sufficient proportion (65%) of patients with thalassemia, at the recommended posology, however tolerability for chronic treatment (> 2 years) is unknown. Hypertransaminasemia is an ADR specific to the thalassemia indication and a specific warning has also been added in section 4.4 of the SmPC for the 100mg strength. The clinical studies did not include patients with active and/or uncontrolled cardiac or pulmonary disease as stated in section 4.4 of the SmPC.

The following warning is already stated in section 4.4 of the SmPC: 'Mitapivat may decrease the systemic exposure of hormonal contraceptives that are sensitive substrates of cytochrome P450 3A4 (CYP3A4) (e.g. ethinylestradiol) (see section 4.5). Women of childbearing potential should be counselled regarding the use of additional or alternative contraception methods (see section 4.6).'

Drug-drug interactions should also be considered considering that mitapivat induces CYP3A4 and may also induce CYP2B6, CYP2C8, CYP2C9, CYP2C19 and uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1). Mitapivat may inhibit CYP3A4. Mitapivat may induce and inhibit P-gp as stated in section 4.5 of the SmPC.

2.7. Risk Management Plan

2.7.1. Safety concerns

The MAH proposed the following summary of safety concerns in the RMP:

Table 90: Summary of safety concerns

Summary of safety concerns	
Important identified risks	Acute haemolysis in PK deficiency
Important potential risks	Embryo-foetal toxicity
Missing information	Long-term use

2.7.2. Pharmacovigilance plan

Table 91: Ongoing and Planned Additional Pharmacovigilance Activities

Title and Study Status	Summary of Objectives	Safety Concerns Addressed	Milestones	Due Dates
Category 1 - Imposed mandatory additional pharmacovigilance activities that are conditions of the marketing authorisation				
None				
Category 2 – Imposed mandatory additional pharmacovigilance activities that are specific obligations in the context of a conditional marketing authorization or a marketing authorisation under exceptional circumstances				
None				
Category 3 - Required additional pharmacovigilance activities				

Title and Study Status	Summary of Objectives	Safety Concerns Addressed	Milestones	Due Dates
AG348-C-017 A Phase 3, Double-Blind, Randomized, Placebo-Controlled Study Evaluating the Efficacy and Safety of Mitapivat in Subjects With Non-Transfusion Dependent Alpha- or Beta-Thalassemia	Evaluate the long-term safety of mitapivat	Long-term use **(thalassemia)**	Long-term safety report with a cutoff date 2 years after the last subject receives the first dose of mitapivat in the OLE Period	Q2 2026
AG348-C-018 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	Evaluate the long-term safety of mitapivat	Long-term use **(thalassemia)**	Long-term safety report with a cutoff date 2 years after the last subject receives the first dose of mitapivat in the OLE Period	Q4 2026
AG348-C-011 A Phase 3, Multicenter, Open-label, Long-term, Extension Study of Mitapivat in Adults with PK Deficiency Previously Treated in Studies AG348-C-006 or AG348-C-007 Ongoing	Evaluate the long-term safety and tolerability of mitapivat	Acute hemolysis Long-term use (PK deficiency)	Final study report	30 November 2025
AG348-C-008 (Peak Registry) An ongoing Agios-sponsored, global, retrospective and prospective, longitudinal observational study of pediatric and adult patients with PK deficiency Ongoing	To understand better the natural history of PK deficiency, including diagnosis, demographic and clinical characteristics, burden of disease, treatment patterns, and clinical outcomes in a real-world setting.	Long-term use for patients receiving mitapivat (PK deficiency)	Final study report for patients who received mitapivat	30 September 2028

2.7.3. Risk minimisation measures

The MAH proposed only routine risk minimisation measures.

Table 92: Summary Table of Pharmacovigilance Activities and Risk Minimisation Activities by Safety Concern

Safety Concern	Risk Minimisation Measures	Pharmacovigilance Activities
Acute hemolysis in PK deficiency	Routine risk minimisation measures: • *Acute haemolysis is listed as a special warnings and precautions for use in the 5, 20, and 50 mg Summary of Product Characteristics (SmPC) Section 4.4* • *Acute haemolysis is described as a selected adverse reaction in the 5, 20, and 50 mg SmPC Section 4.8* • *Acute haemolysis is listed as a warning and precaution in the 5, 20, and 50 mg Patient Information Leaflet (PIL) Section 2* • *Acute haemolysis, after abrupt interruption or discontinuation of Pyrukynd, is described in the 5, 20, and 50 mg PIL Section 4* • *Warning and precaution that acute haemolysis with subsequent anaemia has been observed following abrupt interruption or discontinuation of Pyrukynd in the 5, 20, and 50 mg SmPC Section 4.4* • *Warning that to minimize the risk of acute haemolysis, avoid abrupt interruption or discontinuation of Pyrukynd in the 5, 20, and 50 mg SmPC Sections 4.2 and 4.4* • *Advice on the dose taper schedule to be followed when discontinuing Pyrukynd in the 5, 20, and 50 mg SmPC Section 4.2* • *Warning to monitor patients for signs of acute haemolysis with worsening of anaemia if discontinuing treatment in the 5, 20, and 50 mg SmPC Sections 4.2 and 4.4* • *Warning and precaution for the patient to talk to their doctor if they develop symptoms of acute haemolysis in the 5, 20, and 50 mg PIL Section 4* • *Pack size: Dose taper blister packs, that follow the dose taper schedule, when discontinuing Pyrukynd* • *Description of the dose taper blister packs in the 5, 20, and 50 mg SmPC Section 6.5 and PIL Section 6* Additional risk minimization measures: • *None*	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: • *None* Additional pharmacovigilance activities: • *Long-term safety and tolerability study AG348-C-011; final study report available 30 November 2025.*
Embryo-fetal toxicity	Routine risk minimization measures: • *Information on nonclinical findings in SmPC Section 5.3* • *Advice that Pyrukynd is not recommended during pregnancy and in women of childbearing potential not using contraception in SmPC Section 4.6* • *Advice that contraception should be used by women of childbearing potential during treatment and for at least 1 month after the last dose in SmPC Section 4.6* • *Advice that mitapivat may decrease systemic exposure of hormonal contraceptives that are sensitive substrates of CYP3A4 in SmPC Sections 4.4, 4.5, and 4.6*	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: • *PYRUKYND exposure during pregnancy and lactation follow-up form* Additional pharmacovigilance activities: *None*

