15 September 2022
EMA/792328/2022
Committee for Medicinal Products for Human Use (CHMP)

Assessment report

Pyrukynd

International non-proprietary name: mitapivat

Procedure No. EMEA/H/C/005540/0000

Note

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

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Administrative information

Name of the medicinal product:	Pyrukynd
Applicant:	Agios Netherlands B.V. Zuidplein 36 1077 XV Amsterdam NETHERLANDS
Active substance:	Mitapivat sulfate
International Non-proprietary Name/Common Name:	mitapivat
Pharmaco-therapeutic group (ATC Code):	Other hematological agents, (B06AX04)
Therapeutic indication(s):	Pyrukynd is indicated for the treatment of pyruvate kinase deficiency (PK deficiency) in adult patients (see section 4.4).
Pharmaceutical form(s):	Film-coated tablet
Strength(s):	5 mg + 20 mg, 5 mg, 20 mg, 20 mg + 50 mg and 50 mg
Route(s) of administration:	Oral use
Packaging:	blister (PVC/PCTFE/alu)

Package size(s):	56 tablets, Taper pack: 14 tablets (7 x 20 mg + 7 x 50 mg), Taper pack: 14 tablets (7 x 5 mg + 7 x 20 mg) and Taper pack: 7 tablets

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List of abbreviations

ADME	Absorption, distribution, metabolism, and excretion
AE	Adverse event
Al	Aluminium
ALT	Aminotransferase
AST	Aspartate aminotransferase
BID	Twice daily
BLQ	Below the quantifiable limit
CAD	Charged aerosol detection
CFU	Colony forming units
CL	Plasma clearance
CNS	Central nervous system
CQA	Critical quality attribute
DAD	Diode-array detection
DoE	Design of experiments
EAS	Efficacy analysis set
EC	European Commission
ERA	Environmental risk assessment
FBP	Fructose-1,6-bisphosphate
GC	Gas Chromatography
GC-MS	Gas Chromatography mass spectrometry
HDPE	High density polyethylene
HDW	Haemoglobin distribution width
HPLC	High performance liquid chromatography
HRQOL	Health-related quality of life
HV	Healthy adult volunteers
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICP-MS	Inductively coupled plasma mass spectrometry
IOV	Inter-occasion variability
IR	Infrared
ITT	Individual transfusion trigger
LC-MS/MS	Qualified liquid chromatography with tandem mass spectrometry
LSC	Liquid scintillation counting
IU	International units

KF	Karl Fischer titration
LC	Liquid chromatography
LDPE	Low density polyethylene
M	Missense
MCHC	Mean corpuscular haemoglobin concentration
MRI	Magnetic resonance imaging
MS	Mass spectrometry
NCA	Non-compartimental analysis
NM	Non-missense
NMR	Nuclear magnetic resonance
NMT	Not more than
NTDT	Non-transfusion-dependent thalassaemia
NTD	Non-transfusion-dependent
OECD	Organisation for Economic Co-operation and Development
PCE	Polychromatic erythrocyte
PCTFE	Polychlorotrifluoroethylene
PDE	Permitted daily exposure
PEP	Phosphoenolpyruvate
Ph. Eur.	European Pharmacopoeia
PIP	Paediatric investigation plan
PK	Pyruvate kinase
PKD	Pyruvate kinase deficiency
PKDD	Pyruvate kinase deficiency diary
PKDIA	Pyruvate kinase deficiency impact assessment
PKR	Pyruvate kinase R
PRO	Patient-reported outcome
PVC	Polyvinyl chloride
QbD	Quality by design
QD	Once daily
QOL	quality of life
QTPP	Quality target product profile
QWBA	quantitative whole-body autoradiography
RBC	Red blood count
RH	Relative humidity
TAMC	Total aerobic microbial count
TD	transfusion-dependant

TIBC	total iron-binding capacity
TRR	transfusion reduction responders
TSE	Transmissible spongiform encephalopathy
TYMC	Total combined yeasts/moulds count
UV	Ultraviolet
WT	Wild type
XR(P)D	X-ray (powder) diffraction

1. Background information on the procedure

1.1. Submission of the dossier

The applicant Agios Netherlands B.V. submitted on 25 June 2021 an application for a marketing authorisation to the European Medicines Agency (EMA) for Pyrukynd, through the centralised procedure falling within the Article 3(1) and point 4 of Annex of Regulation (EC) No 726/2004 . The eligibility to the centralised procedure was agreed upon by the EMA/CHMP on 25 February 2021.

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 applicant applied for the following indication:

Treatment of pyruvate kinase deficiency (PK deficiency) in adult patients.

1.2. Legal basis, dossier content

The legal basis for this application refers to:

Article 8.3 of Directive 2001/83/EC - complete and independent application

The application submitted is composed of administrative information, complete quality data, non-clinical and clinical data based on applicants' own tests and studies and/or bibliographic literature substituting/supporting certain test(s) or study(ies).

1.3. Information on paediatric requirements

Pursuant to Article 7 of Regulation (EC) No 1901/2006, the application included an EMA Decision(s) P/0365/2020 on the agreement of a paediatric investigation plan (PIP) and on the granting of a class waiver.

At the time of submission of the application, the PIP P/0365/2020 was not yet completed as some measures were deferred.

The PDCO issued an opinion on compliance for the PIP P/0365/2020.

1.4. Information relating to orphan market exclusivity

Following the CHMP positive opinion on this marketing authorisation, the Committee for Orphan Medicinal Products (COMP) reviewed the designation of Pyrukynd as an orphan medicinal product in the approved indication. More information on the COMP's review can be found in the Orphan maintenance assessment report published under the 'Assessment history' tab on the Agency's website: https://www.ema.europa.eu/en/medicines/human/EPAR/Pyrukynd

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 applicant did not submit a critical report addressing the possible similarity with authorised orphan medicinal products, because there is no authorised orphan medicinal product for a condition related to the proposed indication.

1.5. Applicant's request(s) for consideration

1.5.1. New active substance status

The applicant requested the active substance mitapivat contained in the above medicinal product to be considered as a new active substance, as the applicant claims that it is not a constituent of a medicinal product previously authorised within the European Union.

1.6. Protocol assistance

The applicant received the following Protocol assistance on the development relevant for the indication subject to the present application:

Date	Reference	SAWP co-ordinators
25 February 2015	EMEA/H/SA/3006/1/2015/SME/III	Dr Mario Miguel Rosa, Prof. Brigitte Blöchl-Daum
10 November 2016	EMEA/H/SA/3404/1/2016/SME/III	Dr Pierre Démolis and Dr Jan Sjöberg
25 July 2019	EMEA/H/SA/3006/1/FU/1/2019/PED/III	Dr Walter Janssens, Dr André Elferink

The Protocol assistance pertained to the following:

Non-clinical:

- Duration of repeated-dose toxicity studies to support the proposed Phase 2 clinical trial.
- Acceptability of the toxicology development program to support the clinical development plan and MAA.
- Non-clinical development plan to support the initiation of the clinical paediatric study and for MAA.

Clinical:

- PK deficiency as a serious disease with unmet medical need.
- Design of the proposed phase 2 clinical study, specifically with regards to the key inclusion and exclusion criteria, the clinical safety monitoring plan, the endpoints to assess clinical activity and the proposed duration of treatment, including an extension study.
- Agreement that data from a phase 2 study with AG-348 (AG348-C-003 (DRIVE-PK)) and a phase 1 study in healthy volunteers with AG-519 (AG519-C-001) would support initiation of a pivotal trial with AG-519 in NTD adult patients with PK deficiency.
- Overall clinical development plan for MAA.
- Design of the pivotal clinical trial for a MAA for AG-519 in NTD adult patients with PK deficiency, in particular with regards to the population, comparator, randomised withdrawal phase, the proposed 3-part plan, treatment duration and open-label dose-optimisation
- Primary and secondary endpoints.
- Disease-specific PRO measures for PK deficiency.
- In the light of the chosen primary endpoint, acceptability of the sample size and the proposed analysis methods.
- Acceptability of the safety database for MAA.
- Advice concerning clinical development in the paediatric population.

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: Armando Genazzani

The Rapporteur appointed by the PRAC was:

PRAC Rapporteur: Adam Przybylkowski

The application was received by the EMA on	25 June 2021
The procedure started on	15 July 2021
The CHMP Rapporteur's first Assessment Report was circulated to all CHMP and PRAC members on	4 October 2021
The PRAC Rapporteur's first Assessment Report was circulated to all PRAC and CHMP members on	8 October 2021
The CHMP agreed on the consolidated List of Questions to be sent to the applicant during the meeting on	11 November 2021
The applicant submitted the responses to the CHMP consolidated List of Questions on	19 January 2022
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	28 February 2022
The PRAC agreed on the PRAC Assessment Overview and Advice to CHMP during the meeting on	10 March 2022
The CHMP agreed on a list of outstanding issues in writing and/or in an oral explanation to be sent to the applicant on	24 March 2022
The applicant submitted the responses to the CHMP List of Outstanding Issues on	18 April 2022
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	4 May 2022
The CHMP agreed on a 2nd list of outstanding issues in writing and/or in an oral explanation to be sent to the applicant on	19 May 2022
The applicant submitted the responses to the CHMP List of Outstanding Issues on	21 June 2022
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	7 July 2022
The CHMP agreed on a 3rd list of outstanding issues in writing and/or in an oral explanation to be sent to the applicant on	21 July 2022
The applicant submitted the responses to the CHMP List of Outstanding Issues on	17 August 2022
The CHMP Rapporteurs circulated the CHMP and PRAC Rapporteurs Joint Assessment Report on the responses to the List of Outstanding Issues to all CHMP and PRAC members on	31 August 2022

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	15 September 2022
Furthermore, the CHMP adopted a report on New Active Substance (NAS) status of the active substance contained in the medicinal product (see Appendix on NAS)	15 September 2022

2. Scientific discussion

2.1. Problem statement

2.1.1. Disease or condition

The claimed therapeutic indication is "the treatment of pyruvate kinase deficiency (PK deficiency) in adult patients". Pyruvate kinase deficiency (PKD) is a glycolytic defect causing congenital non-spherocytic haemolytic anaemia.

2.1.2. Epidemiology and risk factors, screening tools/prevention

PKD exhibits autosomal recessive inheritance in nearly all cases and is widely distributed geographically. The frequency is not precisely defined, with a wide estimated prevalence of 3:1 000 000 to 1:20 000 (Beutler & Gelbart, 2000; Carey et al, 2000). The prevalence is higher in the Pennsylvania and Ohio Amish community due to founder effect. The prevalence of this condition may be underestimated due to the lack of specific clinical features and the necessity of highly specialised testing not readily available at many medical institutions.

PKD is an autosomal recessive disorder; thus, the family history is typically unrevealing, with the exception of miscarriages and affected siblings. The differential diagnosis includes the heterogeneous group of both the congenital and acquired haemolytic disorders. The haemolysis is typically exacerbated by acute infections, stress and pregnancy.

2.1.3. Biologic features, aetiology and pathogenesis

Pyruvate kinase converts phosphoenolpyruvate to pyruvate, creating 50% of the red cell total ATP. Red cell longevity is dependent on the ATP produced in glycolysis. Thus, PKD leads to less ATP and a shortened red cell lifespan. The red cells in PKD are variably damaged with the youngest red cells, most dependent on glycolysis and increased ATP levels, at highest risk for destruction, while the older red cells are less severely affected. Travel through the splenic capillaries causes damage to affected red cells, which are variably cleared by both the spleen and liver.

To date, more than 300 mutations in the PKLR gene have been associated with PKD. The majority of these, about 70–80%, are missense substitutions, such as R510Q in Northern Europe and the United States and R486W in Southern Europe. A particularly high frequency of the R479H mutation in homozygous state exists among the Pennsylvania Amish due to a founder effect (Rider et al, 2011). However, not all the mutations detected can be immediately classified as causative until their pathogenic nature is confirmed by other functional tests.

2.1.4. Clinical presentation, diagnosis and stage/prognosis

The clinical features of PKD are highly variable and patients with PKD have a variable laboratory phenotype ranging from mild well-compensated anaemia to transfusion dependent haemolysis. The most frequent symptoms of PKD are those related to anaemia, present in 90–95% of cases, from mild to transfusion dependent (low Hb, reduced haptoglobin, indirect hyperbilirubinaemia, reticulocytosis, hyperferritinaemia, increased LDH), splenomegaly (80–85%), with a variable degree of enlargement,

jaundice (40–70%) and gallstones (30–45%). Less common manifestations include aplastic crisis (2–14%), bone deformities (9%), extramedullary erythropoiesis (9%), delayed puberty (8%), hyperpigmentation (6%), leg ulcers and pulmonary hypertension (2–3%) (Zanella & Bianchi, 2000; Zanella et al, 2005; Grace et al, 2015, 2018a).

The anaemia may also be surprisingly well tolerated because of the increased red cell 2,3-DPG content, which is responsible for a rightward shift in the oxygen dissociation curve of haemoglobin. However, many patients with PKD report fatigue and low energy related to their anaemia, with a negative impact on their daily life.

Iron overload is a common complication of PKD. It is a predictable complication of chronically transfused patients but is also common in patients with PKD and limited or no history of transfusions. In transfusion-independent individuals, additional factors for iron loading include splenectomy, a degree of ineffective erythropoiesis, and co-inheritance of hereditary haemochromatosis mutations. Transfusional haemosiderosis is a predictable complication of chronic transfusion therapy.

The diagnosis of PKD is based on the presence of clinical signs and symptoms and laboratory markers of chronic haemolytic anaemia, 1) on reduced PK enzymatic activity, and 2) on the detection of compound heterozygous and homozygous mutations in the PKLR gene.

PK enzymatic activity is usually determined in red blood cell (RBC) lysates by spectrophotometric assay. Importantly, there is no clear correlation between the severity of the clinical phenotype and the reduction of PK activity in the *in vitro* assay. In fact, clinically mild patients may display significantly reduced PK activity (Bianchi & Zanella, 2000; Zanella & Bianchi, 2000). Therefore, even when testing reveals low PK activity, genotyping of the red cell PK gene, PKLR, is strongly recommended to confirm the diagnosis of PKD.

With over 300 mutations described and compound heterozygous gene mutations in most patients, the relationship between the genotype and clinical symptoms in PKD is difficult to study. This topic was first investigated by describing homozygous patients, and then by studying larger series of compound heterozygous cases grouped according to anaemia severity. Severe anaemia was commonly associated with disruptive mutations, such as stop codon, frameshift, splicing and large deletions, and with missense mutations directly involving the active site or protein stability. However, the clinical manifestations of PKD also include genetic post-translational or epigenetic modifications, along with variations in the degree of ineffective erythropoiesis and splenic function, making it complex to interpret the *in vitro* findings.

More recently, the genotype-phenotype correlation was investigated in patients enrolled in the PKD Natural History Study (Grace et al, 2018a). Patients were grouped according to the presence of two missense mutations (M/M), one missense/ one non-missense (M/NM), or two non-missense mutations (NM/NM); non-missense mutations included nonsense, frameshift, inframe splicing mutations and other disruptive mutation variants. When compared with those patients with at least one missense mutation, the NM/NM group had a more severe phenotype, characterised by earlier diagnosis, lower haemoglobin levels, higher rate of splenectomy, greater transfusion needs and higher ferritin levels. These findings suggest that genetic testing may also be useful in discussing prognosis and establishing a monitoring plan with patients. However, the haemoglobin range overlaps among the genotype groups and the frequency of complications is high across genotypes, suggesting careful monitoring is needed in all patients regardless of the genotype.

2.1.5. Management

Curative therapy for PKD is not currently available.

Until recently, management options for PKD were limited to blood transfusion, splenectomy to maintain an adequate haemoglobin and supportive care. For these patients, haematopoietic stem cell transplant (HSCT) is the only curative option, but it is associated with high rate of mortality and morbidity and is not currently recommended over regular blood transfusions and supportive care.

A common intervention is splenectomy. Approximately 70% of patients diagnosed with PK deficiency undergo a splenectomy procedure between the ages of 5 and 18 years to reduce the need for transfusion and/or to increase Hb. In majority of patients, haemolysis and indirect hyperbilirubinaemia persist after splenectomy, and patients remain at risk for gallstones and jaundice.

Another common intervention is transfusion. A patient's degree of anaemia does not correlate with the symptoms of anaemia. The decision to transfuse a patient with PKD relates to the patient's tolerance of anaemia rather than an arbitrary level of haemoglobin. Because of increased red cell 2,3-DPG content, patients may tolerate moderately severe anaemia with few symptoms due to enhanced oxygen unloading from haemoglobin. In PKD, there is currently no data to support a strategy of transfusing to keep the haemoglobin above a set nadir with a goal of avoiding complications. This strategy exposes patients to risks without clear benefit. Rather, transfusions should be individualised, based on the patient's symptoms, level of activity, and assessment of the impact of the anaemia on their quality of life.