Safety Concern	Risk Minimisation Measures	Pharmacovigilance Activities
	• *Advice that women of childbearing potential should be counselled regarding the use of additional or alternative contraception methods in SmPC Section 4.4* • *Advice that Pyrukynd should be avoided during pregnancy and women of childbearing potential must use reliable contraception and for at least 1 month after the last dose in PIL Section 2* • *Advice that birth control medicines containing hormones may not work as well as expected and pregnancy may occur so a patient should discuss contraception methods with their doctor in PIL Section 2* <u>Additional risk minimisation measures:</u> *None*	
Long-term Use	<u>Routine risk minimisation measures:</u> • *Information that the median duration of treatment with Pyrukynd for patients with PK deficiency was 24.1 weeks in AG348-C-006 and 40.3 weeks in AG348-C-007 in the 5, 20, and 50 mg SmPC Section 5.1* • *Information that the median duration of treatment with Pyrukynd for patients with thalassemia was 24.1 weeks in AG348-C-017 and 48.1 weeks in AG348-C-018 in the 100 mg SmPC Section 5.1* • *For patients with PK deficiency, advice that treatment with Pyrukynd is intended to be long-term and should be discontinued if there is no improvement of haemolytic anaemia at the maximum recommended dose, based on the totality of laboratory results and clinical status of the patient, unless there is another explanation for response failure in the 5, 20, and 50 mg SmPC Section 4.2.* • *For patients with thalassemia, advice that treatment with Pyrukynd is intended to be long-term and should be discontinued if there is no improvement of haemolytic anaemia based on the totality of laboratory results and clinical status of the patient, unless there is another explanation for response failure in the 100 mg SmPC Section 4.2.* Additional risk minimisation measures: • *None*	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: • *None* Additional pharmacovigilance activities: • *Long-term safety and tolerability study AG348-C-011; final study report available 30 November 2025.* • *Longitudinal observational study AG348-C-008 (Peak Registry); final study report for patients that received mitapivat available 30 September 2028.* • ***Open-label Extension Period of Study AG348-C-017 to Assess Long-Term Safety; Long-term safety report with a cutoff date 2 years after the last subject receives the first dose of mitapivat in the OLE Period;Q2 2026.*** • ***Open-label Extension Period of Study AG348-C-018 to Assess Long-Term Safety; Long-term safety report with a cutoff date 2 years after the last subject receives the first dose of mitapivat in the OLE Period; Q4 2026.***

2.7.4. Conclusion

The CHMP considered that the risk management plan version 1.3 is acceptable.

2.8. Pharmacovigilance

2.8.1. Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the MAH fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

2.8.2. Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

2.9. Product information

2.9.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the MAH show that the package leaflet meets the criteria for readability as set out in the *Guideline on the readability of the label and package leaflet of medicinal products for human use.*

2.9.2. Additional monitoring

Pursuant to Article 23(1) of Regulation No (EU) 726/2004, Pyrukynd (Mitapivat) is included in the additional monitoring list as it contains a new active substance which, on 1 January 2011, was not contained in any medicinal product authorised in the EU.

Therefore, the summary of product characteristics and the package leaflet includes a statement that this medicinal product is subject to additional monitoring and that this will allow quick identification of new safety information. The statement is preceded by an inverted equilateral black triangle.

3. Benefit-Risk Balance

3.1. Therapeutic Context

Pyrukynd (mitapivat) is a pyruvate kinase activator and acts by directly binding to the pyruvate kinase tetramer. Pyrukynd is already approved in the EU since 2022 for the treatment of pyruvate kinase deficiency (PK deficiency) in adult patients.

The application relates to a new strength (100-mg film-coated tablet) with an extension of indication: "*Pyrukynd is indicated in adults for the treatment of anaemia associated with transfusion-dependent and non-transfusion-dependent alpha or beta-thalassaemia*". This extension application was also grouped with a variation to update pharmacokinetic information based on final results from study AG348-C-024 listed as a category 3 study in the

RMP; this is a Phase 1, Open-label, Single-dose, Pharmacokinetic Study of Mitapivat in Subjects with Moderate Hepatic Impairment Compared to Matched Healthy Control Subjects with Normal Hepatic Function.

Considering that the data primarily support a significant improvement in anaemia, the following indication wording is agreed to adequately reflect the treatment's objective, and has been agreed by the MAH for Pyrukynd 100 mg film coated tablets:

"*Pyrukynd is indicated in adults for the treatment of anaemia associated with transfusion-dependent and non-transfusion-dependent alpha or beta-thalassaemia*"

3.1.1. Disease or condition

Thalassemia is an inherited, chronic hemolytic anemia caused by mutations in the α- and/or β-globin genes. Based on where the mutation occurs, thalassemia is named α- or β-thalassemia due to the reduced or absent α-globin chains or β-globin chains, respectively, leading to the toxic and unstable accumulation of the other globin chains.

The degree of the anemia in thalassemia is dependent on the magnitude of the globin mismatch and imbalance with larger imbalances resulting in worse anemia, translating into a spectrum of transfusion needs encompassing all genotypes. Some patients require lifelong regular transfusions and are termed patients with transfusion-dependent thalassemia (TDT), and some patients require occasional or intermittent transfusions and are termed patients with non– transfusion-dependent thalassemia (NTDT). In general, patients with β-thalassemia have diverse transfusion requirements and may manifest as either NTDT or TDT, with most patients with β0 /β0 thalassemia having TDT. In general, patients with α-thalassemia are more likely to have NTDT because of the most commonly occurring genotypes but may manifest as TDT (HbH disease). Additional factors affect frequency of transfusions, including access to blood products, physician or patient choice, variability of clinical practices worldwide, and the presence or absence of comorbidities. This stresses the limitation of subdividing patients based on their transfusion frequency as well as using transfusion requirements as a designation of disease severity (Taher *et al,* 2023). Recent evidence shows that patients who do not receive regular transfusions also experience significant morbidity and increased early mortality (Musallam, Cappellini and Taher, 2021).

Over time, with ongoing ineffective erythropoiesis and chronic hemolytic anemia, patients with thalassemia across all genotypes and transfusion burden, suffer from several severe comorbidities and widespread organ damage which ultimately lead to early mortality (Cappellini *et al,* 2014). These complications of thalassemia, in both NTDT and TDT, include iron overload, extramedullary hematopoietic pseudo tumors, leg ulcers, thrombosis, pulmonary hypertension, liver fibrosis/cirrhosis and abnormal function, heart failure, osteoporosis, metabolic and endocrine disorders, and splenomegaly (Bazarbachi *et al,* 2016; Musallam, Cappellini, Daar, *et al,* 2021; Sleiman *et al,* 2018; Taher *et al,* 2018). Patients with thalassemia also have reduced HRQOL including symptoms of fatigue, weakness, shortness of breath, and impaired physical function (Cappellini *et al,* 2019; Taher *et al,* 2019), and report negative impacts on school and career function (Drahos *et al,* 2024; Klaassen *et al,* 2014).

3.1.2. Available therapies and unmet medical need

Globally, the standard of care for alpha and beta thalassemia remains centered on supportive care to address symptoms through transfusions, splenectomy, and/or iron chelators. Regular RBC transfusions are the cornerstone for treatment of patients with thalassemia, despite associated burden and risks. Patients

experience higher rates of premature mortality compared with the general population (Borgna-Pignatti *et al,* 2004; Modell *et al,* 2000). Iron overload caused by regular transfusions is a common complication of thalassemia syndromes which could lead per se to the development of organ damage and increased mortality. Iron overload may occur due to increased intestinal absorption (which occurs in most patients independent of transfusions) or exogenous iron from frequent RBC transfusions (Taher et al, 2011). Thus, iron chelation therapy to remove excess iron is a fundamental supportive therapy.

With regards to splenectomy, this latter 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 surgery (Cappellini et al, 2000), in addition to risks of undergoing a surgical procedure.

TIF acknowledges the need for folic acid supplementation, particularly for patients with un-transfused or low-transfusion regimens and in specific situations like pregnancy. Allogeneic haematopoietic stem cell transplantation (allo-HSCT) is recognised as a potentially curative option for patients with transfusion-dependent β-thalassaemia (TDT). However, its applicability is limited, as fewer than 30% of otherwise eligible patients have access to an HLA-matched related donor. According to TIF recommendations, allo-HSCT should be considered only in young patients with symptomatic TDT who have a matched sibling donor and ideally performed before the development of iron overload. Foetal haemoglobin inducers (e.g. 5-azacytidine, decitabine, hydroxycarbamide, with or without erythropoiesis-stimulating agents [ESAs]), short-chain fatty acids, and thalidomide or its derivatives) have been given as off label use treatments. Collectively, the reported effects in patients were not always consistent and the studies have various limitations: small sample size, heterogenous populations studied, lack of standardised or approved controversial doses used, short duration of follow-up, and inconsistently reproducible results.

To date, there are no authorised medicines to treat α-thalassemia.

Reblozyl is a subcutaneously administered erythroid maturation agent which has been recently approved in adults for the treatment of anaemia associated with transfusion-dependent and non-transfusion-dependent beta-thalassaemia. In patients with NTD β-thalassemia, luspatercept reduced the transfusion burden in patients with TD β-thalassemia and increased Hb levels in NTD β-thalassemia Administration of luspatercept requires a visit to a health care provider every 3 weeks, which can be burdensome for patients and health care systems.

Casgevy a gene editing therapy recently approved is indicated for patients, 12 years of age and older with β-thalassemia but only those transfusions dependent and for whom hematopoietic stem cell (HSC) transplantation is appropriate, but a human leukocyte antigen-matched-related HSC donor is not available. Gene therapies incur acute and long-term toxicities secondary to myeloablative chemotherapy, potential off target mutations and challenges exist with widespread availability.

3.1.3. Main clinical studies

Mitapivat was studied in a global program consisting of 3 studies that enrolled adult patients with thalassemia across genotypes and transfusion needs.

- The 2 global pivotal Phase 3 studies were randomized, double-blind, and placebo controlled and aimed to provide the primary evidence of efficacy of mitapivat in adults with thalassemia, reflective of the real-world thalassemia patient population. Study 018 enrolled subjects with TD α- or β-thalassemia and Study 017 enrolled subjects with NTD α- or β-thalassemia.

- The single-arm Phase 2 study (Study 010) aimed to provide supportive evidence of efficacy in patients with NTD α- or β-thalassemia.

The pivotal study AG348-C-018 was designed to assess the effect of mitapivat on transfusion burden, as measured by the proportion of subjects achieving a transfusion reduction response (TRR) in any consecutive 12-week period through Week 48 compared with the baseline in subjects with α- or β-TDT.

The pivotal study AG348-C-017 was designed to assess the effect of mitapivat on Hb concentration, as measured by the proportion of subjects achieving a ≥10 g/L (1.0 g/dL) increase in average Hb concentration from Week 12 through Week 24 compared with baseline in subjects with α- or β-NTDT.

Study AG348-C-018

The pivotal study AG348-C-018 was a phase 3, double-blind, randomized, placebo-controlled, multicenter study evaluating the efficacy and safety of mitapivat in adult subjects with Transfusion-Dependent Alpha- or Beta-Thalassemia. Subjects were randomly assigned in a 2:1 ratio to receive mitapivat 100 mg BID, or matching placebo BID for up to 48 weeks followed by a 5 years OLE phase.

The design of the study was considered adequate and randomization according to thalassemia genotype and geographic region is endorsed, as they represent recognised covariates in the target population.

The choice of the 100 mg BID dose regimen was based on the results of the phase 2 study AG348-C-010 (supportive study), in which participants started with a 50 mg BID dose, followed by a dose increase to 100 mg BID at week 6 based on tolerability and hemoglobin rate improvement.

Transfusion dependence was defined in this study as receiving 6 to 20 RBC units, with a transfusion-free period lower or equal to 6 weeks during the 24 weeks prior to randomization. This definition is endorsed. At this time, the minimum of 6 RBC was defined based on potential transfusion practices, including a minimum of 1 unit every 4 weeks (or 6 RBC units/24 weeks), which may be observed in countries with limited blood supply.

The primary endpoint of the study was the reduction in transfusion burden defined as ≥50% reduction in transfused red blood cells (RBC) units with a reduction of ≥2 units of transfused RBCs in any consecutive 12-week period through Week 48 compared with baseline.

A 33% reduction in transfusion burden from baseline was previously established as clinically meaningful.

Key secondary endpoints assessed the durability of the effect though different time intervals (rolling period and fixed period) and depth of transfusion reduction rate TRR2 was defined as a ≥33% reduction in transfused RBC units from Week 13 through Week 48 compared with baseline. TRR3 was defined as ≥50% reduction in transfused RBC units in any consecutive 24-week period through Week 48. TRR4 was defined as ≥50% reduction in transfused RBC units from Week 13 through Week 48 compared with baseline

A total of 258 participants were enrolled with 171 subjects randomized in the mitapivat arm and 87 in the placebo arm. Overall, patients had a median age of 35.5 years, only one patient was over 65 years and 70.9% of them had baseline transfusion burden >12 RBC Units in the 24 weeks prior to randomization.

The region with the largest enrolment was Western Europe (38.8%), with Greece, Spain and France leading recruitment. In second place, Asia and the Middle East with similar rates, 18.2% and 18.6% respectively. Currently, thalassemia is the most frequent in the tropical belt, including parts of sub-Saharan Africa, the Mediterranean, the Middle East, South Asia, Southeast Asia, and East Asia. Of the 258 randomized subjects, most subjects were white (60.1%), 30.2% were Asian. Black or African Americans were underrepresented, with 0.8 % of participants.

Patients enrolled are overall representative of patients with TD α- and β-thalassemia. However, subjects with α-thalassemia were primarily captured under the non-β0/β0 population. Non- β0/β0 genotype accounted for 55.8% of the global trial population and β0/β0 genotype accounted for 44.2%.

Considering baseline disease characteristics, the majority of patients had significant transfusion requirements, with 183 patients (70.9 %) having received more than 12 RBC Units during the 24-week pre-randomization period with a pre-transfusion Hb threshold of 8.9 g/dl, representative of a population including α-thalassemia.

To note, according to Guidelines for the Management of α-Thalassaemia pre-transfusion Hb threshold in α-thalassemia is fixed at slightly lower level (8-9 g/dl) in comparison to that fixed for β-thalassemia (9.5-10.5 g/dl) (Amid Ali *et al:* Guidelines for the Management of α-Thalassaemia (2023 Thalassemia International Federation). Baseline markers of hemolysis illustrate a mild hemolysis profile with median indirect bilirubin levels at 23 umol/L, median LDH level of 177.0 U/L, haptoglobin levels slightly decreased with a median value of 0.240 g/L.