In addition to the supportive treatments targeting improvement of haemolytic anaemia, several treatments are used to address other disease complications; cholecystectomy in the case of choledocholithiasis (67.8%), bisphosphonates for improving BMD/preventing further worsening, and supplemental folic acid for rapid red cell turnover.

Iron overload is common in PKD, in both chronically transfused and transfusion-independent individuals, and monitoring through measurements of plasma ferritin, and/or liver, pancreas, and cardiac iron burden by MRI methods is indicated. Chelation therapy is necessary in regularly transfused patients and may be required intermittently in non-transfused patients with PKD as MRI studies are monitored.

2.2. About the product

Mitapivat is an allosteric activator of wild-type PKR (red blood cell [RBC]-specific form of pyruvate kinase) and a range of mutant PKR (mPKR) enzymes. Mitapivat acts by allosterically binding to the PKR tetramer and enhancing its affinity for PEP, thereby increasing the ability of RBCs to convert PEP + ADP to pyruvate + ATP. Thus, by restoring the activity of mutant forms of PKR, mitapivat targets the underlying enzymatic defect that causes haemolysis in PK deficiency.

2.3. Quality aspects

2.3.1. Introduction

The finished product is presented as film-coated tablets containing 5 mg, 20 mg or 50 mg mitapivat. The product contains the hemisulfate sesquihydrate (2:1:3), henceforth abbreviated as 'sulfate hydrate' salt.

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 and indigo carmine aluminium lake (E132);

Printing ink: shellac glaze (E904), isopropyl alcohol, black iron oxide (E172), N-butyl alcohol, propylene glycol (E1520), ammonium hydroxide (E527).

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

2.3.2. Active Substance

2.3.2.1. General information

The chemical name of mitapivat is *N*-(4-((4-(cyclopropylmethyl)piperazine-1)carbonyl)phenyl)quinoline-8-sulfonamide sulfate hydrate (2:1:3) corresponding to the molecular formula $(C_{24}H_{26}N_4O_3S)_2.H_2SO_4.(H_2O)_3$. Mitapivat has a relative molecular mass of 450.56 g/mol (free base) or 1053.23 g/mol (sulfate hydrate salt) and the following structure:

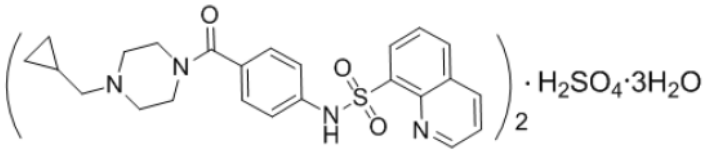


Figure 1: active substance structure

The chemical structure of mitapivat was elucidated by a combination of elemental analysis, infrared spectroscopy, ^{1}H and ^{13}C NMR spectroscopy, mass spectrometry, single crystal X-ray crystallography and UV spectroscopy. The solid-state properties of the active substance were measured by thermogravimetric analysis, differential scanning calorimetry, dynamic vapour sorption and x-ray powder diffraction (XRPD).

The active substance is a white to off-white, non-hygroscopic crystalline solid. Mitapivat shows relatively high solubility at pH ranges from 1.0 - 5.5 and low solubility at pH range from 5.5 - 8.0. Mitapivat is achiral.

Three polymorphic forms have been observed for mitapivat. The mitapivat manufacturing process consistently produces the desired form in the desired stoichiometry.

2.3.2.2. Manufacture, characterisation and process controls

The active substance is synthesised in five main steps using three well defined starting materials with acceptable specifications. Two manufacturers are proposed.

Adequate in-process controls are applied during the synthesis. The specifications and control methods for intermediate products, starting materials and reagents have been presented.

The characterisation of the active substance and its impurities are in accordance with the EU guideline on chemistry of new active substances.

Potential and actual impurities were well discussed with regards to their origin and characterised and a detailed discussion on mutagenic impurities was provided.

During the procedure, a major objection was raised in relation to the description and control of the three active substance starting materials used. The major objection included questions in relation to the control of mutagenic or potentially mutagenic impurities in the starting materials. The applicant provided satisfactory responses to resolve the major objection. Several mutagenic and potentially mutagenic

impurities (class 1, 2 and 3) are controlled via ICH M7 option 3, i.e. by a specification limit in the active substance starting material, supported by purge data to demonstrate sufficiently low levels in the active substance.

The commercial manufacturing process for the active substance was developed in parallel with the clinical development programme. The same route of synthesis has been used throughout the development of mitapivat and this route has been optimised in parallel with the clinical development programme. The quality of the active substance used in the various phases of the development is comparable with that produced by the proposed commercial process.

The active substance is packaged in double low-density polyethylene bags placed within a high-density polyethylene (HDPE) drum. The primary packaging complies with Ph. Eur. 3.1.3 for polyolefins and Regulation EC 10/2011 as amended.

2.3.2.3. Specification

The active substance specification includes tests for: description, identity (IR), assay (HPLC), impurities (HPLC, HPLC/MS and GC), residual solvents (GC), particle size (Ph. Eur.), X-ray powder diffraction (Ph. Eur.) water content by KF (Ph. Eur.), residue on ignition (Ph. Eur.), elemental impurities (ICP-MS) and sulfate content (HPLC-CAD).

The active substance specifications are based on the active substance critical quality attributes (CQA). The CQA identified are: organic related substances, genotoxic impurities, other active substance attributes such as description, identification, assay, residual solvents content, water content, residue on ignition, potential genotoxic impurities content, residual imidazole content, elemental impurities content and the crystalline form of mitapivat (hemisulfate sesquihydrate salt). Microbiological purity is not a CQA.

The control of mutagenic or potentially mutagenic impurities is adequate.

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

Batch analysis data (5 validation/commercial batches) of the active substance are provided. The results are within the specifications and consistent from batch to batch. The batch data provided includes batches from each of the two active substance manufacturers proposed for commercial manufacturing, which are considered equivalent.

2.3.2.4. Stability

Stability data from 3 batches manufactured at commercial scale from each of the two manufacturers proposed for marketing stored in the intended commercial package for up to 36 months (manufacturer 1) and 18 months (manufacturer 2) under long term (25ºC / 60% RH) and intermediate (30ºC, 65 % RH) conditions, and for up to six months under accelerated conditions (40ºC / 75% RH) according to the ICH guidelines were provided. The following parameters were tested: description, assay, related impurities, water content, XRPD, particle size and microbial purity. The analytical methods used were the same as for release (with the exception of microbiological purity which is not tested at release) and are stability indicating. All tested parameters were within the specification limits.

Photostability testing following the ICH guideline Q1B was performed on one batch. Mitapivat is photostable.

Results from stress tests (acidic, basic, oxidative, simulated sunlight, thermal and humidity) were also provided for one batch (solid active substance and in solution). Formation of a degradation product was observed under acidic and basic conditions and formation of another degradation product was observed under oxidative conditions. In solution, formation of degradation impurities was observed upon exposure to simulated sunlight while the solid active substance remained stable.

The stability results indicate that the active substance is sufficiently stable. The stability results justify the proposed retest period 48 months when stored at or below 30 °C in the proposed container.

2.3.3. Finished Medicinal Product

2.3.3.1. Description of the product and pharmaceutical development

The finished product is presented as blue film-coated tablets for oral administration containing 5 mg, 20 mg or 50 mg of mitapivat (as hemisulfate sesquihydrate) as the active substance. The 5 mg tablet is round, approximately 5 mm in diameter and imprinted on one side with "M5" in black ink. The 20 mg tablet is round, approximately 8 mm in diameter and imprinted on one side with "M20" in black ink. The 50 mg tablet is oblong, approximately 16 mm in length and 6.8 mm in width and imprinted on one side with "M50" in black ink. The three different strengths are sufficiently distinguishable from each other by tablet size and/or shape, and by the use of a unique product identification imprint on one tablet face. The composition is quantitatively proportional between the three strengths, the formulation being manufactured from a common blend and the same coating agent.

The aim of development was an immediate release dosage form to deliver the therapeutic dose of mitapivat for twice daily administration as defined in the quality target product profile (QTPP). This allowed identification of potential critical quality attributes (CQAs: description, identification, assay, degradation products, uniformity of dosage units, water content, microbiological quality and dissolution) of the finished product which were then investigated during development studies.

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

The polymorphic form of the active substance is controlled in the specification of the active substance and has been shown to be stable.

An excipient compatibility study was conducted with binary blends of excipients that directly come into contact with mitapivat and the active substance has been shown to be compatible with the selected excipients. Functional attributes of the excipients have been adequately described. There are no overages in the formulation. All excipients are well known pharmaceutical ingredients and their quality is compliant with Ph. Eur. or other relevant 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. As the finished product contains contains less than 1 mmol (23 mg) sodium per tablet, it is essentially “sodium-free”.

Two formulations of mitapivat finished product have been used during clinical development: 5 mg, 20 mg, and 50 mg tablets and 5 mg, 25 mg, and 100 mg capsules.

Mitapivat active substance absorption is potentially limited by solubility and dissolution. Mitapivat demonstrates relatively high solubility at pH ranges from 1.0 - 5.5 and low solubility at pH range from 5.5 - 8.0. The sulfate hydrate form of the active substance affords sufficient solubility overall at physiologically relevant pH values.

The manufacturing process for Pyrukynd tablets used in clinical trials is essentially the same as the commercial process in terms of process and control strategy. The choice of manufacturing process has been justified. Dry granulation and direct compression formulations were initially considered.

The tablet batches used for clinical development as well as the primary registration stability batches were manufactured at the manufacturing site proposed for commercial manufacturing and following the proposed commercial process with the exception of imprinting, as all tablets used in clinical studies were plain-faced. The imprinting does not alter the physical properties of the tablets and the trace amount of imprinted ink on the tablet is not expected to impact the performance and bioavailability of the tablets. Therefore, no formal bioequivalence studies were conducted to compare the plain-faced and imprinted tablets.

The relative bioavailability following administration of the direct compression tablet formulation intended for commercial manufacture and the capsule formulation used in phase 1 safety and phase 2 efficacy studies was investigated in a clinical study and found to be similar.

The development of the QC dissolution method is described. The discriminatory power of the dissolution method has been studied and sufficient discriminatory capacity has been demonstrated for meaningful changes in process parameters or composition.

For QC testing, a method utilizing a USP Apparatus 2 with 900 mL of 100 mM phosphate buffer, pH 6.8 containing 0.05% SDS with a paddle speed of 70 rpm was selected.

The critical process parameters of the manufacturing process have been identified, adequately studied and are appropriately defined and controlled.

A bulk holding time study was conducted. Stability data justify a maximum hold time of 3 months for the final blend mixture, core tablets, and bulk film-coated tablets.

The start of shelf-life of the finished product is determined in accordance with Note for Guidance on Start of Shelf-life of the Finished Dosage Forms (CPMP/QWP/072/96).

The primary packaging is PVC/PCTFE/Al blisters. 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.

2.3.3.2. Manufacture of the product and process controls

The manufacturing process consists of several steps which include blending, tabletting and packaging.

The process is considered to be a standard manufacturing process.

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 3 consecutive production scale batches prior to commercialisation.

2.3.3.3. Product specification

The finished product release specifications include appropriate tests for this kind of dosage form: description (visual), identification (HPLC/UV and HPLC/DAD), assay (HPLC/UV), impurity AGI-80276 (LC/MS/MS), degradation products (HPLC/UV), uniformity of dosage units (Ph. Eur.), dissolution (in house), water content (Ph. Eur.) and microbiological examination (Ph. Eur.).

Limits for impurities are set in line with ICH Q3B. 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.

During the procedure, a major objection was raised in relation to the risk of presence of nitrosamines. The major objection is resolved.

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.

Batch analysis results are provided for 6 batches of each strength manufactured with active substance from both manufacturers confirming the consistency of the manufacturing process and its ability to manufacture to the intended product specification. Batch results for clinical batches and for the registration batches were also provided.

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

2.3.3.4. Stability of the product

Stability data from 18 finished product batches (6 batches per strength) produced according to the commercial manufacturing process and using different active substance batches from both active substance manufacturers stored for up to 12-24 months under long term conditions (25ºC / 60% RH), for up to 12-24 months under intermediate conditions (30ºC / 75% RH) and for up to 6 months under accelerated conditions (40ºC / 75% RH) according to the ICH guidelines were provided. The batches of medicinal product are representative to those proposed for marketing and were packed in the primary packaging proposed for marketing.

Samples were tested for description, assay, degradation products, dissolution, water content, microbial purity and XRPD. All results were within specification.

In addition, two batches (5 mg and 50 mg strength) were exposed to light as defined in the ICH Guideline on Photostability Testing of New Drug Substances and Products. The results indicate that the finished product is not light sensitive.

Results from forced degradation studies were also provided. 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.

Based on available stability data, the proposed shelf-life of 24 months with the special precaution for storage 'store below 25ºC' as stated in the SmPC (section 6.3 and 6.4) is acceptable.

2.3.3.5. 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.

2.3.4. Discussion on chemical, pharmaceutical and biological aspects

Information on development, manufacture and control of the active substance and finished product has been presented in a satisfactory manner. 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. A Major objection raised during the procedure in relation to the description and control of the three starting materials used to manufacture the active substance has been satisfactorily resolved. A Major Objection raised in relation to the risk of presence of nitrosamines is also satisfactorily resolved. Suitable limits at release and shelf-life have been included in the finish product specification. The shelf life of 2 years with storage condition 'store below 25 ºC' ensures that the nitrosamine impurity remains below the acceptable intake until the end of shelf life.

2.3.5. 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.

2.3.6. Recommendations for future quality development

Not applicable.

2.4. Non-clinical aspects

2.4.1. Pharmacology

Primary pharmacodynamics

Pyruvate kinase deficiency is an autosomal recessive enzymopathy in red cells that is caused by mutations in PKLR. These genetic alterations lead to a deficit of pyruvate kinase activity in red cells and to haemolytic anaemia of variable severity. The main mutation found in Caucasian patients with PK deficiency are R510Q (40%) and R486W (30%) approximately. Pyruvate kinase R (PKR) is one of 4 pyruvate kinase isoenzymes expressed in human tissues by 2 different 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. The isoforms are expressed either in the lungs, adipocytes or epithelial cells (PKM2), either in the brain, muscle or heart (PKM1), either in liver and small intestine (PKL) or erythroblasts or matures erythrocytes (PKR). PKM2 et PKLR are regulated by fructose-1,6-bisphosphate [FBP] [activating] and several amino acids [both activating and inhibitory]) and phosphorylation [inhibitory]. Mitapivat is an oral, small-molecule allosteric activator of wild type (WT) and a range of mutant isoforms of pyruvate kinase in red cells that binds to an allosteric pocket to stabilise the active tetrameric form of the enzyme and enhance its affinity for its substrate, phosphoenolpyruvate (Grace RF, et al. and Kung C, et al. 2017).

In vitro studies

Mitapivat and its predominant metabolite AGI-8702 were tested to evaluate their activation potential. It was demonstrated that while the WT PKR was activated by mitapivat with AC50 0.013 µM the 10 mutant PKR isoforms evaluated were activated with AC50 values ranged approximately 0.009 to 0.059 µM.

Mitapivat also activated PKM2 and PKL, with AC50 values of 0.038 and 0.037 μM, respectively, and maximum percent activation of 587% and 293%, respectively. In contrast to mitapivat, AGI-8702 had low potency mixed activity (activation, inhibition, or no effect) against WT and the mutant isoforms.

Mitapivat also demonstrated the ability to stabilise mutant PKR isoforms that are hypersensitive to thermal denaturation such as R510Q, G364D and R532W, especially towards R510Q.

In human red blood cells, mitapivat activates PKR with AC50 and AC90 for PKR activation of 0.0619±0.0346 μM and 1.002±1.109 μM, respectively (n=16). The average maximum percent PKR activation was 274%±56%. AGI-8702 is a weak activator of PKR in RBCs from human whole blood compared to mitapivat.

To evaluate mitapivat's effect, the rate of carbon flow through the PKR enzyme reaction (PKR flux) was measured in WT C57BL/6 mouse whole blood. Mitapivat increased the PKR flux by 80% and also induced enhanced glycolytic pathway activity in RBCs. In human RBCs isolated from fresh whole blood from healthy donors: mitapivat increased ATP levels in human RBCs, consistent with activation of cellular PKR with an average absolute of 1.59-fold change and a mean AC50 0.0109±0.007 μM. The metabolite of mitapivat, has a low potency (0.5% of mitapivat) to increased ATP Production.

In human RBCs isolated from 4 patients with distinct genotypes of PK deficiency (Patient A: R510Q/G511R; Patient B: R486W/D390N; Patient C: A495V/E241stop; Patient D: R510Q/E241stop), mitapivat increased ATP levels and decreased 2.3-DPG and phosphoenolpyruvate (PEP) after 3 (Patient C) to 24 hours (Patient A and B) of exposure to mitapivat. Patient D was not evaluated in this experiment because the number of available cells was limited.

Additionally, dose-response curves assessed for 2 of the 4 patients (B and D) demonstrated that Mitapivat treatment (concentration range was 0.000153 to 10 μM for Patient B and 0.000508 to 10 μM for Patient D) resulted in a dose-dependent increase in PKR activity.

In vivo studies

The objective of PK/PD studies was to investigate the relationship between mitapivat pharmacokinetics and target engagement (PKR activation) and pharmacodynamics parameters (2,3-DPG and ATP concentrations, and calculated ATP/2,3-DPG ratios) after single or multiple oral doses of mitapivat.

After single dose of 1, 10, 50 or 150mg/kg of mitapivat, an increase of PKR activity in RBC (24.1% to 145%) and 2,3 DPG levels (by 6.53% to 23.3%) was observed. No change of ATP level was noted. The increase of ATP level was observed after 3 days BID of treatment with an augmentation of 14.8 to 45%. The decrease of 2.3 DPG levels and the increase of PKR activity were observed with the same changes than single dose (0.777% to 22.5% and 39.9% to 196%, respectively). After 7 days of BID oral dosing of mitapivat, results were similar to the ones after 3 days of treatment: ATP exposure increased (by 15.6% to 43.7%) and 2,3- DPG exposure decreased (by 1.78% to 18.7%) in mouse blood, and PKR activity effectively increased (by 29.4% to 141%) in RBCs in a dose-proportional manner. Therefore, results were pooled to determinate the curve of pharmacokinetic/pharmacodynamics relationships.

Mitapivat was evaluated in anaemia mice (female Hbbth3/+ mice were used in all studies since they exhibit a more severe phenotype than males [Nai et al, 2012]versus WT C57BL/6J mice during 21 days at 50 mg/kg BID oral gavage or 100 mg/kg diet and 56-day study diet: 100 mg/kg. In anaemic mice at 50 mg/kg, an increase of haemoglobin and mean corpuscular volume was observed as well as a decrease of absolute reticulocyte count.

An increase of ATP levels was observed in peripheral blood after 21 days in Hbbth3/+ mice (50 mg/kg BID). Moreover, a decrease of apoptotic erythroblasts from spleen and bone marrow and a decrease of

levels of reactive oxygen species in erythroblasts in bone marrow (P<0.05 vs vehicle control) were observed in anaemic mice.

Secondary pharmacodynamics

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).

On the other hand, no aromatase inhibition activity was observed with AGI-8702 at the highest concentration tested (10 µM).

Safety pharmacology

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. Otherwise, CNS (central nervous system) effects observed in the Irwin study in male rats, were more frequent in a proportional manner in the treated groups (30, 150, or 300 mg/kg) compared to the control, in particular at the highest doses tested of 150 and 300 mg/Kg. Consequently, a relationship with the test article cannot be excluded at all. Anyway, considering the exposure values at 16 and 33-fold the human Cmax (965 ng/mL) and 26 and 53-fold the human AUC0-12hr (3,580 hr•ng/mL) at the recommended dose of 50mg BID, respectively, the occurred effects were suggested to not represent a safety risk from a clinical point of view.

Following observed effects in repeat dose toxicology studies in Cynomolgus monkeys, the emetic activity was evaluated in ferrets. Mitapivat effects 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. The mitapivat $AUC_{0\text{-}12hr}$ and Cmax values associated with the 30 mg/kg dose were 23,752 hr•ng/mL (6.6-fold the human $AUC_{0\text{-}12hr}$ at the recommended dose) and 10,183 ng/mL (11-fold the Cmax at the recommended dose), respectively. The mitapivat AUC0-12hr and Cmax values associated with the 60 mg/kg dose were 96,105 hr•ng/mL (27-fold the human AUC0-12hr at the recommended dose) and 20,200 ng/mL (21-fold the Cmax at the recommended dose), respectively. No pharmacodynamic drug interaction studies have been conducted; according to the applicant it is not anticipated that mitapivat will be administered in combination with other therapies that may alter the pharmacological effect of the test article, or with therapies whose pharmacological effects may be altered by co-administration with mitapivat. This is agreed.

Pharmacodynamic drug interactions

No pharmacodynamic drug interaction studies have been conducted, which is acceptable give the product-characteristics.

2.4.2. Pharmacokinetics

Pharmacokinetic studies

Mitapivat is a small molecule activator of the red blood cell–specific form of pyruvate kinase (PKR). A comprehensive series of *in vitro* and *in vivo* absorption, distribution, metabolism, and excretion (ADME) studies has been conducted with mitapivat. *In vitro* studies of mitapivat were also performed to evaluate the potential for pharmacokinetic (PK) drug-drug interactions.

The single-dose PK of mitapivat was characterised by rapid oral absorption, moderate to high plasma clearance (CL), high volume of distribution at steady-state (Vss), moderate to long half-life ($T_{½}$), and moderate to high oral bioavailability in Sprague Dawley rats, Beagle dogs, and Cynomolgus monkeys.

Toxicokinetics was evaluated in several repeat-dose toxicology studies. The TK of mitapivat and its metabolite, AGI-8702, was evaluated in mice, rats, monkeys, and rabbits after twice-daily (BID) oral doses of mitapivat ranging from 10 to 2,000 mg/kg/day for up to 9 months.

Methods of analysis

Qualified liquid chromatography with tandem mass spectrometry (LC-MS/MS) bioanalytical assays were used for all single-dose PK and *in vitro* studies. Method details are included in the individual reports. Metabolism and excretion studies using [^{14}C]mitapivat used LC-MS/MS techniques for metabolite identification. Quantitative determination of total radioactivity and metabolites was made either by liquid scintillation counting (LSC) or in-line radioactivity monitoring (β-radioactivity monitoring [β-RAM]) techniques. The tissue distribution study conducted in rats after administration of [^{14}C]mitapivat used quantitative whole-body autoradiography (QWBA) methodology. All radiolabelled studies were conducted with [^{14}C]mitapivat of 97% purity or greater.

In the toxicokinetic studies plasma concentrations of mitapivat and its metabolite, AGI-8702, were determined using validated LC-MS/MS assays.

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.

Absorption

It was shown by a permeability test that mitapivat is actively transported across Caco-2—cells. The bidirectional permeability of Mitapivat appeared greater in the basal-apical direction than the A-B direction resulting in efflux ratios greater than 2 and was reduced by 92 and 98% in the presence of the P-gp inhibitors, valspodar and verapamil respectively, confirming that mitapivat is actually a substrate of P-gp while it is not actively transported across MDCKII-BCRP cells. After a single PO dose, the oral bioavailability of mitapivat was 50%, 82%, and 24% in rats, dogs, and monkeys, respectively. 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.

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 higher in female than male rats. At 1000 and 2000 mg/kg, the dose proportional was less important (only 2/2.5-fold).

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.

Distribution

The brain versus plasma distribution of mitapivat was assessed in fed male rats after a single dose 300 mg/kg PO or during 5 days of once-daily PO dosing at 300mg/kg. Low brain penetration was observed, with brain-to-plasma ratios of 0.0912 after five days of daily oral administration. After 24 hours, the plasma concentration of mitapivat decreased from approximately 10,000 ng/ml to <10 ng/ml after following the single-dose treatment while decreased to <100ng/ml during the 5-day dosing. Conversely, in the brain, the mitapivat concentration seemed to keep constant (around 1000 ng/ml) and similar either at a single dose or after 5 days of treatment (no accumulation). Indeed, the mitapivat plasma and brain AUC0-24hr from time 0 to 24 hours post-dose were 253619 and 14394 hr*ng/mL, respectively following single dose administration and 192010 and 17506 hr*ng/mL, respectively after 5 days administration.

The tissues distribution was assessed in male Long-Evans rats by [14C]radioactivity using validated QWBA techniques. The radioactivity after a single oral 100-mg/kg dose of [14C]mitapivat after 168 post-dose were below the quantifiable limit (BLQ) in most tissues. By 504 hrs, mosti tissues were BQL except aorta, eye, abdominal fat, liver, muscle, spleen, thyroids and uveal tract. The 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 mean values for mitapivat (0.2, 1, and 10 µM) was moderate to high (89%, 93.3%, 98% and 97.7% in dog, monkey, rat and human, respectively) and was 53.76% for the metabolite AGI-8702 (0.1, 0.3 and 1 µM) 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. No Placental transfer of mitapivat has been evaluated.

Metabolism

Microsome tests have shown that mitapivat presents a 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%, excepted metabolites M396 or AGI-8702 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.

Excretion

In male rats, the dosed radioactivity was excreted via biliary, urinary, and fecal 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 feces and urine, the mean cumulative excretion was 30.4% and 13.9%, respectively. AG-348 recovered unchanged in urine was negligible (0.0458% of dose). The total amount of mitapivat recovered in the urine of monkeys was negligible (0.267% of dose) as well.

Excretion of mitapivat in milk has not been evaluated.

Pharmacokinetic drug interactions

Please refer to the section on Clinical Pharmacology below.

2.4.3. Toxicology

2.4.3.1. Single dose toxicity

The applicant provided 3 non-GLP compliant studies in rats, dogs and monkeys.

In the rat study, no early mortality was observed nor mitrapivat-related clinical observation, effects on body weight or macroscopic findings at the dose of 100 to 2000 mg/kg by oral gavage.

In dogs, mitapivat was administrated from 10 up to 125 mg/kg; swelling (ear, eyelid, mouth, paw) and/or discoloration (mouth, thoracic region, abdominal region, or whole body) were observed from 30 mg/kg. Emesis was noted from 62.5 mg/kg; lethargy, prostration and reduced activity from 125 mg/kg. The clinical observations appeared at 0.5 to 4 hr postdose and were almost completely resolved by 8 hours postdose.

In monkey, mitapivat was administrated from 100 up to 1000 mg/kg; no early mortality or mitapivat-related effects were observed at ≤250 mg/kg. Only a non-adverse emesis was observed at 500 mg/kg.

2.4.3.2. Repeat dose toxicity

The applicant performed GLP-compliant regulatory studies such as 28-days, 3-months and 6-months pivotal studies in rats and 28-days, 3-months and 9-months pivotal studies in monkeys.

RATS

Regarding the variability of exposure between male and female rats, different dose levels were used to attain similar drug exposure.

In the first pivotal study in rats, mitapivat was administrated at 60, 300 and 600 mg/kg/day in males and at 20, 100 and 200 mg/kg/day in female by oral gavage during 28 days. One male was found dead on day 8 in group at 60 mg/kg/day, the cause of death was dark area on all lobes of the lung correlated with microscopic moderate haemorrhage. This death was considered likely traumatic in origin and was not considered test article related.

The clinical observation, such as clear material around the mouth, was only observed 1-2 hours post dose at highest dose and was fully resolved during the recovery period. The body weight and food consumption were decreased of 7.7% in males at 600 mg/kg and was not reversible during the recovery period, whereas the effects on food consumption was reversible. Mitapivat exposure increased with increasing dose, and dose proportionality varied across doses. The mean mitapivat accumulation ratios ranged from 0.63 to 2.20 in males and from 0.68 to 1.85 in females.

The haematology and coagulation parameters were impacted by mitapivat but were related to the mechanism of action. The absolute reticulocyte counts, minimally lower MCV, and minimally higher mean corpuscular haemoglobin concentration (MCHC) were decreased in rat at 600 mg/kg. In females, at all doses, the haemoglobin distribution width (HDW) was decreased and non-reversible after 14 days recovery. Excepted the lower mean glucose value in male at the highest dose, all serum chemistry changes were reversible during the recovery period. The alterations of serum chemistry parameters were lower serum chloride, sodium, glucose, and higher cholesterol, albumin and albumin/globulin ratio in male from 300 mg/kg/d and lower serum chloride, blood urea nitrogen and higher cholesterol in females at 200 mg/kg/d. The incidence of urinary ketones was higher in the 300 and 600 mg/kg/day males at the Day 28/29 interval. This finding was considered to correlate to decreased food consumption and

body weight in the 600 mg/kg/day males. The urine chemistry changes were an increase of corrected total sodium and chloride in male from 300 and 600 mg/kg/d respectively and an increase of corrected total chlorid and creatinine at 200mg/kg/d, and an increase of corrected total potassium from 100mg/kg/d. All those changes were reversible.

In general in the 28-day study in rats, the incidence of microscopic findings increased with dose and effect were more pronounced in males than in females. An exception was at highest doses the incidence and impact in liver and adrenal gland that were similar in both sexes. Microscopic findings from 60 mg/kg/day included vacuolation in adrenal zona glomerulosa in male and was reversible.

From 300/100 mg/kg/d hepatocellular and centrilobular hypertrophy correlated with higher liver weight were observed in both sexes, a lower thickness in adrenal zona fasciulata correlated with lower adrenal weight and a decrease of zymogene granule in pancreas was observed in males. In females, an incomplete corpora lutea and follicular cyst were observed in ovary correlated with higher ovary weight, as well as a hypertrophy of uterus and changes in oestrous cycle consisting of persistent dioestrous and vaginal mucification. This impact on female reproductive organ was related to the inhibition of CYP19 aromatase by mitapivat. It is known that aromatase inhibitor can change the oestrus cycle.

At 600/200 mg/kg, all effects noted previously were observed, as well as, tubular vacuolation in kidneys correlated with higher kidney weight in both sexes, in males myocardium vacuolation correlated with lower heart weight, and a decreased secretion in prostate correlated with lower prostate and seminal vesicles weights and in females, increased basophilia in pituitary gland. All effects were reversible except decreased prostate weight (-20% absolute) and decreased secretion prostate weight (-13.8% absolute). Only microscopic findings in the ovaries were considered adverse at 100 mg/kg/d. The tubular vacuolation in kidney corresponds to multiple, non-staining, often non-discrete vacuoles within the cytoplasm of the renal tubular epithelium primarily in the outer medulla.

Therefore, the NOAEL was considered to be 600 mg/kg/day for males and 20 mg/kg/day for females. Dosage levels of 600 mg/kg/day for males and 20 mg/kg/day for females resulted on day 27 mitapivat AUC0-12hr values of 118,000 and 10,400 hr.ng/mL (33- and 2.9-fold the human AUC0-12hr value at 50 mg BID), respectively, and mitapivat Cmax values of 27,500 and 4,730 ng/mL, respectively. On day 27 AGI-8702/mitapivat AUC0-12hr ratios at these dosage levels were 0.37 and 0.07, respectively.

The second pivotal study in rats was a 3-month with 28-day recovery period study at doses 40/10, 60/20, 150/50 and 300/100 mg/kg/day in males/females.

There were no test article–related deaths during the study. One control group male was found dead on day 42. The cause of death was not determined from the macroscopic or microscopic examinations. One 50 mg/kg/day group female was euthanised on day 62. The cause of the deteriorating condition necessitating early euthanasia as determined from the microscopic examination was ascending chronic active pyelonephritis. One TK Group 2A male died on day 19 and one TK Group 5A male died on day 63. The cause of death for both TK males as determined from the macroscopic examination was undetermined. No early mortalities were considered mitapivat related because of their isolated incidence and lack of dose response.

No effect was observed at 40/10 mg/kg/day except haematology changes (decrease reticulocytes in female) and a decrease of adrenal weight in male which were reversible during the recovery period. Effects observed were similar to effects on the 28 days study and correlated with organ weights changes (vacuolation in adrenal ZG and decreased thickness in ZF adrenal, centrilobular hypertrophy in liver, incomplete CL in ovaries as wells as a persistent dioestrus during cycle and uterus atrophy and mucification). These effects were reversible at 150/50 mg/kg/d. At 300/100 mg/kg/day the vacuolation in ZG adrenal in F was not reversible. In males treated from 150 mg/kg/day, changes in testis were observed: tubular degeneration, spermatid retention and Leydig cell hypertrophy correlated with a higher testis weight, as well as cell debris in epididymis. Those changes were reversible at 150 but not at 300 mg/kg/d. However, changes in kidney and prostate weight (increase and decrease respectively) were observed at 300 mg/kg/d in males without microscopic findings correlated. In this study, effects on serum chemistry have a low incidence: only decreased chloride in female from 20 mg/kg/day was observed and it was not persistent after the recovery period.

Effects observed on kidney, heart, prostate and pituitary gland in the 28-day study at 600 and 200 mg/kg/day did not occur at lower doses with longer duration. Based on the results of this study, the NOAEL was considered to be 60 mg/kg/day for males and 50 mg/kg/day for females. Dosage levels of 60 mg/kg/day for males and 50 mg/kg/day for females resulted on day 90 mitapivat AUC0-12hr values of 16,800 and 39,400, respectively (4.7 and 11-fold the human AUC0-12hr value at 50 mg BID) and mitapivat Cmax values of 5,550 and 13,600, respectively.