The majority of patients had no prior hydroxyurea treatment (248/258, 96.1%). Prior chelation therapy concerned 97.7% of subjects. Over half the overall population (141; 54.7%) had prior splenectomy. The most frequently reported concomitant medication in both treatment arms was Deferasirox (>70% subjects, 74.4% in the mitpaivat arm and 74.1% in the placebo arm), followed by Folic acid (>50% subjects, 55.8 in the mitapivat arm and 52.9% in the placebo arm).

Study AG348-C-017

The pivotal study AG348-C-017 is a phase 3, double-Blind, randomized, placebo-controlled, multicenter study evaluating the efficacy and safety of mitapivat in adult subjects with non-Transfusion-Dependent Alpha- or Beta-Thalassemia (NTDT). Patients were randomly assigned in a 2:1 ratio to receive mitapivat 100 mg BID, or matching placebo BID for up to 24 weeks, followed by a 5-year OLE period.

NTDT encompasses three clinically distinct forms: β-thalassaemia intermedia, haemoglobin E/β-thalassaemia (mild and moderate forms), and α-thalassaemia intermedia (haemoglobin H disease).

The primary endpoint of the study was Hb response defined as a ≥1.0-g/dL increase in average Hb concentration from Week 12 through Week 24 compared with baseline. The use of surrogate endpoints is acceptable if they reliably predict a positive effect on clinical outcomes such as mortality or morbidity. The MAH relies on recent evidence linking hemoglobin levels >10 g/dL with improved survival in NTDT patients. This is endorsed. Data also supports a decrease in development of multiple morbidities for each 1 g/dL Hb increase, with greater benefits seen in patients with lower Hb levels. Key secondary endpoints are generally endorsed and included the change in average FACIT-fatigue subscale scores, according to a pre-defined meaningful within-person change threshold, and change from baseline in average Hb concentration.

A total of 194 participants with NTDT were enrolled with 130 subjects randomized in the mitapivat arm and 64 in the placebo arm. Randomization was stratified by baseline Hb concentration (≤ 9.0 g/dL versus 9.1-10.0 g/dL), with 73.2% of participants with Hb ≤ 9.0 g/L and β-thalassemia accounting for 68.8% of the population. Patients with both baseline Hb ≤ 9.0 g/dL and β-thalassemia genotype accounted for the most part of the trial global population (50.5%). Patients with α-thalassemia/HbH disease represented 32% of the overall study population and 22.7% when combined to Hb levels ≤ 9.0 g/dL. Overall, patients had a median age of 41.2 years, 8 patients were older than 65 years. The mean baseline Hb was 8.334 g/dL. Prior iron chelators were reported in 63 (32.8%) subjects across both treatment arms.

The majority of patients (86.6%) had no RBC unit transfused in the 24-week period prior to randomization. Only 13.4% of patients had received between 1 and 5 RBC units in the prior 24 weeks before randomization.

This is consistent with the characteristics of the target population who, despite a moderate anemia profile, may require occasional transfusions. Baseline markers of hemolysis show a mild haemolytical profile with median indirect bilirubin levels at 23 umol/L, median LDH level of 265.0 U/L, haptoglobin levels slightly decreased with a median value of 0.100 g/L.

The study enrolled mainly Western Europe patients (46.9%), with higher recruitment in Mediterranean countries (Italy and Greece), and Asian patients (22.2%). Of the 194 subjects, most subjects were white (56.2%), 39.2% were Asian. Black or African Americans were underrepresented with 1 % of the population being Black or African American. There were a higher proportion of women than men (63.4% vs 36.6%). The median age was 41.0 years. 8 subjects were older than 65 years and the large majority of patients had no prior hydroxyurea treatment (91.2%).

3.2. Favourable effects

Study AG348-C-018 (transfusion-dependent α- or β thalassemia)

Mitapivat demonstrated a significant difference in transfusion reduction response (TRR) compared to placebo. The adjusted difference in TRR rate, defined as the proportion of subjects achieving a ≥50% reduction in transfused RBC units with a reduction of ≥2 transfused RBC units in any consecutive 12-week period compared with baseline, was of 17.6% [CI 95% (8.0; 27.2) 2-sided p-value 0.0003]. These results were encouraging despite the modest rate of responders. Indeed, the number of responders was relatively low in the mitapivat arm with an observed rate of 30.4% vs 12.6% in the placebo arm.

The TRR assessment across different time intervals and depth of reduction assessed by the 3 key secondary endpoints TRR2, TRR3 and TRR4 demonstrate a relative long-lasting effect of mitapivat, extended beyond the 12 weeks rolling period of the primary endpoint. TRR2, defined as ≥50% reduction in Transfused RBC Units in any consecutive 24-week period TRR3 was defined as ≥33% and ≥50 % Reduction in Transfused RBC Units respectively, from Week 13 through Week 48 and TRR4 as ≥50 % Reduction in Transfused RBC Units from Week 13 through Week 48.

Within a 24 week rolling period, 13.5% participants demonstrated a ≥50% reduction in transfused RBC Units in the mitapivat arm versus 2.3% in the placebo arm, leading to an adjusted difference in TRR rate of 11.1% [CI 95% (5.1, 17.0)].

From Week 13 through Week 48, 14.6% of participant in the mitapivat arm had a ≥33% reduction in transfused RBC units (adjusted difference versus placebo 13.4% [CI 95% (7.7, 19.1)] versus 1.1% and 7.6% had a ≥50% reduction in transfused RBC units (adjusted difference versus placebo 6.4% [CI 95% (1.9, 10.9)].Overall, mitapivat led to a significant greater reduction in RBC units transfused from Week 13 through Week 48 compared to baseline leading to a difference in LS mean of 7.63% versus placebo [CI 95% (1.06, 14.20)].

Overall, 12 of the 258 subjects enrolled had transfusion dependent α-thalassemia including, 9 in the mitapivat arm and 3 in the placebo arm. Among the 9 mitapivat treated subjects, 7 (77.8%) met the primary endpoint TRR compared to 0 in the placebo arm, leading to difference in response rate of 77.8%; 95% CI (2.6, 97.2). Difference in response rate for TRR2 was 77.8 % (CI 95% 2.6, 97.2).

The reduction in transfusion volumes led to transfusion independence in 9.9% of participant (17/171) receiving mitapivat over a specified interval of 8 consecutive weeks through Week 48, performing better than placebo (1.1% subjects; 1/87) with an adjusted difference in response rate versus placebo of 8.8% [CI 95% (3.8, 13.8)]. It is to be noted that 3 subjects in the mitapivat arm did not receive any transfusions during the 48-week Double-blind Period.

An overall 10.96% reduction of RBC units from Week 13 through Week 48 was observed in the mitapivat arm, leading to a difference of 7.63% [95% CI (1.06, 14.20)] with the placebo arm. With continued exposure to mitapivat in the OLE Period, the mean percent reduction from baseline in RBC transfusion burden was 13.86%, increasing from 10.96% observed during the Double-blind Period which translates in a reduction of approximately 4 transfusion visits per year.