At the Week 13 primary necropsy, test article–related microscopic observations were observed at doses from 50 mg/kg/day on. Test article–related changes in the female reproductive tract were seen at ≥50 mg/kg/day. A single incidence of luteal cysts in the ovary at 50 mg/kg/day and 2 incidences of follicular cyst and of incongruence of ovarian morphology with oestrous stage at 20 mg/kg/day were of uncertain test article relationship.

After the 4-week recovery period, an increased severity of vacuolation of the zona glomerulosa persisted in females at 100 mg/kg/day; single incidences of tubular degeneration/atrophy in the testis and luminal cellular debris in the epididymis were noted in 1 male at 300 mg/kg/day. The lower incidences of these findings compared to those at the primary necropsy suggested partial recovery. Increased large atretic follicles and decreased corpora lutea in 1 female at 50 mg/kg/day (associated with an abnormal oestrous phase of the oestrous cycle) was consistent with spontaneous reproductive senescence, but a relationship to test article administration cannot be ruled out. All other test article–related microscopic findings observed at the Week 13 primary necropsy were no longer present, were present at similar incidences to the control groups, or were present in low incidences consistent with spontaneous findings. All the organ weight changes observed at the Week 13 primary necropsy were no longer present.

Mitapivat was rapidly absorbed after oral dosing, with a tmax of 1.0 to 2.0 hours. Mitapivat exposure increased with increasing dose, and dose proportionality varied across doses. Exposure ratios over the dosing intervals can be found in Module 2.6.5. The mean mitapivat accumulation ratios ranged from 1.81 to 3.82 in males and from 1.56 to 2.60 in females. AGI-8702 was rapidly formed after mitapivat dosing, with tmax of 1.0 hours (except on day 90 in females in the 50 mg/kg/day group, in which tmax was 8.0 hours). AGI-8702 exposure increased with increasing dose, and dose proportionality varied across doses. The mean ratio of AGI-8702 exposure from day 90 to day 0 ranged from 1.32 to 4.01 in males and from 2.84 to 9.46 in females.

The third study in rats was a 6-month with 28 days of recovery study at doses of 40/10, 60/20, 150/50 and 300/200 mg/kg/d in males/females.

There were no test article–related deaths; the mortality observed in TK group was justified and none of the early mortalities were considered test article related.

Administered doses were similar to the 3-month study except in females for which the highest dose has been increased to 200 instead of 100 mg/kg/d.

Test article-related clinical observations were sporadic and limited to clear, red, and/or yellow material around the mouth and/or nose and on the forelimb(s) in all test article-treated male groups and in the 50 and 200 mg/kg/day group females during the dosing period.

Test article-related lower body weight gains were noted in the 150 and 300 mg/kg/day group males and higher body weight gains were noted in the 200 mg/kg/day group females throughout the dosing period, which correlated with higher food consumption beginning from study week 2 to 3.

Test article-related alterations in clinical pathology parameters included minimally lower absolute reticulocyte counts with consequently lower mean haemoglobin distribution width values in females at ≥ 10 mg/kg/day, and a minimally lower chloride value in the 300 mg/kg/day group males at study week 26. Although these alterations were of minimal magnitude and non-adverse, they were considered by the applicant test article-related because they have been noted consistently in other studies with this test article and another test article with the same mode of action (Bultman, 2014, Kappeler, 2015). The lower reticulocyte count was likely related to the mechanism of action of the test article, while the cause of the lower serum chloride value was uncertain.

Effects observed were similar to those observed in the 3-month study at same doses such as haematology changes (decreased reticulocytes in females), serum chemistry changes (decreased chloride in female), vacuolation in ZG adrenal, centrilobular hypertrophy correlated with a higher liver weight from 60 mg/kg/d in male and at 200 mg/kg in female, spermatid retention, tubular degeneration and Leydig cell hypertrophy in testis, incomplete CL in ovaries, uterus atrophy and mucification in vagina. All effects were reversible excepted from 50 mg/kg/d in female, liver changes (hepatocellular and centrilobular hypertrophy) and adrenal vacuolation, at 300 mg/kg/d spermatid retention. At 200 mg/kg/day, an increase of luteinised follicles was observed in ovaries, as well as some changes in organs weight without microscopic findings (kidneys, and thyroid).

All test article-related findings recovered or partially recovered during the recovery period with the exception of mean body weights which remained similar to the end of the dosing period; however, body weight gains during the recovery period were similar to those of the control group animals.

There were sex-related differences in exposure to mitapivat on all days

There were sex-related differences in exposure to mitapivat on all days. Mitapivat exposure increased with increasing dose, and dose proportionality varied across doses. The mean mitapivat accumulation ratios ranged from 0.804 to 4.00 in males and females. AGI-8702 was rapidly formed after oral dosing with mitapivat, with a mean tmax of 1.0 hour for all groups across all days. AGI-8702 exposure increased with increasing dose, and dose proportionality varied across doses. There were sex-related differences in AGI-8702 exposure. The mean ratio of AGI-8702 exposure from day 90 or day 180 to day 0 ranged from 0.869 to 5.08 in males and females.

MONKEYS

In monkeys, mitapivat was well tolerated in the 28-day study at all doses (0, 20, 50 and 150 mg/kg/day). No test article–related clinical observations or effects on body weight or food consumption were noted. There were no test article–related alterations in clinical pathology parameters or ophthalmic, electrocardiography, macroscopic, or microscopic findings. There were no test article-related microscopic observations noted in the examined tissues from animals at the primary or recovery necropsies. All microscopic observations noted were considered spontaneous and incidental, as stated by the applicant; many of them have been well described as background changes in cynomolgus monkeys (Chamanza et al., 2010; Wood 2008; Drevon-Gaillot et al., 2006).

Only a reversible increase of liver weight at 150 mg/kg/day was observed in male and female. The NOEL was 50 mg/kg/day and the NOAEL was 150 mg/kg/day in both sexes. Mitapivat was rapidly absorbed after oral dosing, with mean tmax ranging from 1.00 to 2.40 hours. There were small sex differences observed at the 20 mg/kg/day dose level on day 0 and day 27, and at the 50-mg/kg/day dose level on day 0. Mitapivat exposure increased with increasing dose, and dose proportionality varied across doses. Accumulation ratios for mitapivat ranged from 0.65 to 1.61 in males, and from 0.67 to 1.63 in females.

The mean tmax of AGI-8702 ranged from 1.00 to 2.40 hours. There were no notable sex-related differences in the exposure of AGI-8702 on day 0 or day 27. The mean ratio of AGI-8702 exposure from day 27 to day 0 ranged from 0.77 to 1.34 in males, and from 0.86 to 1.16 in females.

In the 3-month study at 0, 50,100 and 200 mg/kg, mitapivat increased the incidence of emesis at all doses during the dosing period (reversible). Body weight loss was observed in males and females at 100 and 200 mg/kg/day compared to the control group and was reversible.

The hepatocellular hypertrophy was observed in both sexes from 50 mg/kg/day. This effect was correlated with higher liver weight in male from 50 mg/kg/day and from 100 mg/kg/day in female, it was reversible only at 50 mg/kg/day and in female at all doses (persisting in male at 100 and 200 mg/kg/day.) The NOAEL was 200 mg/kg/day. The dosage level of 200 mg/kg/day resulted in Day 90 mean Cmax values of 2,420 ng/mL and 3,180 ng/mL and mean AUC0-12hr values of 10,400 hr•ng/mL and 14,600 hr•ng/mL (2.9- and 4.1-fold the human AUC0-12hr value at 50 mg BID) for males and females, respectively.

There were no consistent sex-related differences in the TK of mitapivat. Mitapivat exposure increased with increasing dose, and dose proportionality varied across doses. Accumulation ratios ranged from 0.414 to 1.24 in males, and from 0.642 to 1.27 in females. AGI-8702 was rapidly formed after mitapivat dosing, with mean tmax ranging from 1.2 to 3.2 hours. There were no consistent sex-related differences in the TK of AGI-8702. AGI-8702 exposure increased with increasing dose, and dose proportionality varied across doses. The mean ratio of AGI-8702 exposure from day 90 to day 0 ranged from 0.456 to 0.804 in males and from 0.746 to 1.03 in females.

During the 9-month study in monkey, mitapivat increased the incidence of emesis at all doses. A reversible white area in liver was observed in females at 200 mg/kg. From 50 mg/kg/day, hepatocellular hypertrophy and minimal pigment in the Kupffer cells; minimal subcapsular hepatocellular inflammation/necrosis), lung (minimal to mild pigmented macrophages), and lymph nodes (minimal to mild pigmented macrophages) in the 50, 100, and/or 200 mg/kg/day group males and/or females, and in the adrenal cortex (increases in incidence and severity of diffuse hypertrophy of the zona fasciculata correlating with higher adrenal gland weights) in the 100 and 200 mg/kg/day female group at the week 39 primary necropsy. The pigment nature was not determined from examination of special stains, although the pigment stained similarly in the same tissue between the control and test animals, suggesting it was present in the control animals but exacerbated by administration of mitapivat.

Mitapivat was rapidly absorbed after oral dosing, with mean tmax ranging from 1.0 to 3.0 hours. There were no consistent sex-related differences in mitapivat exposure. Increases in dose from 50 to 100 mg/kg/day and from 100 to 200 mg/kg/day resulted in approximately dose-proportional increases in mitapivat exposure on all days. Accumulation ratios ranged from 0.593 to 0.998 in males and from 0.696 to 1.39 in females. There were no consistent sex-related differences in AGI-8702 exposure. Increases in dose from 50 to 100 mg/kg/day and from 100 to 200 mg/kg/day resulted in approximately dose-proportional increases in AGI-8702 exposure on all days. The mean ratio of AGI-8702 exposure from day 90, 180, or 270 to Day 0 ranged from 0.704 to 1.04 in males and from 0.738 to 1.06 in females.

Based on the results of this study, the NOAEL was considered to be 50 mg/kg/day for males and 100 mg/kg/day for females. These dosage levels corresponded to mean AUC0-12hr values were 4,370 and 8,040 ng•hr/mL (1.2- and 2.2-fold the human AUC0-12hr value at 50 mg BID) and mean Cmax values were 1,570 and 2,350 ng/mL for males and females, respectively, on Day 270.

The applicant provided a comparison between animal exposure and human exposure. Margins of exposure were determinate on AUC0-12hours in blood on the final TK sampling day in each study and the exposure in blood at the maximum dose recommended in human (50 mg BID). The safety margins are acceptable even if they are low. Indeed, the severity was of relative significance and effects were reversible in most of cases.

2.4.3.3. Genotoxicity

Mitapivat and AGI-8702 were tested in a non–GLP-compliant bacterial reverse mutation assay using S. typhimurium tester strains TA98, TA100, TA1535, and TA97a, and E. coli tester strain WP2 uvrA in the presence and absence of rat liver S9 at the doses of 0.075, 0.25, 0.75, 2.5, 7.5, 25, 75, and 250 µg per well. Under the conditions of this assay, mitapivat and AGI-8702 were nonmutagenic.

Mitapivat was tested in a GLP-compliant bacterial reverse mutation assay using S. typhimurium tester strains TA98, TA100, TA1535, and TA1537 and E. coli tester strain WP2 uvrA in the presence and absence of rat liver S9. Mitapivat dose levels tested were 50, 150, 500, 1,500, and 5,000 µg per plate. No mutagenic responses were observed with any of the tester strains in either the presence or absence of S9 activation. The metabolite of mitapivat, AGI-8702, was identified in a non–GLP-compliant analysis to be present over the concentrations tested in the S9 portion of the treatment at 298 to 3,840 ng/mL, which corresponds to 0.77 to 10 µg per plate, respectively. Under those conditions of the study, mitapivat was negative in the bacterial reverse mutation assay.

Mitapivat was tested in a GLP-compliant *in vitro* mammalian cell micronucleus test using HPBLs in both the absence and presence of a S9 activation system at doses ranged from 35 to 500 µg/mL. Based on

the findings of this study, mitapivat was negative for the induction of micronuclei in both nonactivated and S9-activated test systems in the *in vitro* mammalian cell micronucleus test using HPBLs.

In this GLP-compliant study mitapivat was evaluated for its clastogenic activity and/or disruption of the mitotic apparatus by detecting micronuclei in polychromatic erythrocyte (PCE) cells in rat bone marrow. Test and/or control article formulations were administered at a dose volume of 10 mL/kg by oral gavage twice within a 24-hour period. The administration of mitapivat at doses up to and including 2,000 mg/kg did not induce a significant increase in the incidence of MPCEs, and mitapivat was negative in the micronucleus assay.

2.4.3.4. Carcinogenicity

In a 2-year carcinogenicity study in male and female rats some neoplastic and nonneoplastic findings were observed. The target organs are liver, pancreas, thyroid and ovary but most of those effects were minimal and observed in control group as well. However, neoplastic lesions in the liver and thyroid at 300 mg/kg and preneoplastic lesions in the liver of males at all dose levels and females at 200 mg/kg/day were observed and are most likely related to known effects of mitapivat on CYP enzyme induction and associated with hepatocellular changes (centrilobular hypertrophy). In the pancreas of males, acinar adenoma and hyperplasia were observed at an increased incidence and/or severity at ≥30 mg/kg/day, however pancreatic findings were within the range of historical control data at ≤100 mg/kg/day, and were outside the historical control data range only at 300 mg/kg/day. The ovary atrophy was observed in control group and treatment group thus this effect was not significant. However, there was an increased incidence and severity of granulosa and/or luteal/granulosa cell hyperplasia as well as a slightly increased incidence of corpus luteum atrophy at 200 mg/kg/day.

Twice daily oral administration of AG-348 for up to 95 weeks to Sprague-Dawley rats resulted in neoplastic lesions in the liver and thyroid of males at 300 mg/kg/day and pre-neoplastic lesions in the liver of males at all dose levels and females at 200 mg/kg/day most likely related to known effects of AG-348 on cytochrome p450 enzyme induction and associated hepatocellular changes (centrilobular hypertrophy). Beyond this effect, there was no evidence that AG-348 treatment caused *de novo* tumour types or promoted rare tumour types within the liver.

Additionally in the pancreas of males acinar adenoma and hyperplasia were observed at an increased incidence and/or severity at ≥ 30 mg/kg/day, however the pancreatic findings were within the range of historical control data at ≤ 100 mg/kg/day, and were outside the historical control data range only at 300 mg/kg/day.

The applicant provided three studies of 5 and 28 days in CByB6F1 mice and 26 weeks in CByB6F1/Tg rasH2 Hemizygous mice.

The 5-day study was conducted at doses of 0, 1100, 300, 1000 and 2000 mg/kg. The highest dose led to the death of 3 animals in main cohort (30%). Clinical signs of decreased motor activity, labored breathing, thin, hunched, and/or ruffled fur were noted at 1000 mg/kg/dose BID in males and females at the detailed observation. Mitapivat was associated with lower mean body weight parameters in males and females at 1000 mg/kg/dose BID.

The 28-day study was conducted at mitapivat doses of 0, 300, 750 and 1500 mg/kg/day in 10 animals /sex/group. Mortality was observed at the highest dose at day 2 and 12 (20% M and 30% F) and was related to mitapivat without micro or macroscopic findings. At 300 mg/kg/dose in males and 750 mg/kg/dose in both sexes, liver organ weight increased and centrilobular hypertrophy were observed. Female reproductive tract changes included increased incidence of metestrus noted at 750 mg/kg/dose. Uterine atrophy, decreased incidence of corpora lutea, and increased incidence of metestrus were noted at 300 and 750 mg/kg/dose.

The 26-week study in CByB6F1/Tg rasH2 hemizygous has not shown carcinogenicity at ≤ 500mg/kg/day in male and at ≤ 250mg/kg/day in females. No mortality was observed and no test article–related neoplastic or non-neoplastic changes were observed microscopically in mice administered mitapivat.

Microscoping findings observed in mice receiving mitapivat (spleen, lung or papilloma/squamous cell) were not significant because they were also observed in control group. The various other neoplastic lesions observed in other tissues/organs were generally typical of those commonly encountered in rats of this strain and age range and were not considered AG-348-related. The tumour incidences were within the range of spontaneous occurrence reported in aged SD rats and/or lacked a dose relationship.

2.4.3.5. Reproductive and developmental toxicity

A full programme of reproductive and developmental toxicity studies was performed in rats and rabbits. In rats, treatment-related effects male reproductive organs organs were observed at doses ≥150 mg/kg/day. They consisted in bilateral small testes, degeneration of seminiferous tubules, spermatid retention and residual bodies as well as an increased incidence of cellular debris in the epididymides. These pathological findings correlated with decreased sperm motility and density and increased numbers of abnormal sperm (detached or no head)). These changes were reversible following a recovery period corresponding to one spermatogenic cycle, and were not associated with adverse outcomes on mating and fertility when male animals were mated with untreated females. Since fertility assessment in test animals has limited sensitivity as a measure of reproductive injury, the paternal NOAEL for effects on fertility is considered at 60 mg/kg/day based on the adverse effects on sperm parameters and testes. In female rats, reversible decreases in the number of oestrous stages and in progesterone levels were seen at 200 mg/kg/day; these effects were not associated with adverse outcomes on mating, fertility, histology of reproductive tissues, or early embryonic development.

In the embryo-foetal toxicity conducted in rats, mitapivat was demonstrated to induce embryo-fetolethality, fetotoxicity and teratogenicity at the maternotoxic dose of 200 mg/kg/day inducing exposure levels well-above those reached at the MRHD (x63). Treatment-related external malformations were reported in 5 fetuses from 5 litters, and included notably omphalocele (2 fetuses from 2 litters) with other malformations affecting the whole fetus, face, trunk, ear, head, vertebrae, and eye. Visceral and skeletal malformations were also reported in one fetus each (narrow pulmonary trunk, skull anomaly). The developmental NOAEL was determined at 50 mg/kg/day. In the rabbit embryo-foetal development study, fetotoxicity (decreased fetal weights) was noted in presence of maternotoxicity at the high dose level of 125 mg/kg/day. The developmental NOAEL was determined at 60 mg/kg/day.

In the rat pre- and post-natal development study, increased perinatal mortality was reported at 200 mg/kg/day in relation to drug-induced dystocia/prolonged parturition and associated maternal mortality at ⩾50 mg/kg/day. No treatment-related effect on the postnatal development of F1 offspring, notably on their sexual maturation, learning and memory, and reproductive capacity was reported at doses up to 50 mg/kg/day. This was the highest dose level evaluated for such endpoints due to excessive mortality of F1 animals at 200 mg/kg/day during the preweaning phase. Based on these results, the no-observed-adverse effect level (NOAEL) for general toxicity in the F0 generation females was 20 mg/kg/day and reproductive toxicity in the F1 generation males and females was considered to be 50 mg/kg/day.

In the definitive juvenile rat toxicity study designed to support trial in children above 1 year of age, 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. Reduced mating, fertility and pregnancy indices were also observed at ≥150 mg/kg/day when males were mated to untreated females, with additional decreased implantations and increased postimplantation losses 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. In treated females mated to untreated males, 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, oestrous cycle length was prolonged. These findings were shown to be reversible. Regarding growth, it is noted that the body weight of males at the high dose level of 300 mg/kg/day was decreased vs. controls, and this effect was not clearly reversed after a 13-week recovery period. There were also changes in bone

densitometry parameters at all dose levels in femur metaphysis and diaphysis, with significant differences vs. controls still noted in diaphysis at the end of recovery at ≥150 mg/kg/day. The applicant explains that they 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%). 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. There was no adverse treatment-related effect on memory, learning, ambulation, fine movement, and habituation at any dose level, i.e. up to 300 mg/kg/day in males and 200 mg/kg/day in females. On PND 97, the AUC(0-12) was 5430, 79000, and 223000 hr.ng/mL in males at 30, 150 and 300 mg/kg/day, respectively, and 6480, 72000, 215000 hr.ng/mL in females at 10, 50, and 200 mg/kg/day, respectively.

2.4.4. Ecotoxicity/environmental risk assessment

According to current guideline on the environmental risk assessment of medicinal products for human use (2006- EMEA/CHMP/SWP/4447/00 corr 21*), the applicant performed a phase I for the ERA. The partition coefficient of mitapivat was determined at 3 pH levels and all values were below 4.5 (ie, logDow=0.3 [pH 5], 1.8 [pH 7], and 1.6 [pH 9]). Therefore, mitapivat is not considered to be a PBT or a vPvB substance. The Phase I PECSURFACEWATER of mitapivat (0.0026 µg/L) does not exceed the action limit of 0.01 µg/L.

As mitapivat has known off-target aromatase inhibitory properties, it was evaluated whether this activity could cause potential adverse ecological effects at the PECSURFACEWATER. This was performed in 2 ways: the lowest NOAEL observed in reproductive studies in any species was compared with the expected tissue concentration in fish, and the potential for a pharmacological response in fish was estimated using the fish plasma model.

The environmental assessment based on nonclinical data indicates that the predicted tissue concentration in fish, at the PECSURFACWATER, is 6 orders of magnitude lower than the mammalian NOAEL. The ER derived by the fish plasma model is far above 1, indicating a large safety margin and a low risk potential to fish.

Despite of the fact that mitapivat has off-target aromatase inhibitor activity, no environmental effects are expected at the PECSURFACEWATER and mitapivat is not expected to affect the reproduction of vertebrate or lower animals at concentrations lower than 0.01 µg/L. Therefore, no Phase II environmental assessment is needed.

However, although the fish plasma model may be acceptable from a scientific point of view, this test is not validated (OECD) and the applicant has committed to perform the regulatory tests according to the requirements of the ERA/OECD guidelines (234), and submit the results of the study as a post approval measure.

2.4.5. Discussion on non-clinical aspects

Pharmacology

Pyruvate kinase deficiency is an autosomal recessive disease with hundreds of mutant alleles described. The main mutation found in Caucasian patients with PK deficiency are R510Q (40%) and R486W (30%) approximately.

In an *in vitro* study, in human RBCs (patients with distinct genotypes of PK deficiency), mitapivat increased ATP levels and decreased 2.3-DPG and PhosphoEnolPyruvate (PEP) after 3 to 24 hours of exposure to mitapivat in patients with PKR mutations (Patient A: R510Q/G511R; Patient B: R486W/D390N; Patient C: A495V/E241stop; Patient D: R510Q/E241stop). Mitapivat treatment of pyruvate kinase-deficient RBCs resulted in a dose-dependent increase in PKR activity.

Mitapivat was evaluated in anaemia mice (Hbbth3/+) versus WT mice during 21 days at 50 mg/kg BID oral gavage or 100 mg/kg diet and 56-day study diet: 100 mg/kg. In anaemic mice at 50 mg/kg, an increase of haemoglobin and mean corpuscular volume was observed as well as a decrease of absolute reticulocyte count. An increase of ATP levels was observed in peripheral blood after 21 days in Hbbth3/+ mice (50 mg/kg BID). Moreover, a decrease of apoptotic erythroblasts from spleen and bone marrow and a decrease of levels of reactive oxygen species in erythroblasts in bone marrow ($P<0.05$ vs vehicle control) were observed in anaemia mice.

The pertinence of anaemia mice model is questionable, it would have been interesting to assess mitapivat (and its metabolite) *in* an *in vivo* model of deficiency PK mice if available. However, assessment of NFS and haematology parameters sufficiently proves efficacy of mitapivat on anaemia.

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 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%, excepted metabolites M396 or AGI-8702 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.

The applicant provided 3 non-GLP compliant single dose studies in rats, dogs and monkeys. The dogs were a hypersensitive species, thus the applicant has preferred the use of rat and monkeys only for the repeat dose toxicity studies.

The applicant performed GLP-compliant regulatory studies such as 28-days, 3-months and 6-months pivotal studies in rats and 28-days, 3-months and 9-months pivotal studies in monkeys. Regarding the variability of exposure between male and female rats, different dose levels were used to attain similar drug exposure. As there is no difference between men and women in the clinical data in humans, it suggests that this is an effect related to the species without clinical relevance.

In the first pivotal study in rats, the death was considered likely traumatic in origin and was not considered test article related. The clinical observation, such as clear material around the mouth, was only observed 1-2 hours post dose at highest dose and was fully resolved during the recovery period. The haematology and coagulation parameters were impacted by mitapivat but were related to the mechanism of action. All changes observed on serum chemistry were reversible.

In studies in rats, the incidence of microscopic findings generally increased with dose and effect and was more pronounced in males than in females. The main target organs are adrenal gland, liver, reproductive organ male and female, kidney and thyroid. The impact on male and female reproductive organs was related to the inhibition of CYP19 aromatase by mitapivat. Findings on reproductive organs seen in repeat dose toxicity studies have been reflected in the SmPC Section 5.3.

In monkeys, mitapivat was well tolerated in the 28-day study at all doses (0, 20, 50 and 150 mg/kg/day). No test article–related clinical observations or effects on body weight or food consumption were noted. There were no test article–related alterations in clinical pathology parameters or ophthalmic, electrocardiography, macroscopic, or microscopic findings. Only a reversible increase of liver weight at 150 mg/kg/day was observed in male and female. In the 3-month study at 0, 50,100 and 200 mg/kg, mitapivat increased the incidence of emesis at all doses during the dosing period (reversible). Body

weight loss was observed in males and females at 100 and 200 mg/kg/day compared to the control group and was reversible.

The hepatocellular hypertrophy was observed in the 3-month study in both sexes from 50 mg/kg/day. This effect was correlated with higher liver weight in male from 50 mg/kg/day and from 100 mg/kg/day in female, it was reversible only at 50 mg/kg/day and in female at all doses (persisting in male at 100 and 200 mg/kg/day.)

During the 9-month study in monkey, mitapivat increased the incidence of emesis at all doses. A reversible white area in liver was observed in females at 200 mg/kg. From 50 mg/kg/day, hepatocellular hypertrophy and minimal pigment in the Kupffer cells; minimal subcapsular hepatocellular inflammation/necrosis), lung (minimal to mild pigmented macrophages), and lymph nodes (minimal to mild pigmented macrophages) and in the adrenal cortex (increases in incidence and severity of diffuse hypertrophy of the zona fasciculata correlating with higher adrenal gland weights).

The applicant provided a comparison between animal exposure and human exposure. Margins of exposure were determinate on AUC0-12hours in blood on the final TK sampling day in each study and the exposure in blood at the maximum dose recommended in human (50 mg BID). The safety margins are acceptable even if they are low. Indeed, the severity was of relative significance and effects were reversible in most of cases.

Mitapivat was not mutagenic in an *in vitro* bacterial reverse mutation (Ames) assay. Mitapivat was not clastogenic in an *in vitro* human lymphocyte micronucleus assay, or in an *in vivo* rat bone marrow micronucleus assay.

Mitapivat was not carcinogenic in transgenic rasH2 mice in male mice at 500mg/kg/day (6.4-fold difference in human exposure) and 250 mg/kg/day (2.6-fold difference in human exposure) in female mice twice daily for a minimum of 26 weeks. In a 2-year carcinogenicity study in male and female rats some neoplastic and non-neoplastic findings were observed. The target organs are liver, pancreas, thyroid and ovary but most of those effects were minimal and observed in control group as well. However, neoplastic lesions in the liver and thyroid at 300 mg/kg and preneoplastic lesions in the liver of males at all dose levels and females at 200 mg/kg/day were observed and are most likely related to known effects of mitapivat on CYP enzyme induction and associated with hepatocellular changes (centrilobular hypertrophy). In the pancreas of males, acinar adenoma and hyperplasia were observed at an increased incidence and/or severity at ≥30 mg/kg/day, however pancreatic findings were within the range of historical control data at ≤100 mg/kg/day, and were outside the historical control data range only at 300 mg/kg/day. The ovary atrophy was observed in control group and treatment group thus this effect was not significant. However, there was an increased incidence and severity of granulosa and/or luteal/granulosa cell hyperplasia as well as a slightly increased incidence of corpus luteum atrophy at 200 mg/kg/day.

Reproductive and developmental toxicity

In reproductive and developmental toxicity studies, reversible effects on sperm quality and male reproductive tissues were reported in rats, hence the safety margin for male reproductive capacity is considered to amount to 6 based on a NOAEL of 60 mg/kg/day for the abovementioned parameters. No effects were reported on female fertility at exposure levels nearly 50-fold higher than those reached in humans. As regards embryo-fetal development, a safety margin of 13 can be derived based on the rat study considering the adverse effects (malformations and embryo-fetal lethality) reported at 63-fold the human exposure. Effects reported in rabbits were less severe, however lower exposure levels could be attained in that species with foetotoxicity (decreased fetal weight) noted at 3-fold the human exposure levels, and a developmental NOAEL determined at exposure levels similar to those reached in humans. In the rat pre- and post-natal development study, increased perinatal mortality was reported at 200 mg/kg/day in relation to drug-induced dystocia/prolonged parturition and associated maternal mortality; no treatment-related effect on the postnatal development of F1 offspring, notably on their sexual maturation, learning and memory, and reproductive capacity was reported at doses up to 50 mg/kg/day which represents approximately 13-fold the human exposure based on TK data obtained in the embryofoetal development study. It is noted that rat seems to be more sensitive than humans to

inhibition of aromatase based on 4-fold lower IC50 values determined *in vitro* for the rat aromatase. However, a cautious approach is suggested in the clinical setting since some adverse effects reported in patients would be in line with aromatase inhibition (see SmPC section 4.8: changes in oestradiol, oestrone, and testosterone levels; as well as hot flush).

A programme of juvenile toxicity studies was conducted in rats in line with the PIP adopted for development of mitapivat for the treatment of pyruvate kinase deficiency in children aged 1 year and above. In general, the toxicological profile of mitapivat (target organs) in this study was in line with that defined in the adult rat studies with aromatase-related adverse effects noted on male and female animals. 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. Changes in bone densitometry values were also reported, and it is not certain that they should be viewed as non-adverse and non-relevant to humans considering also the adverse bone effects reported in the clinical setting with other aromatase inhibitors (bone loss) and the data suggesting aromatase inhibition in patients treated with mitapivat (see SmPC section 4.8), respectively. The main findings of the juvenile rat toxicity study were reported in SmPC section 5.3.

Environmental risk assessment

Mitapivat PEC $_{surfacewater}$ value is below the action limit of 0.01 μg/L. and is not a PBT substance as log K_{ow} does not exceed 4.5. Therefore, mitapivat is not expected to pose a risk to the environment.

As mitapivat has off-target aromatase inhibitor activity an evaluation was performed to determine the potential impact on fish. It is to be noted that the applicant provided a fish plasma model that may be acceptable from a scientific point of view. However, this test is not validated (OECD).

Therefore, the applicant has committed to performing the Organisation for Economic Co-operation and Development (OECD) 234 Fish Sexual Development Test and providing the results as a post approval measure, which is acceptable.

2.4.6. Conclusion on the non-clinical aspects

The CHMP considers that Pyrukynd can be granted a marketing authorisation from a non-clinical point of view.

2.5. Clinical aspects

2.5.1. Introduction

Mitapivat (AG-348), a new chemical entity, 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 targets the underlying enzymatic defect that causes haemolysis in pyruvate kinase deficiency by restoring the activity of mutant forms of PKR.

In the current submission, the applicant seeks marketing approval for mitapivat, for the treatment of adult patients with pyruvate kinase deficiency.

The proposed recommended starting dose is 5 mg taken orally twice daily, which should be increased sequentially every 4 weeks to 20 mg twice daily and then 50 mg twice daily. Such titration phase (from 5 to 50 mg BID) is haemoglobin (Hb) level dependent. Hb level should be assessed before increasing to the next dose level as some patients may reach and maintain normal Hb levels at 5 mg twice daily or 20 mg twice daily. The maximum recommended dose is 50 mg twice daily.

The drug product for registration is a film coated tablet containing mitapivat sulfate hydrate proposed at three strengths 5 mg, 20 mg and 50 mg.

GCP aspects

The Clinical trials were performed in accordance with GCP as claimed by the applicant.

The applicant 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**

Table 1 Listing of Clinical Studies Contributing to the Characterisation of Clinical Pharmacology of Mitapivat in This Application

Study Number	Key Clinical Pharmacology Objectives
Mitapivat Clinical Studies in Healthy Adults	
AG348-C-001	To characterize the PK of mitapivat and AGI-8702 after single oral doses of mitapivat. To characterize the PD of 2,3-DPG and ATP in blood after single oral doses of mitapivat. To characterize the PK/PD relationship of mitapivat in plasma and 2,3-DPG and ATP in blood. To evaluate the effect of food on mitapivat (presented in Module 2.7.1).
AG348-C-002	To characterize the PK of mitapivat and AGI-8702 after multiple oral doses of mitapivat. To characterize the PD of 2,3-DPG and ATP in blood after multiple oral doses of mitapivat. To characterize the PK/PD relationship of mitapivat in plasma and 2,3-DPG and ATP in blood.
AG348-C-004	To characterize the PK of mitapivat and AGI-8702 after single oral doses of mitapivat in healthy Japanese and non-Asian subjects. To evaluate the potential effect of oral mitapivat on QTc in healthy Japanese and non-Asian subjects.
AG348-C-005	To assess the relative bioavailability of the tablet and capsule formulations of mitapivat (presented in Module 2.7.1).