In the mitapivat arm, the translated benefit of transfusion reduction into lower iron indices was observed with serum iron (difference in LS mean -6.63 umol/L; CI 95% (-9.53, -3.72)) and total iron binding capacity (difference in LS mean -20.57 umol/L; CI 95% (-30.46, -10.69)). The decrease in both parameters within the mitapivat arm was meaningful (serum iron LS mean change -4.76 umol/L 95% CI (-6.59,-2.94) and -4.57 umol/L 95 % CI (-6.44, -2.69) at week 24 and week 48 respectively with a total iron binding capacity LS mean change -27.98 CI (-34.80, -21.16) and -21.80 CI (-28.92, -14.68) at week 24 and week 48 respectively).

Exploratory analyses showed a trend towards thalassemia symptom improvement measured by the PGIC-Thalassemia Symptoms questionnaire with mitapivat compared to placebo. A higher proportion of participants in the treatment arm reported feeling "much better" compared to those in the placebo arm (13.5% vs 4.6%) at Week 48 compared with baseline

Considering haemolysis biomarkers, a slight favourable trend was observed on indirect bilirubin levels in the mitapivat arm, with a slightly greater reduction compared to placebo, of about 5 umol/L observed from week 4 and which seems to remain stable throughout study visits. Preliminary efficacy data from the ongoing OLE period also showed sustained effect of mitapivat in decreasing indirect bilirubin. The interpretation of these markers is however limited by concomitant transfusions and the exploratory nature of the analysis.

Regarding reticulocyte %, a slight favourable trend was observed in the mitapivat arm.

Study AG348-C-017 (non-transfusion-dependent α- or β thalassemia)

With respect to primary endpoint, more subjects treated with mitapivat had an increase from baseline in Hb levels ≥ 1 g/dL compared to placebo: 42.3% and 1.6 % respectively at week 24, leading to an adjusted difference in response rate of 40.9% [CI 95% (32.0, 49.8)].

Among α-NTDT subjects, 23.8% of subjects in the mitapivat arm met the primary Hb response endpoint compared to 0 in the placebo arm, leading to difference in response rate of 23.8%;95% CI (2.2, 39.5).

The least-squares (LS) mean change in Hb from baseline at 24 weeks was 8.57 g/L (95% CI 7.26, 9.88) in the mitapivat arm and -1.06 g/dl(95% CI -2.77, 0.65) in the placebo group, with a difference in LS mean between arm of 9.63 g/dl (95% CI 7.80, 11.46).

Other secondary endpoints included hemoglobin increase of ≥1.5 g/dL from baseline. An increase in Hb of more than 1.5 g/dl was observed in 24.6% (32/130) in the mitapivat arm and 0% (0/65) in the placebo arm with an adjusted difference between arms of 24.7% (95% CI 17.3, 32.1).

The treatment with mitapivat resulted in a significant improvement in average FACIT-Fatigue subscale, with a LS mean change from baseline of 4.85 [CI 95% (3.41, 6.30)], slightly outperforming the established meaningful change threshold of 4.5 points.

A higher proportion of patients in the mitapivat arm (36.2%) experienced a meaningful within-person change threshold for the FACIT-Fatigue score compared to placebo (21.9%), defined as a ≥4.5-point improvement from baseline in average FACIT-Fatigue score from Week 12 through 24, compared to the placebo arm [adjusted difference in response rate 14.2% (95% CI: 1.1, 27.2)].

Among α-NTDT subjects, significant improvement in FACIT-Fatigue score was observed from Week 12 through Week 24 in the mitapivat arm with a LS mean change of 5.28 points (95% CI (2.69, 7.87)] versus 0.78 ;95% CI (-2.73, 4.29) in the placebo arm. The difference in LS mean was 4.50; 95% CI (0.13, 8.87).Regarding haemolysis parameters, treatment with mitapivat resulted in a significant decrease in indirect bilirubin (LS mean difference −10.62; 95% CI: −13.74, −7.50) and LDH (LS mean difference 24.28 95% CI: −45.40, −3.15) compared to placebo.

Efficacy data from the ongoing OLE period equally showed sustained treatment effect of mitapivat in increasing Hb and FACIT-fatigue and decreasing markers of haemolysis.

Although no improvement could be demonstrated in blood markers of iron metabolism, monitoring of hepatic iron concentration, including data from the ongoing OLE period, revealed a visual trend towards lower hepatic iron concentration after 48 weeks of mitapivat treatment compared to baseline. In addition to the statistically significant improvement observed in the FACIT-Fatigue questionnaire, several other exploratory QoL measures—including PROMIS® SF-8 Physical Function, FACT-G, PGIS/PGIC-Thalassemia Symptoms and Walking Capacity, and EQ-5D-5L—demonstrated consistent numerical trends favouring the treatment arm

The Week 24 mean (SD) change from baseline for the FACT-G total score was higher in the mitapivat arm (3.25 [11.343]) than in the placebo arm (0.84 [13.190]). According to PGIS-Thalassemia symptoms questionnaire, a higher frequency of subjects in the mitapivat arm compared with the placebo arm reported feeling much better (28.5% vs 12.5%) or a little better (39.2% vs 20.3%) at Week 24 compared with baseline. A higher frequency of subjects in the mitapivat arm compared with the placebo arm reported their walking capacity as much better (24.6% vs 12.5%) or a little better (30.8% vs 15.6%) at Week 24 compared with baseline. The Week 24 mean (SD) change from baseline for the EQ-5D-5L VAS score was higher in the mitapivat arm (4.2 [15.78]) than in the placebo arm (1.6 [14.70]).

AG348-C-010 study, is an ongoing phase 2 supportive study aiming to assess the efficacy, safety, and PK and PD of mitapivat given 50mg BID followed by optional dose increase to 100mg BID in adult patients with NTDT. This study consisted of a Core Period (up to 24 weeks) followed by an Extension Period (up to 10 years) which is ongoing. Twenty patients were enrolled. Efficacy data from this supportive study did not allow to draw robust conclusions given the very low number of patients enrolled and the different recommended dosing schedule (dose increase from 50 mg BID to 100 mg BID during the core period). The rate of responders was high and amounted to 80 % (100% α-thalassemia subjects and 73.3% β-thalassemia) 95% IC (59.90, 92.86) p-value <0.0001. The mean for the secondary endpoint of average change from baseline in Hb concentrations from Week 12 to Week 24 was approximately 13 g/L. The Week 4-6 average change in Hb from baseline was 10.74 g/dL when subjects received 50 mg BID in Study 010 and, after dose escalation to 100 mg BID, the Week 12-24 average change in Hb from baseline increased to 13.01 g/dL.