Study Number	Key Clinical Pharmacology Objectives
AG348-C-009	To characterize mitapivat mass balance and routes of elimination, the PK of mitapivat and total radioactivity, and metabolite profiling and identification. To determine absolute bioavailability of oral mitapivat.
AG348-C-012	To determine the effect of multiple dose itraconazole (a strong CYP3A4 and P-gp inhibitor) and rifampin (a strong CYP3A4 and P-gp inducer) on the single-dose PK of mitapivat after a single oral dose of mitapivat.
AG348-C-014	To evaluate the effect of food on mitapivat (presented in Module 2.7.1). To evaluate the effect of oral mitapivat on QTc in healthy subjects.
Mitapivat Clinical Studies in Adults With Pyruvate Kinase Deficiency	
Pivotal Studies	
AG348-C-006	To evaluate the PK of mitapivat in not regularly transfused subjects with pyruvate kinase deficiency. To evaluate the relationship between mitapivat PK and safety parameters. To evaluate the relationship between mitapivat PK and indicators of clinical activity.
AG348-C-007	To evaluate the PK of mitapivat in regularly transfused subjects with pyruvate kinase deficiency. To evaluate the relationship between mitapivat PK and indicators of clinical activity To evaluate the relationship between mitapivat PK and safety parameters.
Supporting Studies	
AG348-C-011	To evaluate the PK of mitapivat in not regularly transfused subjects with pyruvate kinase deficiency (Cohort 1 only)[1]. To evaluate the relationship between mitapivat PK and safety parameters (Cohort 1 only). To evaluate the relationship between mitapivat PK and indicators of clinical activity (Cohort 1 only).
AG348-C-003	To characterize the PK of mitapivat and AGI-8702 after multiple oral doses of mitapivat in subjects with pyruvate kinase deficiency. To characterize the PD of 2,3-DPG and ATP in blood after multiple oral doses of mitapivat.

Abbreviations: 2,3-DPG = 2,3-diphosphoglycerate; ATP = adenosine triphosphate; CYP = cytochrome P450; PD = pharmacodynamics; P-gp = P-glycoprotein; PK = pharmacokinetics; QTc = heart rate–corrected QT interval.
[1] Cohort 1 of the open-label extension Study AG348-C-011 included subjects who previously received placebo in Study AG348-C-006; subjects received active treatment with mitapivat in Study AG348-C-011.

2.5.2. Clinical pharmacology

Introduction

Mitapivat sulfate hydrate is a small molecule, with a molecular weight of 1053.23 Da (450.6 Da as free base).

Additionally to non-clinical pharmacokinetics studies (*in vitro* metabolite profiling, CYP inhibition and induction, P-gp substrate evaluation, and protein binding), the clinical pharmacology investigations of mitaprivat consisted of 7 clinical studies in healthy adult volunteers (HV). These studies were designed to provide further information on mitaprivat including ADME properties, food interaction, drug interactions, effect on QT.

Additional information was collected from studies performed in adult patients with two supporting ongoing studies (Phase 2 study AG348-C-003, and Phase 3 Extension Study AG348-C-011) and two pivotal completed Phase 3 studies (studies AG348-C-006 and AG348-C-007).

PK data from both adult patients and HV were used to develop a Population PK model, from which only PK/PD data from adult patients were used for exposure-response analysis. PBPK models were developed to predict DDI.

Pharmacokinetics

Methods

Bioanalysis

Throughout the clinical development, two bioanalytical methods were developed to quantify, mitapivat and its main metabolite (N-dealkylated metabolite: AGI-8702) in human K2EDTA plasma. Overall, the used methods appears to be adequate and comply with acceptance criteria of the bioanalytical method validation following the CHMP guideline.

Pharmacokinetic analyses

PK data were analysed using non-compartmental analysis (NCA) and population PK modelling.

Standard non-compartmental (model-independent) pharmacokinetic methods were used to calculate PK parameters.

Pharmacokinetics of mitapivat were investigated by population pharmacokinetic modelling using a nonlinear mixed effects modelling approach.

Pharmacokinetic-pharmacodynamic (PK-PD) and exposure-response analyses were performed using model-derived pharmacokinetic metrics, assessing the relationship between mitapivat concentration and haemoglobin measure, and parameters for efficacy and safety, respectively.

Model evaluation and selection were based on commonly used statistical and graphical criteria.

Absorption

Following single dose of mitapivat between 30 mg to 2500 mg in healthy volunteers (Study AG348C-001), absorption was reasonably rapid with Cmax approximately achieved at median Tmax of 0.77-1.01 hours, for doses up to 360 mg. Above the 360 mg dose observed median Tmax ranged from 1.49 to 4.07 hours (700 to 2500 mg). Mean Cmax ranged from 461 to 25477 ng/mL (30 mg to 2500 mg).

Following multiple-dosing of mitaprivat at doses between 15 mg to 700 mg BID in healthy volunteers (Study AG348C-002), absorption was reasonably rapid with Cmax approximately achieved at median Tmax ranging from 0.5 to 1.02 hours after 14 days, Cmax ranged from 255 to 5830 ng/mL.

Absolute bioavailability

The absolute bioavailability of mitapivat has been investigated as part of Study AG348-C-009 and was estimated at 72.7%

Relative bioavailability/ Bioequivalence

Throughout the clinical development, two formulations were used, a capsule (strength 5, 25 and 100 mg), and a film-coated tablet (strength at 5, 20 and 50 mg). The commercial formulation is the same as the tablet formulation used in the pivotal Phase 3 studies (AG348-C-006 and AG348-C-007), differing only in the nonfunctional printing.

A relative bioavailability study (AG348-C-005) was performed in a crossover design between the two formulations at a 50 mg dose. Results of this study indicated that compared to capsule, with the tablet formulation Tmax was shorter with a median difference of -0.5 h, Cmax and AUCs were 19% and 5% higher, other PK parameters were similar (CL/F, Vz/F).

Influence of food

The effect of a standardised high fat meal on mitapivat PK was investigated in healthy subjects using the capsule formulation (Study AG348-C-001) or the tablet formulation (Study AG348-C-014).

In Study AG348-C-001, the effect of a high fat meal on mitapivat PK was investigated in 5 healthy volunteers who were administered a single oral dose of 700 mg mitapivat (capsule formulation) in the fasted and the fed states. PK results indicated that administration of a high fat meal decreased Tmax by 0.56 h decreased Cmax by 7.7% and slightly increased AUC0-∞ by 4.3%.

In Study AG348-C-014, the effect of a high fat meal on mitapivat PK was investigated in 31 healthy volunteers who were administered a single oral dose of 100 mg mitapivat (film-coated tablet) in the fasted and the fed states. PK results indicated that administration of a high fat meal delayed Tmax by 2.25 h decreased Cmax by 42% with similar AUC0-∞.

Distribution

Mitapivat has a 97.7% protein binding, a B/P below 1. Mitapivat is extensively distributed in tissue with Vz estimated at 135 L.

Elimination

Across clinical studies in healthy volunteers, after single oral dose from 30 mg to 2500 mg (Study AG348-C-001), estimated half-life ranged from 17.8 to 79.3 h. After multiple doses from 15 mg BID to 700 mg BID, estimated half-life ranged from 32.2 to 194 h.

In healthy volunteers, CL/F ranged from 10.3 to 14.4 L/h. Mitapivat clearance increased in a time and dose dependent manner after multiple doses. An auto-induction of clearance modelled as an exponential increase to steady-state was considered in the PopPK analysis. In patients, based on the results from this analysis, the estimated CL/F on Day 1 was 10.6 L/h and at steady-state was 11.5, 12.7 and 14.4 L/h respectively at the 5 mg BID, 20 mg BID and 50 mg BID.

The main elimination route was hepatic metabolism via CYP3A4/5 enzymes and excretion of metabolites in both urine and feces.

- Mass balance

The excretion and biotransformation of a ^{14}C-radiolabeled mitapivat dose of 120 mg was investigated in 8 healthy volunteers (Study AG348-C-009).

The total recovery of radioactivity in this mass balance study was approximately 89.1 % with individual recovery ranging from 85.5 to 91.2%. Approximately 39.6 % and 49.6 % of the radioactive dose was recovered in feces and urine respectively, unchanged mitapivat was found at trace levels in feces (less than <1%) and approximately 2.62% in urine.

The arithmetic mean CL_R of mitapivat was 0.335 L/h (3.4% of total clearance of 9.87 L/h).

- Metabolism

Metabolite profiling was performed and up to 17 metabolites were identified in the three matrix (all representing < 10% of the total administered radioactivity). Mitapivat accounted for 57.2 % of the total radioactivity in plasma and no major metabolites were identified. AGI-8702 (a weak active metabolite) accounted for only 5.47 % of the total radioactivity in plasma, but remain the main compound excreted in both feces and urine, with 18.5 % and 24.9 % of the recovered radioactivity. Metabolite M642a accounted for 8.48% of the total radioactivity in human plasma. Approximately, 88% and 91.6% of the recovered radioactivity in urine and feces respectively was identified.

Based on *in vitro* investigations mitapivat was found to be predominantly metabolised by CYP3A4 and CYP3A5, AGI-7802 formation is mediated by both.

- Interconversion

Mitapivat is achiral.

- Pharmacokinetic of metabolites

No major plasma metabolites (≥ 10% of total compound-related material) were detected based on Study AG348-C-009, nevertheless a thorough assessment of AGI-8702 PK was performed throughout the clinical pharmacology programme as AGI-8702 was thought as an active metabolite. Non-clinical data suggests that AGI-8702 is a weakly active metabolite.

Dose proportionality and time dependency

Mitapivat is dose proportional over the clinically relevant dose range of 5 to 50 mg BID in healthy subjects and in patients with PK deficiency. Across all the available clinical studies in both healthy and adult patients, mitapivat show no or minimal accumulation. Steady state is generally reached by Day 7-10 after BID administration.

Pharmacokinetics in target population

Population pharmacokinetic analysis was performed using pharmacokinetic data of healthy volunteers from five Phase 1 studies AG348-C-001, AG348-C-002, AG348-C-004, AG348-C-005, and AG348-C-012 and patients with PK deficiency from studies AG348-C-003 (Phase 2), AG348-C-006, AG348-C-007, and AG348-C-011 (Phase 3). Data from Phase 1 studies AG348-C-009 and AG348-C-014, and Phase 2 Study AG348-C-010 were not included in the population pharmacokinetic analysis. The model was developed directly using all data from healthy volunteers and patients included Phase 1 (AG348-C-001, AG348-C-002, AG348-C-004, AG348-C-005, and AG348-C-012), Phase 2 (AG348-C-003), and Phase 3 (AG348-C-006, AG348-C-007, and AG348-C-011).

In total, 4686 (95.4% of total) pharmacokinetic samples from 341 volunteers (186 healthy volunteers and 155 patients with PK deficiency) were used in the final model. The 155 patients with PK deficiency were aged 18 to 78 years (mean = 36.1 years), and 54.8 % of the patients were female and 45.2 % male. Overall, patients with PK deficiency had slightly lower body weight (mean 69 vs 77 kg), lower haemoglobin (mean 8.85 vs 14.2 g/dL), and lower haematocrit (mean 28.4% vs 41.9%) compared to healthy volunteers. Patients with PK deficiency had much higher baseline total bilirubin levels than healthy volunteers (mean 5.58 vs 0.567 mg/dL). The majority of the healthy volunteers were of Black (41%) or White (41%) race, while the majority patients with PK deficiency were of White race (77%). The percentage of male (69% vs 45%) and Hispanic ethnicity (24% vs 2%) participants were higher in the healthy compared to the PK deficiency population. Strong CYP3A4 inhibitors and CYP3A4 inducers were prohibited in the Phase 2/3 studies. Thus, concomitant medications of CYP3A4 inhibitors or inducers were uncommon in patients with PK deficiency.

The final mitapivat population pharmacokinetic model was a 3-compartment model with first-order absorption and a lag-time on absorption. Clearance of mitapivat was modeled as exponentially increasing to steady state, as a function of time (DAY) and dose (DOSE), to account for the auto-induction observed after multiple doses; CL/F of mitapivat increases in a dose- and time-dependent manner. IIV were found on CL (29 %CV, shrinkage=6.6 %), V2 (21.2 %CV, shrinkage=34.9 %), Q3 (71.8 %CV, shrinkage=20.4 %), V3 (46.6 %CV, shrinkage=52.1 %) and Ka (89.8 %CV, shrinkage=21.1 %). Interoccasion variability (IOV) was estimated for Ka. ALAG was fixed to the estimated values ($ALAG_{Tablet}$ = 0.249 h, $ALAG_{Capsule}$ = 0.227 h). A proportional error model was chosen each, for healthy volunteers (δ =0.277, RSE=3.82 %, shrinkage = 9.1 %) and patients (δ =0.425, RSE=4.64 %, shrinkage = 9.1 %). Volumes of distribution (V2, V3, and V4) increased with increasing body weight, females had a slightly lower V2 (central compartment) relative to males, and CL increased with increasing haematocrit level. The impact of these covariates on mitapivat exposure seem limited with no clinical relevance. The PK of mitapivat seems similar between healthy volunteers and patients. Table 2 gives the post-hoc pharmacokinetic parameters of patients with PK deficiency.

Table 2 Steady-state exposure and clearance in patients with pyruvate kinase deficiency

Mitapivat Regimen	Median [5th and 95th Percentiles]				
	CL/F (L/hr)	Ctrough (ng/mL)	Cmax (ng/mL)	Daily AUC (ng·hr/mL)	$AUC_{0\text{-}12,ss}$ (ng·hr/mL)
5 mg BID	11.5 [6.735, 16.73]	10.3 [4.015, 33.66]	104 [75.03, 131.1]	899 [617.8, 1538]	449 [309.4, 770.4]
20 mg BID	12.7 [7.429, 18.53]	33.9 [12.74, 115.9]	404 [288.1, 503.3]	3270 [2247, 5613]	1640 [1127, 2803]
50 mg BID	14.4 [8.387, 20.86]	63.3 [23.15, 233.6]	965 [679.7, 1200]	7150 [4921, 12250]	3580 [2457, 6139]
100 mg BID	16.4 [9.602, 23.93]	90.1 [32.6, 354.5]	1840 [1270, 2289]	12300 [8482, 21140]	6170 [4241, 10620]
300 mg BID	22.5 [13.15, 32.66]	129 [46.88, 549.2]	5020 [3338, 6286]	26800 [18370, 45840]	13400 [9202, 22940]

Source: pk.sim.r.
Abbreviations: AUC = area under the concentration-time curve; $AUC_{0\text{-}12,ss}$ = AUC from time 0 to 12 hours at steady state; BID = twice daily; CL/F = apparent clearance; Cmax = maximum concentration; Ctrough = trough concentration; N = number of subjects; PK = pharmacokinetic.
Note: Median and 5th to 95th percentile of the parameters were simulated from the post-hoc PK parameters of subjects with pyruvate kinase deficiency (N=155).

Special populations

Covariate effects on model-predicted steady-state mitapivat exposure were assessed. Median and 5th and 95th percentiles of the reference exposure were summarised from 1000 simulated PK deficiency patients with typical covariate values (male, body weight of 70 kg, and baseline haematocrit of 28%) treated with mitapivat 50 mg BID. In the simulation of mitapivat exposure in healthy volunteers, a typical body weight of 77 kg and a baseline haematocrit of 42% was used. A statistical summary of the simulated exposures is given in

Table 3.

Table 3 Statistical summary of simulated exposures and doses in patients with PK deficiency

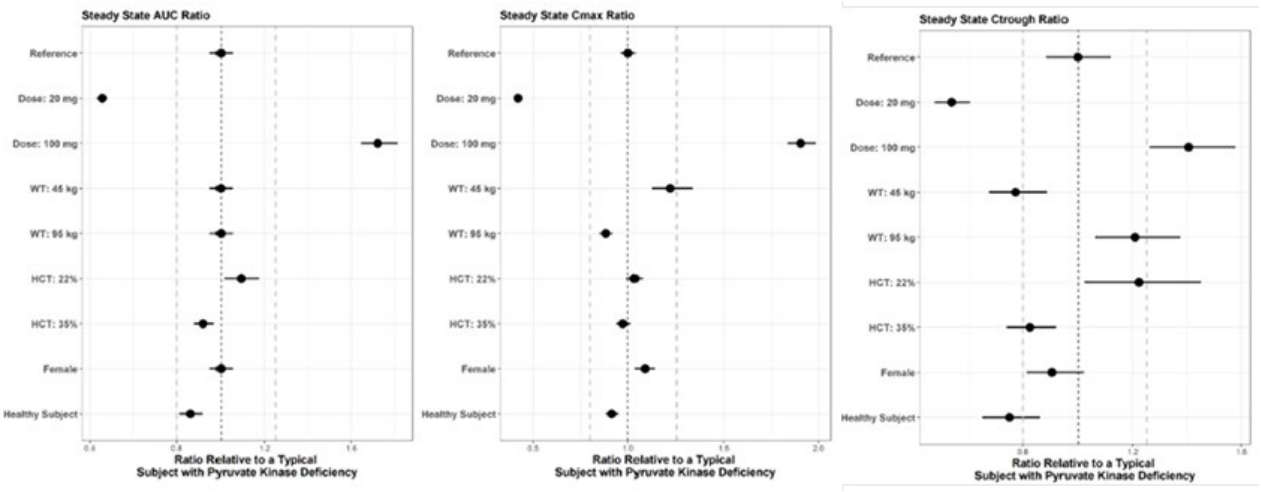
Exposure Metric	Scenario	Median [5th and 95th Percentiles]	Mean (SD)	Fold/Ref[1]
Daily AUC-ss (ng·hr/mL)	Reference	7220 [4530, 11500]	7560 (2190)	1
	Dose: 20 mg	3300 [2070, 5230]	3460 (1000)	0.457
	Dose: 100 mg	12400 [7810, 19700]	13000 (3780)	1.72
	WT: 45 kg	7220 [4530, 11500]	7560 (2190)	1
	WT: 95 kg	7220 [4530, 11400]	7560 (2190)	1
	HCT: 22%	7880 [4950, 12500]	8260 (2400)	1.09
	HCT: 35%	6650 [4180, 10600]	6970 (2020)	0.922
	Female	7220 [4530, 11500]	7560 (2190)	1
	Healthy subject	6230 [3910, 9880]	6520 (1890)	0.863
Cmax-ss (ng/mL)	Reference	883 [550, 1250]	897 (216)	1
	Dose: 20 mg	369 [236, 519]	376 (88)	0.418
	Dose: 100 mg	1680 [1020, 2390]	1710 (424)	1.9
	WT: 45 kg	1070 [630, 1530]	1080 (277)	1.21
	WT: 95 kg	778 [504, 1090]	792 (182)	0.88
	HCT: 22%	910 [576, 1280]	925 (219)	1.03
	HCT: 35%	859 [526, 1220]	873 (214)	0.972
	Female	957 [579, 1360]	971 (240)	1.08
	Healthy subject	807 [495 - 1140]	820 (201)	0.913
Ctrough-ss (ng/mL)	Reference	67.6 [20.9, 191]	82.2 (55.3)	1
	Dose: 20 mg	36.7 [11.5, 97.1]	43.3 (27.7)	0.543
	Dose: 100 mg	96.5 [29.5, 289]	120 (84.7)	1.43
	WT: 45 kg	53 [16.4, 158]	65.6 (46.5)	0.784
	WT: 95 kg	81.6 [25.3, 216]	96.2 (61.8)	1.21
	HCT: 22%	82.8 [25.9, 224]	98.7 (64.2)	1.22
	HCT: 35%	56.2 [17.2, 164]	69.2 (47.9)	0.831
	Female	61.5 [19.3, 178]	75.6 (51.5)	0.91
	Healthy subject	51.1 [15.4 - 151]	63.1 (44.2)	0.755

Source: pk.sim.r.
Abbreviations: AUC-ss = area under the concentration-time curve at steady state; BID = twice daily; Cmax-ss = maximum concentration at steady state; Ctrough-ss = trough concentration at steady state; HCT = hematocrit; PK = pharmacokinetic; SD = standard deviation; WT = body weight.
Note: Reference is defined as a typical male subject with pyruvate kinase deficiency, weighing 70 kg with baseline HCT of 28% and receiving mitapivat 50 mg BID. The 5th to 95th percentile presented for the exposure of 1000 simulated subjects are based on the typical PK parameters, between-subject variability, and the dose or covariate values. Healthy subject was simulated as a male with WT=77 kg and HCT=42%.
[1] Relative to the median value of the reference.

In

Figure 1 the exposures relative to the reference typical patient with PK deficiency are expressed as ratios for various doses and covariate settings. The 90% CIs of the ratios for the impact of body weight and haematocrit on AUC and C_{max} were contained within the range of 0.8 and 1.25 except for the C_{max} ratio at 45 kg body weight. The 90% CIs of the ratios for the impact of body weight and baseline haematocrit on C_{trough} were slightly outside the window of 0.8 and 1.25; the point estimate of the C_{trough} ratio for 45 kg body weight was less than 0.8.

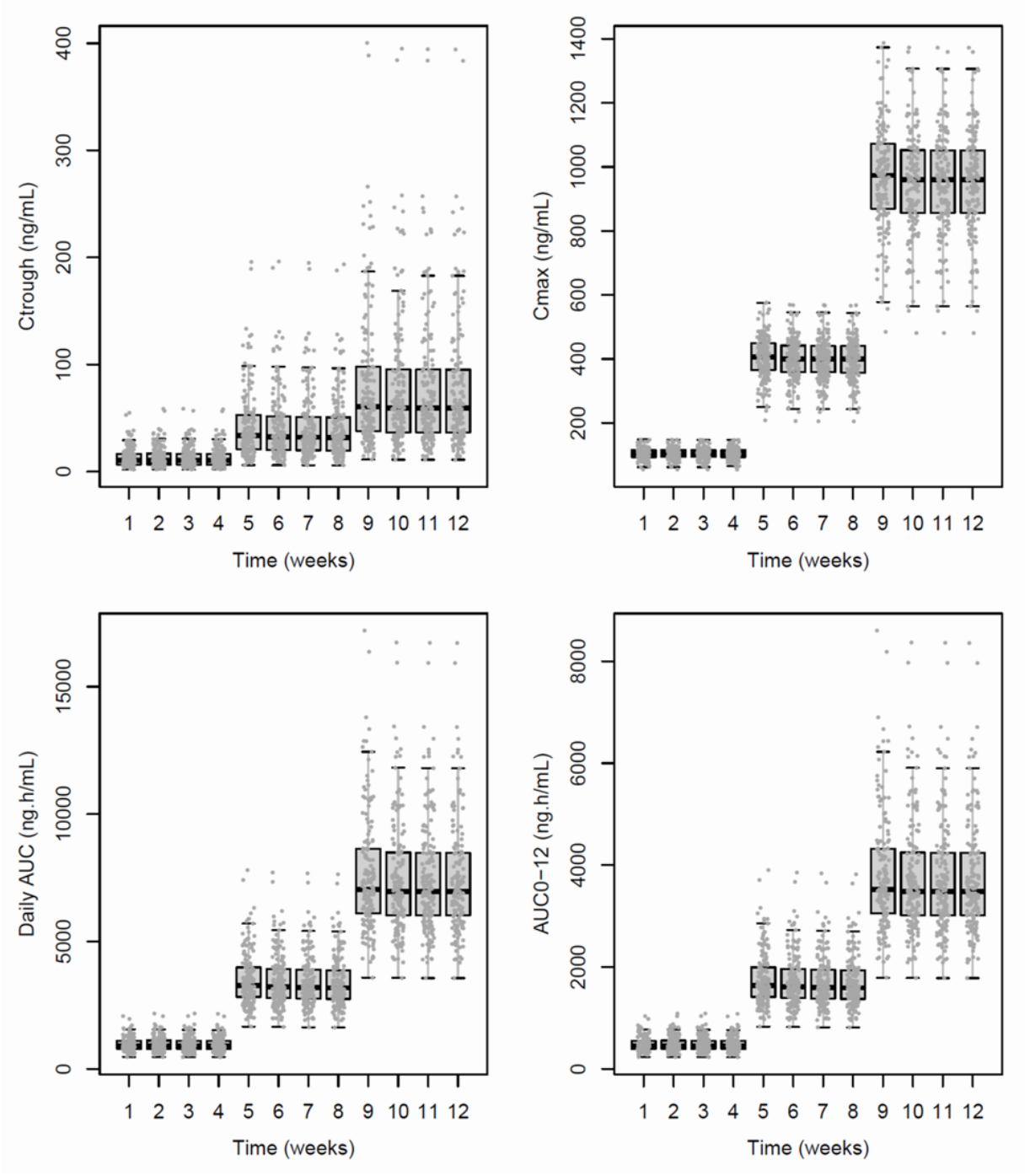
Figure 2 Impact of dose and covariates on steady-state exposure



Expected exposures (AUC, C_{max}, and C_{trough}) for all three doses that are planned to be given through titration (i.e. 5 mg, 20 mg for a 4-week titration scheme, and 50 mg maintenance) were provided (

Figure 2). Overall, after administration of 5 mg BID (weeks 1 - 4), C_{trough} values of approximately 4 to 34.5 ng/mL, C_{max} of 75 to 132 ng/mL, and $AUC_{0-24,ss}$ of 620 to 1555 h*ng/mL are expected. After administration of 20 mg BID (weeks 5 – 8) C_{trough} values of approximately 12 to 116 ng/mL, C_{max} of 284 to 510 ng/mL, and $AUC_{0-24,ss}$ of 2187 to 5605 h*ng/mL are expected. Administration of 50 mg BID (weeks 9 onwards) are expected to result in C_{trough} values of approximately 22 to 228 ng/mL, C_{max} of 671 to 1183 ng/mL, and $AUC_{0-24,ss}$ of 4791 to 12190 h*ng/mL.

Figure 3 Mitapivat exposure in patients with pyruvate kinase deficiency (titrated 5-20-50 mg BID)



Source: pk.sim.bp.r.

Abbreviations: AUC=area under the concentration-time curve; AUC_{0-12}=area under the concentration-time curve from 0 to 12 hours; BID=twice daily; C_{max}=maximum concentration; C_{trough}=concentration at the end of a dosing interval, before the next dose.

Note: Subjects received 5-mg BID doses during Weeks 1 through 4, 20-mg BID doses during Weeks 4 through 8, and 50-mg BID doses during Weeks 9 through 12.

- 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 Population-PK approach.

Model-based steady-state AUC among patients with PK deficiency in different renal categories were compared after administration of 50 mg BID. Most participants had normal renal function (n=127, 81.9%), while 24 (15.5%) had mild renal impairment and 4 (2.6%) had moderate renal impairment. None had severe renal impairment. Steady-state AUC was similar between participants with normal renal function and those with mild renal impairment. Steady-state AUC of participants with moderate renal impairment were numerically higher, but still within the range in subjects with normal renal function.

- Hepatic impairment

No formal PK study investigating hepatic impairment on mitapivat PK was performed, but a study is planned to investigate the hepatic impairment effect on mitapivat pharmacokinetics. Besides the effect of hepatic impairment on the PKs of mitapivat was investigated in patients based on the Population-PK approach.

Model-based steady-state AUC in patients with PK deficiency stratified by liver function parameter categories were compared after administration of 50 mg BID.

Most patients in the dataset had normal AST, ALT, and ALP at baseline. Only limited data are available for patients with AST values from 2 to > 3-times ULN (n=5), ALT values 2 to > 3-times ULN (n=1), and ALP values 1 to 2-times ULN (n=6). No apparent difference in steady-state AUC was observed in volunteers with normal AST, ALT, or ALP levels versus those with elevated levels.

Most patients with PK deficiency had elevated total bilirubin level at baseline. Post-hoc steady-state AUC appeared similar among the PK deficiency patients with total bilirubin of 1 to 2 × ULN, 2 to 3 × ULN, and >3 × ULN.

- 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 Population-PK approach.

Sex was a statistically significantly covariate on the volume of distribution of the central compartment. Females are expected to have lower volume of distribution. Simulations reveal that female patients receiving 50 mg mitapivat BID are expected to have similar exposure compared to male patients, and relative ratios compared to the reference patients were within the 0.8 to 1.25 range. No dose adjustment is warranted.

- Race

A formal dedicated PK study (AG348-C-004) was performed to investigate a potential race /ethnicity effect on PKs of mitapivat between Japanese and non-Asian subjects. Besides, race/ethnicity have been tested as a covariate in the population-PK analysis.

From Study AG348-C-004, in Japanese subjects, at the recommended dose of 50 mg, compared to non-Asian subjects, Cmax was increased by 15% and AUCs remain generally similar.

Race was not a statistically significantly covariate. No dose adjustment is warranted.

- Weight

Weight was a statistically significantly covariate on all volumes of distribution. Simulations for 50 mg BID dosing reveal that $AUC_{steady\text{-}state}$ is comparable for patients weighing 45 or 95 kg compared to the typical patient (70 kg). C_{trough} at steady-state is expected to be about 21 % lower and 21% higher in patients weighing 45 and 95 kg, respectively. Overall, the median exposures were within the 0.8 to 1.25 range. The expected mitapivat exposure in patients with pyruvate kinase deficiency was in addition simulated over a borader body weight range of 40 kg to 140 kg. $C_{max,ss}$ is expected to be about 27 % higher in patients with lower body weight (40 kg) and 24 % lower in patients with higher body weight

(140 kg). C_{trough} is expected to be about 26 % and 22 % lower in patients with lower body weight (40 kg and 45 kg, respectively) and 49 % higher in patients with higher body weight (140 kg).

- Elderly

The 155 patients with PK deficiency included in the population pharmacokinetic analysis were aged 18 to 78 years (mean = 36.1 years). Among the 155 patients included in the population PK analysis, only five patients were older than 65 years (n=4 [2.6] % aged 65 to 74 years and one patient [0.6 %] in the age group 75 – 84 years). Details on the number of participants per range per clinical study are provided in **Table 4**. In the population pharmacokinetic analysis, all 155 subjects with pyruvate kinase deficiency were included. Age was not a statistically significantly covariate.

Table 4 Number of patients per study and age range (population pharmacokinetic analysis set)

Study ID	Number (%) of Subjects				
	<65 years	**65-74 years**	**75-84 years**	**85+ years**	**Total**
AG348-C-003	52 (100%)	0	0	0	52
AG348-C-006	38 (95%)	2 (5%)	0	0	40
AG348-C-007	26 (96.3%)	1 (3.7%)	0	0	27
AG348-C-011	34 (94.4%)	1 (2.8%)	1 (2.8%)	0	36
Total	150 (96.8%)	4 (2.6%)	1 (0.6%)	0	155

- Laboratory parameters

In the analysed Phase 3 studies, haematocrit in patients varied between 19.4 and 39.2 %. In Phase 1 and Phase 2 studies, haematocrit values of up to 52.4 % were measured.

Haematocrit was a statistically significantly covariate on CL/F. Simulations reveal that patients with haematocrit of 22 % are expected to have similar $AUC_{steady\text{-}state}$ and C_{max} values, while median C_{trough} is expected to be about 22 % higher compared to patients with a haematocrit of 28 %. For patients with a haematocrit of 35 %, median C_{trough} is expected to be about 17 % lower compared to patients with a haematocrit of 28 %. Overall, the expected exposures are mostly within the within the 0.8 to 1.25 range.

In addition, model-based expected exposures over a broader range of haematocrit levels of 19 to 52 % were provided. AUC (predictor for safety measures) is expected to decrease by about 20 % with increasing haematocrit (of up to 52 % haematocrit). The decrease in exposure with increasing haematocrit is more pronounced for C_{trough}, which is expected to decrease by 41 % for patients reaching 52 % haematocrit levels, and by 24 % for patients reaching 39 % haematocrit levels.

The baseline haematocrit in patients with PK deficiency was generally lower than in healthy volunteers. Therefore, the CL in patients with PK deficiency was generally lower. Those who responded to mitapivat treatment had haematocrit increasing toward normal range.

Haemoglobin, albumin, total bilirubin Aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, blood urea nitrogen were non statistically significantly covariates. Patients had lower haemoglobin levels compared to healthy volunteers (mean 8.85 vs 14.2 g/dL).

- Children

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

- Pyruvate kinase deficiency mutations

Pyruvate kinase deficiency mutation type was not statistically significantly covariate on the pharmacokinetics of mitapivat and seem not have an impact on mitapivat exposure.

Pharmacokinetic interaction studies

In vitro, mitapivat was identified as CYP3A4 and P-gp substrate (CYP3A4 metabolism responsible for AGI-8702 metabolite formation N-dealkylated metabolite). Mitapivat was demonstrated to be auto-inhibited and auto-induced through CYP3A4 / P-gp pathway.

The multiple-ascending dose study showed increase in mitapivat clearance and reduction in its exposure suggesting the overall net effect of mitapivat as (auto-) inducer of CYP3A4.

In vivo, mass balance study in healthy subjects (Study AG348-C-009) underlined that [^{14}C] mitapivat was extensively metabolised in humans after a single oral dose, as <3% and <1% of the dose was excreted unchanged in urine and feces, respectively. Mitapivat metabolite, on the other hand, AGI-8702 represented 44.4% of the total radioactivity collected in excreta (18.5% in feces and 24.9% in urine) but contributed to less than 25% of the parent-drug AUC. There is, thus, no need to conduct clinical DDI study to investigate its interaction potential.

In addition to *in vivo* clinical studies conducted with itraconzole, and rifampin, mitapivat drug-drug interactions was predicted based on in-silico approaches. The model accounted for mitapivat metabolism as followed:

- CYP3A4 substrate: CYP3A4 metabolised fraction was estimated based on clinical stady with itraconazole;

- CYP3A4 mechanism-based inhibition: inhibition parameter were set to *in vitro* values;

- CYP3A4 induction: induction parameter were set based on best fitting of mitapivat PK at steady-state.

The model is not considered qualified to predict mitapivat interactions with other drugs with mitapivat being as victim drug or perpetrator.