3.3. Uncertainties and limitations about favourable effects

Study AG348-C-018 (transfusion-dependent α- or β thalassemia)

In the mitapivat arm, the translated benefit of transfusion reduction into lower iron indices was observed with serum iron (difference in LS mean -6.63 umol/L; CI 95% (-9.53, -3.72)) and total iron binding capacity (difference in LS mean -20.57 umol/L; CI 95% (-30.46, -10.69)). However, no statistical difference from baseline in ferritin levels, could be evidenced, neither at week 24 (LS mean -104.6 ug/L, [95% CI (-248.5, 39.4), nor at week 48 (LS mean 45.3 [95% CI (-129.9, 220.5)]. Similarly, no notable effect of mitapivat on transferrin saturation could be demonstrated (LS mean 0.108 [95%CI (0.049, 0.166)] at week 24 and 0.079 [95% CI (0.027, 0.130)] at week 48). Quantification of iron in the liver (exploratory analysis) did not show a

trend toward improvement, and data from the open-label extension (OLE) indicated stabilisation of hepatic iron concentration values over time, with no further improvement observed with extended treatment duration. Therefore, data regarding iron overload parameters are insufficiently robust to establish a definitive effect on iron burden. Additionally, hemolysis biomarkers (indirect bilirubin, LDH and haptoglobin) and quality of life were assessed as exploratory endpoints, with results that did not demonstrate consistent or statistically significant improvement.

Study AG348-C-017 (non-transfusion-dependent α- or β thalassemia)

The reduction in fatigue measured by the FACIT-Fatigue questionnaire was accompanied by an improving trend on walking capacity assessed through 6MWT functional measurement but no threshold for the clinical relevance of the test was defined, complicating data interpretation. It would also have been more informative to assess specific NTDT-related patient relevant outcomes. The impact of mitapivat treatment on patients' quality of life is therefore only assessed in terms of fatigue, which has its limitations.

A favourable effect was observed in liver iron concentration with new data from the OLE period, with a decrease from baseline at week 48, suggesting a potential therapeutic benefit on iron overload. However, this result is limited by the fact that liver iron was only assessed at a single timepoint, providing no information on the progression over time. Moreover, this effect is tempered by the lack of statistically significant reductions in blood biomarkers of iron overload. At this stage, the overall evidence remains unclear regarding the consistency and robustness of the treatment’s impact on iron overload.

Study AG348-C-017 and Study AG348-C-018

One important limitation is the small number of participants with alpha-thalassemia included in these studies. While a similar therapeutic effect is expected in this population based on the underlying pathophysiology, the conclusions that can be drawn remain limited due to the low sample size. As such, any observed trends in this subgroup should be interpreted cautiously, as they may not fully capture the variability or generalisability of the response in individuals with alpha-thalassemia. To account for the limited number of α-thalassemia patients, information on the population characteristics (demographic and other baseline characteristics) are included in section 5.1 of the SmPC, with reference to 5.1 included in section 4.1

Subjects with active and/or uncontrolled cardiac or pulmonary disease were excluded from both studies and this is reported in the SmPC in section 4.4.

Results from the supportive study AG348-C-010 were consistent with those observed in pivotal study AG348-C-017, with a rapid Hb responses within the first few weeks of mitapivat treatment. However, the sequential dose escalation and absence of control does not allow for clear attribution of efficacy observations to one or other of the dosages. The recommended dose is 100 mg taken orally twice daily based on data from the 2 pivotal studies.

3.4. Unfavourable effects

The safety analysis dataset included 301 patients treated with mitapivat 100mg BID and 148 patients treated with placebo in two pivotal double-blind studies in thalassemia and an additional 19 patients treated with mitapivat for 207 weeks in open-label extension study. In general, the studied population was representative of the target population in terms of disease characteristics. The size of the safety database is considered sufficient to address the safety profile in the thalassemia indication. Long-term safety (>12 months, up to 2 years) is available for 65% of subjects.

In the pivotal studies, the overall frequency of any TEAE was comparable between mitapivat and placebo: 87.0% and 81.8%. The most common TEAE by PT were headache (24.9%), initial insomnia (14.0%), upper respiratory tract infections (13.6%), diarrhoea, fatigue (10% each), and nausea (9.3%).

The most common treatment related TEAE were by PT insomnia (19.2% vs 6.1%) and headache (15.0% vs 6.1%). In general, Grade ≥ 3 TEAE occurred in one patient each, except ALT increased, AST increased, and insomnia in 4, 3, and 3 patients respectively. Headache and insomnia are known effects of mitapivat, while ALT/AST increased is an effect in thalassemia only and management is described in the SmPC.

Serious adverse events occurred in 9% of patients treated with mitapivat and 8.8% patients treated with placebo. The SAE considered related to study treatment were Grade 3 Arrhythmia supraventricular, supraventricular tachycardia, lower respiratory tract infection, and Grade 4 renal mass and, in the OLE period, one event of hypertransaminasaemia. Hypertransaminasemia is an ADR specific to the thalassaemia indication and a specific warning has been included in section 4.4 of the SmPC. Hepatocellular injury (in the form of transaminases increased) is identified as a risk for patients treated for thalassemia. In studies 017 and 018, discontinuation of mitapivat treatment due to events of hepatocellular injury occurred in 5 subjects: 2 in the double-blind period and 3 in the open label extension (OLE).

Mitapivat is a weak inhibitor of aromatase activity. Consistent with the observation in the PK deficiency indication, a decrease of oestrogens and an increase in androgens were observed in male subjects. The change is reversible upon treatment discontinuation. The following warning is already stated in section 4.4 of the SmPC: 'Mitapivat may decrease the systemic exposure of hormonal contraceptives that are sensitive substrates of cytochrome P450 3A4 (CYP3A4) (e.g. ethinylestradiol) (see section 4.5). Women of childbearing potential should be counselled regarding the use of additional or alternative contraception methods (see section 4.6).'

Discontinuations and treatment modifications due to AE were rather infrequent and occurred mostly in mitapivat treated patients (4.7%) than placebo (0.7%). An updated safety analysis with a more recent data cut-off included 464 patients exposed to mitapivat for a median duration of 14.8 months and 309 patients exposed for more than 12 months (median 18.8 months). The safety profile for the extended exposure is overall comparable to initial 12 months. In addition, a pooled safety analysis of all indications where patients were treated with mitapivat is supportive of the safety profile of mitapivat. In general, a lower incidence of adverse events is observed in thalassemia than PK deficiency. Safety data in patients with renal impairment is limited and safety profile in these patients is not sufficiently characterised, however sufficiently addressed in the SmPC.

Mitapivat has an aromatase inhibitory activity (off-target), which led to an advanced sexual development test in fish (OECD234). An updated environmental risk assessment, including the full set of Phase II studies and any potential required follow-up on persistence testing will be provided by December 2026 as committed by the MAH.

3.5. Uncertainties and limitations about unfavourable effects

The clinical studies did not include patients with active and/or uncontrolled cardiac or pulmonary disease as stated in the SmPC. In the context of long-term treatment intended for thalassemia, long-term (> 24 months) safety in patients treated with mitapivat 100mg BID in thalassemia indication is unknown; the patients included in the pivotal studies are being followed for safety in the open-label long-term follow-up (LTFU) studies for up to 5 years from randomization. Cardiac disorders, BMD and osteoporosis should be monitored in the LTFU studies.

3.6. Effects Table

Table 93. Effects Table for Pyrukynd in adults for the treatment of anaemia associated with transfusion-dependent and non-transfusion-dependent alpha- or beta-thalassaemia

Effect	Short Description	Unit	Mitapivat	Placebo	Uncertainties/ Strength of evidence	References
Favourable Effects						
TRR (Transfusion Reduction Response)	≥50% reduction in transfused RBC units with a reduction of ≥2 units of transfused RBCs in any consecutive 12-week period through Week 48	Percentage (95% CI)	30.4%	12.6%	The higher responder rate in the mitapivat arm indicate a significant benefit of mitapivat on transfusion burden reduction compared to placebo. The effect is however of low amplitude given the quite low rate of responders, particularly over longer assessment periods (TRR2 TRR3 and TRR4). Investigations of subgroups were unable to recapitulate the significant improvements from the ITT population in several sub-population, in particular in β^0/β^0 genotype (difference 7.1% CI -8.3,19.7)	Study AG348-C-018
			Adjusted difference in TRR rate 17.6% (8.0, 27.2)			
TRR2	≥50% reduction in transfused RBC units in any consecutive 24-week period through Week 48	Percentage (95% CI)	13.5%	2.3%		
			Adjusted difference in TRR2 rate 11.1% (5.1, 17.0)			
TRR3			14.6%	1.1%		

Effect	Short Description	Unit	Mitapivat	Placebo	Uncertainties/ Strength of evidence	References
	≥33% reduction in transfused RBC units from Week 13 through Week 48 compared	Percentage (95% CI)	Adjusted difference in TRR3 rate 13.4% (7.7, 19.1)			
TRR4	≥50% reduction in transfused RBC units from Week 13 through Week 48	Percentage (95% CI)	7.6%	1.1%		
			Adjusted difference in TRR3 rate 6.4% (1.9, 10.9)			
						Study AG348-C-017
Hb response	≥10 g/L increase in average Hb concentration from Week 12 through Week 24	Percentage (95% CI)	42.3%	1.6%	Treatment with mitapivat was associated with early and robust Hb responses with 42.3% of pts achieving a≥10 g/L increase in average Hb	
			Adjusted difference in response rate 40.9% (32.0, 49.8)			
Change in FACIT-Fatigue sub-scale score	13-item measure that assesses self-reported fatigue	LS mean	4.85	1.46	The treatment with mitapivat resulted in a significant improvement in average FACIT-Fatigue subscale	
			Difference in LS Mean: 3.4 (1.21, 5.59)			

Unfavourable Effects						
Headache	All grades	Incidence %	24.9%	10.8%		Study 017 and 018
Insomnia	All grades	Incidence %	26.7%	8.8%		Study 017 and 018
Upper respiratory tract infection	All grades	Incidence %	13.6%	12.2%		Study 017 and 018
Diarrhoea	All grades	Incidence %	10.0%	8.8%		Study 017 and 018
Fatigue	All grades	Incidence %	10.0%	4.1%		Study 017 and 018
Investigations changes in sex hormones		Incidence %			Aromatase inhibition	Study 017 and 018
Hypertransaminasaemia	All grades	Incidence %	6.5%	2.7%		Thalassemia pool

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

The agreed indication is the following: 'Pyrukynd is indicated in adults for the treatment of anaemia associated with transfusion-dependent and non-transfusion-dependent alpha- or beta-thalassaemia.'

The conclusions are based on two pivotal placebo-controlled, randomized phase 3 studies, study AG348-C-017 in adults with non–transfusion-dependent thalassemia (NTDT) and study AG348-C-018 in adults with transfusion-dependent thalassemia (TDT), and one supportive phase 2 open-label study AG348-C-010 in adults with non–transfusion-dependent thalassemia.

Study AG348-C-018 (TDT) demonstrated that mitapivat can lead to a reduction in transfusion requirements. Within a 12-week rolling period, 30.4 % of subjects achieved a ≥50% reduction in transfused RBC units with a reduction of ≥2 transfused RBC units. The reduction in transfusion volumes led to transfusion independence in 9.9% of participants (17/171) receiving mitapivat over a specified interval of 8 consecutive weeks. Overall, a 13.86% reduction in Transfusion Burden was observed among mitapivat-treated subjects, which translates to a reduction of approximately 4 transfusion visits per year. These results are particularly meaningful in the context of chronic transfusion-dependent conditions, where transfusions are associated with cumulative risks and increase morbidity, even if achieved in a subset of subjects.

There are no authorised medicines in the α-thalassemia setting. Even though α-thalassemia subjects are poorly represented, the shared pathophysiology between α- and β-thalassemia and the mechanistic rationale considering mitapivat MoA, supports the assumption that efficacy should *a priori* not differ between α-and β genotypes.

Although not the primary focus of the study and investigated as additional secondary endpoints, the reduction in transfusion requirements also translated into significant improvement in serum iron and TIBC. However, no significant effects were observed on serum ferritin and transferrin levels, nor on hepatic iron concentrations assessed by MRI, although exploratory, considered more relevant to assess the effect on iron overload.

Additionally, quality of life was assessed as an exploratory endpoint however no definitive conclusions could be drawn from these data due to the lack of consistent or statistically significant improvement.

Study AG348-C-017 (NTDT) demonstrated that mitapivat-treated patients exhibited clinically significant and sustained increases in haemoglobin, with 42.3% of subjects reaching an increase from baseline in Hb levels ≥ 1 g/dL which led to a significant improvement in fatigue, objectified by the FACIT-fatigue questionnaire scores (mean increase of 4.85 points in the FACIT-fatigue score). Additionally, the significant improvement in haemolysis markers alongside the benefits observed in hepatic iron concentration suggests a meaningful clinical benefit of mitapivat. Long-term data provided during the OLE period strengthen these results. The Thalassemia International Federation (TIF) guidelines provide comprehensive recommendations for monitoring iron overload in thalassemia patients, emphasising the importance of both serum ferritin and liver iron concentration (LIC) measurements. In study 017, a small and similar reduction in ferritin concentrations was observed in both treatment arms leading to no significant difference (LS mean (SE) difference was −2.26 (49.529) (95% CI: −100.04, 95.52)). However, a favourable effect was observed in liver iron concentration with new data from OLE period, where liver iron concentration appears to decrease steadily from baseline to Week 48, with an approximate reduction of 1 mg/g. However, the exact numerical values are not provided, and the data are presented only graphically with two time points baseline and Week 48.

Overall, considering both populations, TD and TDT thalassemia patients, pivotal studies AG348-C-017 and AG348-C-018 demonstrated that mitapivat can lead to a durable increase in haemoglobin levels and reduce transfusion burden in patients suffering from thalassemia.

Subjects with α-thalassemia were primarily captured under the non-β0/β0 population and were quite underrepresented, particularly TD- α-thalassemia. Although the globin imbalance differs, α-and β-thalassemia share the same pathophysiology. Therefore, considering mitapivat MoA, there is a mechanistic rationale to support the assumption that efficacy should not differ *a priori* between α- and β-genotypes. This supports the generalisability of the results from the ITT population to α- thalassemia genotype, especially given the consistency of the efficacy data. Given the limited treatment options, particularly for α-thalassaemia, these findings are meaningful and support a positive benefit/risk for the new indication, irrespective of genotype.

Considering that the data primarily support a significant improvement in anaemia, the following indication wording is agreed to adequately reflect the treatment's objective: "*Pyrukynd is indicated in adults for the treatment of anaemia associated with transfusion-dependent and non-transfusion-dependent alpha or beta-thalassaemia*".

The safety profile of mitapivat in thalassemia is considered acceptable in the target indication and manageable. Most of adverse events were low grade, with a reasonably low incidence of Grade ≥ 3 treatment related TEAE, serious adverse events, treatment discontinuation, and dose modifications. Long term (>12 months) safety is available for 65% of patients in the target population.

Five events of hypertransaminasaemia (including 2 SAE) led to treatment discontinuation; treatment modifications are recommended in section 4.4 of the SmPC.

The following warning is already stated in section 4.4 of the SmPC: 'Mitapivat may decrease the systemic exposure of hormonal contraceptives that are sensitive substrates of cytochrome P450 3A4 (CYP3A4) (e.g. ethinylestradiol) (see section 4.5). Women of childbearing potential should be counselled regarding the use of additional or alternative contraception methods (see section 4.6).'

3.7.2. Balance of benefits and risks

Mitapivat is intended to constitute a new therapeutic option for anaemia in adults for the treatment of thalassemia irrespective of genotype (β-thalassemia and alpha–thalassemia) and transfusion requirements (transfusion dependent and non-transfusion dependent).

The pivotal Study AG348-C-018 (TDT) demonstrated a significant difference in transfusion reduction response (TRR) in mitapivat-treated patients when compared to placebo:

- TRR (≥50% reduction with reduction of ≥2 units in any consecutive 12-week period): 30.4% versus 12.6%; Adjusted difference in TRR rate 17.6 % 95% CI (8.0, 27.2)

TRR assessment across different time interval demonstrated a relative long-lasting effect of mitapivat, extended beyond the 12 weeks rolling period of the primary endpoint:

- TRR2 (≥50% reduction in any consecutive 24-week period): 13.5% versus 2.3%; Adjusted difference in TRR rate 11.1 % 95% CI (5.1, 17.0).
- TRR3 (≥33% reduction from Week 13 through Week 48): 14.6 % versus 1.1%; Adjusted difference in TRR rate 13.4 % 95% CI (7.7, 19.1)
- TRR4 (≥50% reduction from Week 13 through Week 48): 7.6% versus 1.1%; Adjusted difference in

TRR rate 6.4 % 95% CI (1.9, 10.9).

Mitapivat demonstrates a meaningful improvement in transfusion burden, also leading to transfusion independence achievement in some subjects, which is a crucial clinical outcome in the management of TDT. However, it seems that the clinical the benefit only concerns a subset of patients, particularly over longer assessment periods. It is therefore relevant to identify sufficiently early patients who may not benefit from treatment with mitapivat and discontinue treatment in these patients to avoid unnecessary exposure as stated in section 4.2, 4.4 and 4.5 of the SmPC.

While acknowledging measurement limitations, consistent improvement across key iron overload markers could not be clearly evidenced. The same concerns are formulated for quality-of-life data.

The pivotal Study AG348-C-017 (NTDT) demonstrated that mitapivat-treated patients exhibited clinically significant and sustained increases in haemoglobin, with 42.3% of subjects reaching an increase from baseline in Hb levels ≥ 1 g/dL compared to 1.6 % of placebo arm treated patients.

Treatment with mitapivat also resulted in a significant improvement in average FACIT-Fatigue subscale, with a LS mean change from baseline of 4.85 [CI 95% (3.41, 6.30)], slightly outperforming the established meaningful change threshold of 4.5 points, in haemolysis markers and LIC.

Overall, the combined analysis of the two studies indicates that a_meaningful effect on anaemia has been demonstrated in adult patients with thalassaemia, irrespective of transfusion status (i.e., both transfusion-dependent [TDT] and non-transfusion-dependent [NTDT] populations).

The safety profile of mitapivat in thalassemia patients is acceptable and consistent with the known mitapivat safety profile. Most adverse events were of low grade. Hypertransaminasaemia is reflected as a warning in section 4.4 of the SmPC related to mitapivat. Long-term (up to 5 years exposure) safety in the context of chronic treatment has been added as missing information in the safety specifications and is monitored in the open label portion of the clinical studies.

3.7.3. Additional considerations on the benefit-risk balance

Pyrukynd has an aromatase inhibitory activity (off-target), which led to an advanced sexual development test in fish (OECD234). An updated environmental risk assessment, including the full set of Phase II studies and any potential required follow-up on persistence testing should be provided by December 2026 as committed by the MAH.

3.8. Conclusions

The overall benefit/risk balance of Pyrukynd 100 mg film coated tablet in the treatment of anaemia in adult patients with non-transfusion-dependent and transfusion-dependent alpha- or beta-thalassemia is considered positive, subject to the conditions stated in section 'Recommendations'.

4. Recommendations

Similarity with authorised orphan medicinal products

The CHMP by consensus is of the opinion that Pyrukynd is not similar to Casgevy and Reblozyl within the meaning of Article 3 of Commission Regulation (EC) No. 847/2000. See appendix on similarity.

Outcome

Based on the CHMP review of data on quality, safety and efficacy, the CHMP considers by consensus that the benefit-risk balance of, Pyrukynd 100mg film coated tablets is favourable in the following indication:

Pyrukynd is indicated in adults for the treatment of anaemia associated with transfusion-dependent and non-transfusion-dependent alpha- or beta-thalassaemia (see section 5.1).

The CHMP therefore recommends the extension of the marketing authorisation for Pyrukynd subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to medical prescription.

Conditions and requirements of the marketing authorisation

Periodic Safety Update Reports

The requirements for submission of periodic safety update reports for this medicinal product are set out in the list of Union reference dates (EURD list) provided for under Article 107c(7) of Directive 2001/83/EC and any subsequent updates published on the European medicines web-portal.

Conditions or restrictions with regard to the safe and effective use of the medicinal product

- **Risk Management Plan (RMP)**

The Marketing authorisation holder (MAH) shall perform the required pharmacovigilance activities and interventions detailed in the agreed RMP presented in Module 1.8.2 of the marketing authorisation and any agreed subsequent updates of the RMP.

An updated RMP should be submitted:

- At the request of the European Medicines Agency;
- Whenever the risk management system is modified, especially as the result of new information being received that may lead to a significant change to the benefit/risk profile or as the result of an important (pharmacovigilance or risk minimisation) milestone being reached.

Conditions or restrictions with regard to the safe and effective use of the medicinal product to be implemented by the Member States.

Not applicable.

Additional Data exclusivity/Marketing protection

Furthermore, the CHMP reviewed the data submitted by the Agios Netherlands B.V., taking into account the provisions of Article 14(11) of Regulation (EC) No 726/2004 and considers that the new therapeutic indication brings significant clinical benefit in comparison with existing therapies (see appendix on Article 14(11)).