Mitapivat effects on other drugs

In vitro, mitapivat was shown to be metabolism-dependent inhibitor of CYP3A4/5, CYP2C19, inhibitor of P-gp, and inducer of enzymes and transporters which expressions are regulated by CAR/PXR pathways, i.e. CYP3A4, 2B6, 2C8, 2C9 and 2C19, and P-gp. Mitapivat may thus decrease CYP2B6, 2C8, 2C9 and 2C19 drug substrate concentrations.

Clinical net effects of mitapivat on CYP3A4/P-gp substrates are considered unknown (mitapivat being both TDI and inducer), but it is expected mitapivat to decrease CYP3A4/P-gp substrate concentrations including CYP3A-sensitive hormonal contraceptives. This may also affect the latter pharmacodynamics.

In vitro results showed mitapivat was a weak aromatase inhibitor *in vitro* which induced in male subjects increases in testosterone above normal range (2/16, 12.5%, in ACTIVATE study) and decreases in oestrone and oestradiol below normal range (9/16, 56.3%, in ACTIVATE study and 1/7, 14.3 % in ACTIVATE-T study). These effects were reversible when Pyrukynd treatment was discontinued. No data were presented in female subjects, due to, according to the applicant, physiologic variations in hormone levels expected throughout the normal menstrual cycle and the various types of hormonal contraceptives used by patients.

Other drugs effects on mitapivat

Identified *in vitro* signal (*i.e.* mitapivat identified as CYP3A4 and P-gp substrate) were further assessed through both *in vivo* study and in silico simulations. Mitapivat showed pH-dependent solubility, the previously built PBPK model was also used to predict mitapivat interactions with gastric acid reducing agent.

CYP3A inhibitor and inducer effect on mitapivat (*in vivo / in silico*)

In vivo DDI study results showed that following mitapivat 20 mg single dose admistration with strong CYP3A4 (and P-gp) inhibitor and inducer increase and decrease mitapivat plasma exposure by respectively approximately 5-fold and 91% following mitapivat 20 mg administration.

Gastric acid reducing agent effect on mitapivat (*in silico*)

A combined mitapivat PBPK model with a PD model on gastric pH was used to assess the impact of ranitidine co-administration on mitapivat exposure. The model is not considered qualified for this purpose. The outcome from interactions between mitapivat with gastric acid reducing agent is considered unknown.

Pharmacodynamics

Mechanism of action

Mitapivat is an allosteric activator of wild-type PKR (red blood cell [RBC]-specific form of pyruvate kinase) and a range of mutant PKR (mPKR) enzymes.

The presence of mPKR in patients with PK deficiency leads to a disruption in the glycolytic pathway, causing accumulation of PEP and 2,3-diphosphoglycerate (2,3-DPG) and a reduction in ATP. This glycolytic defect and subsequent reduction in ATP leads to shortened reticulocyte and RBC life spans.

Mitapivat acts by allosterically binding to the PKR tetramer and enhancing its affinity for PEP, thereby increasing the ability of RBCs to convert PEP + ADP to pyruvate + ATP.

Primary and Secondary pharmacology

Study AG348-C-001 (Single Ascending Dose)

Study AG348-C-001 was a Phase 1, randomised, double-blind, placebo-controlled study designed to assess the safety, tolerability, PK, and PD of single ascending oral doses of mitapivat administered using a capsule formulation in sequential cohorts of healthy subjects.

Six cohorts of 8 subjects each were randomised to receive a single dose of either mitapivat (n=6) or placebo (n=2). The doses of mitapivat studied were 30, 120, 360, 700, 1,400, and 2,500 mg. All doses were administered after a 10-hour fast, and subjects continued to fast until 4 hours postdose.

2,3-Diphosphoglycerate Pharmacodynamic Results

Table 5 Summary of 2,3-DPG Pharmacodynamic Parameters After Single Oral Doses of Mitapivat and Placebo Under Fasted Conditions (Study AG348-C-001)

Pharmacodynamic Parameters	Placebo (N=12)	Mitapivat Dose					
		30 mg (N=6)	120 mg (N=6)	360 mg (N=6)	700 mg (N=6)	1,400 mg (N=6)	2,500 mg (N=6)
Baseline (µg/mL)	600 (9.3)	567 (11.6)	630 (4.8)	580 (14.0)	600 (5.2)	652 (9.2)	647 (7.8)
AUC_Net_$B_{0\text{-}t}$ (hr·µg/mL)	-199 (1,004.6)	-4,590 (29.3)	-11,577 (15.3)	-13,934 (5.7)	-18,210 (24.7)	-21,729 (37.3)	-21,684 (15.0)
BR_{min} (µg/mL)	-40.1 (23.6)	-105 (22.3)	-216 (14.8)	-276 (5.3)	-292 (14.6)	-281 (18.1)	-297 (7.9)
$\%BR_{min}$ (%)	-6.70 (22.8)	-18.4 (15.5)	-34.3 (15.6)	-48.2 (12.6)	-48.7 (13.6)	-42.9 (12.8)	-46.2 (12.4)
t_{min} (h)	9.03 (0.517, 96.0)	24.1 (12.0, 48.1)	24.0 (24.0, 24.1)	24.1 (24.1, 24.2)	24.1 (24.0, 24.2)	24.2 (24.1, 24.6)	24.0 (24.0, 24.1)

Source: CSR AG348-C-001, Table 15 and Table 14.5.6.1.
Abbreviations: $\%BR_{min}$ = maximum percent decrease from baseline response value; 2,3-DPG = 2,3-diphosphoglycerate; AUC_Net_$B_{0\text{-}t}$ = net area of the response curve above and below the baseline effect value from 0 to the last quantifiable; BR_{min} = maximum decrease from baseline response value; R_{min} = minimum observed response value; RSD = relative standard deviation; t_{min} = time of observed R_{min}.
Note: Values are arithmetic mean (RSD%), except for t_{min}, which is median (minimum, maximum).

Adenosine Triphosphate Pharmacodynamic Results

Table 6 Summary of ATP Pharmacodynamic Parameters after Single Oral Doses of Mitapivat and Placebo under Fasted Conditions (Study AG348-C-001)

Pharmacodynamic Parameters	Placebo (N=12)	Mitapivat Dose					
		30 mg (N=6)	120 mg (N=6)	360 mg (N=6)	700 mg (N=6)	1,400 mg (N=6)	2,500 mg (N=6)
Baseline (µg/mL)	296 (11.4)	262 (10.0)	255 (9.8)	267 (16.8)	261 (10.6)	271 (19.1)	283 (9.5)
AUC_Net_$B_{0\text{-}t}$ (hr·µg/mL)	-374 (356.1)	748 (95.7)	1,441 (36.5)	1,259 (88.2)	2,403 (105.8)	4,279 (48.3)	4,125 (32.0)
BR_{max} (µg/mL)	28.7 (93.6)	31.4 (26.9)	37.6 (29.7)	50.9 (39.4)	59.3 (63.9)	79.1 (38.8)	59.3 (23.8)
$\%BR_{max}$ (%)	9.88 (91.6)	12.2 (31.3)	14.7 (29.6)	19.9 (44.1)	22.8 (62.3)	31.1 (48.0)	20.8 (16.4)
t_{max} (h)	3.51 (0.25, 96.40)	36.05 (0.58, 72.07)	48.04 (24.02, 72.00)	18.15 (3.03, 72.02)	84.03 (48.00, 120.00)	48.30 (2.03, 96.03)	96.03 (0.50, 120.02)

Source: CSR AG348-C-001, Table 16 and Table 14.5.6.2.
Abbreviations: $\%BR_{max}$ = maximum percent increase from baseline response value; ATP = adenosine triphosphate; AUC_Net_$B_{0\text{-}t}$ = net area of the response curve above and below the baseline effect value from 0 to the last quantifiable concentration; BR_{max} = maximum increase from baseline response value; R_{max} = maximum observed response value; RSD = relative standard deviation; t_{max} = time of observed R_{max}.
Note: Values are arithmetic mean (RSD%), except for t_{max}, which is median (minimum, maximum).

Study AG348-C-002 (Multiple Ascending Dose)

Study AG348-C-002 was a Phase 1, randomised, double-blind, placebo-controlled study designed to assess the safety, tolerability, PK, and PD of multiple ascending oral doses of mitapivat administered using a capsule formulation in sequential cohorts of healthy subjects.

Six cohorts of 8 subjects each were randomised to receive multiple doses of either mitapivat (n=6) or placebo (n=2). Mitapivat dose regimens were studied in the following order: 120 mg BID, 360 mg BID, 700 mg BID, 120 mg QD, 60 mg BID, and 15 mg BID for 14 days.

2,3-Diphosphoglycerate Pharmacodynamic Results

Figure 4 Mean (SD) Observed and Change From Baseline Blood Concentrations of 2,3-DPG Versus Time After Single (Day 1) and Multiple (Day 14) Oral Doses of Mitapivat (Study AG348-C-002)

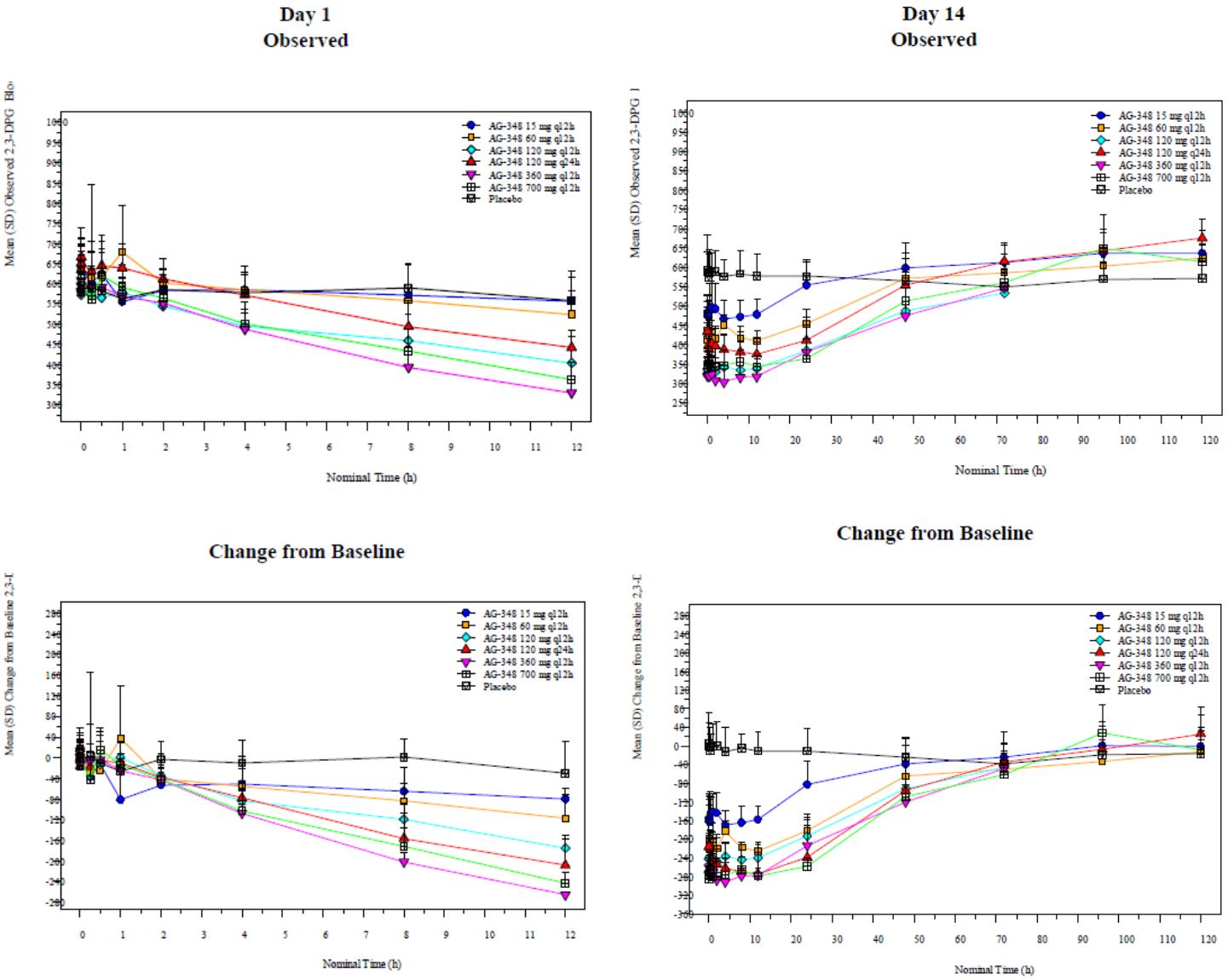
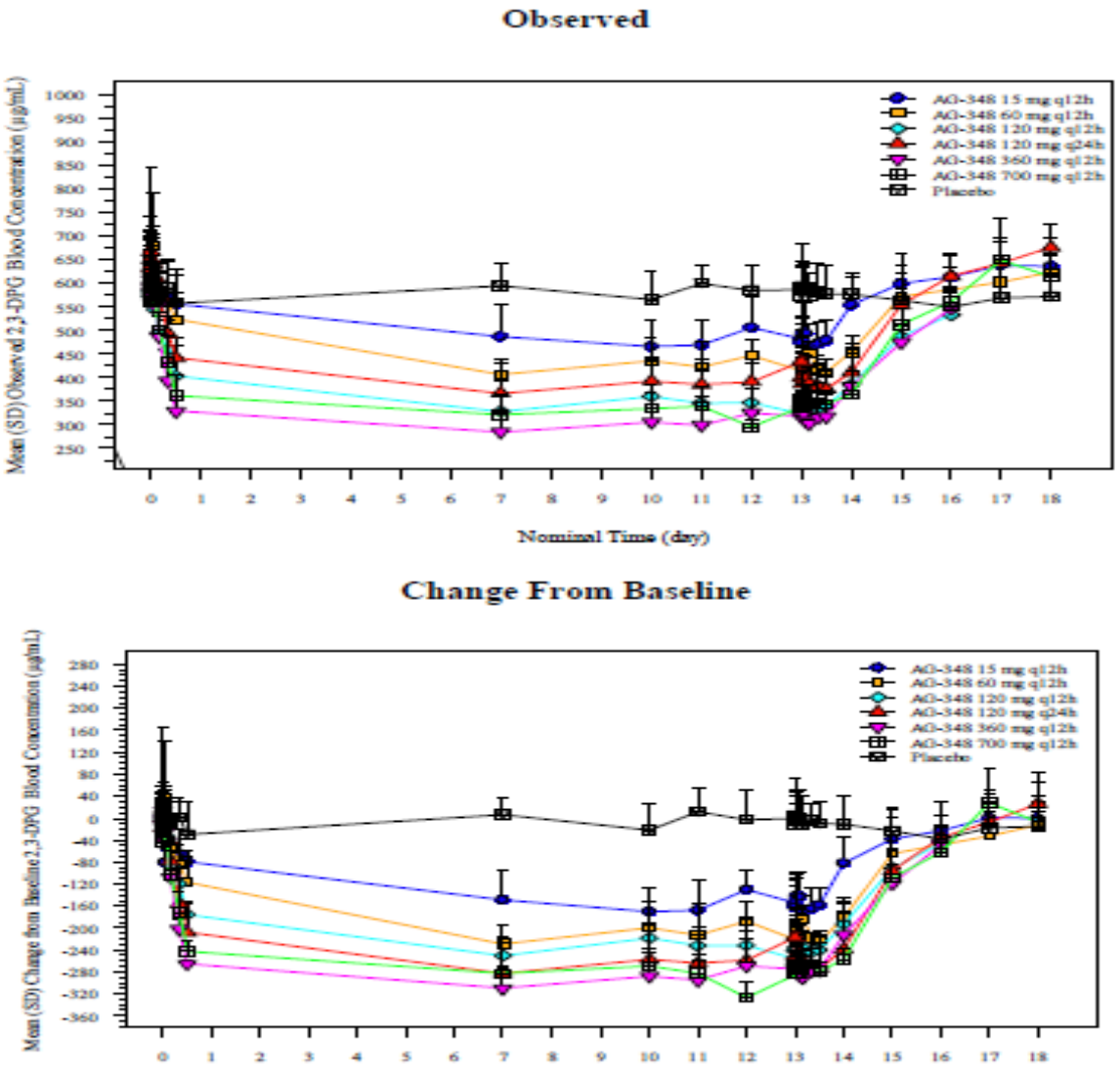


Figure 5 Mean (SD) Observed and Change From Baseline in Trough Blood Concentrations of 2,3-DPG Versus Time After Multiple Oral Doses of Mitapivat (Study AG348-C-002)



Adenosine Triphosphate Pharmacodynamic Results

Figure 6 Mean (SD) Observed and Change From Baseline Blood Concentrations of ATP Versus Time After Single (Day 1) and Multiple (Day 14) Oral Doses of Mitapivat and Placebo (Study AG348-C-002)

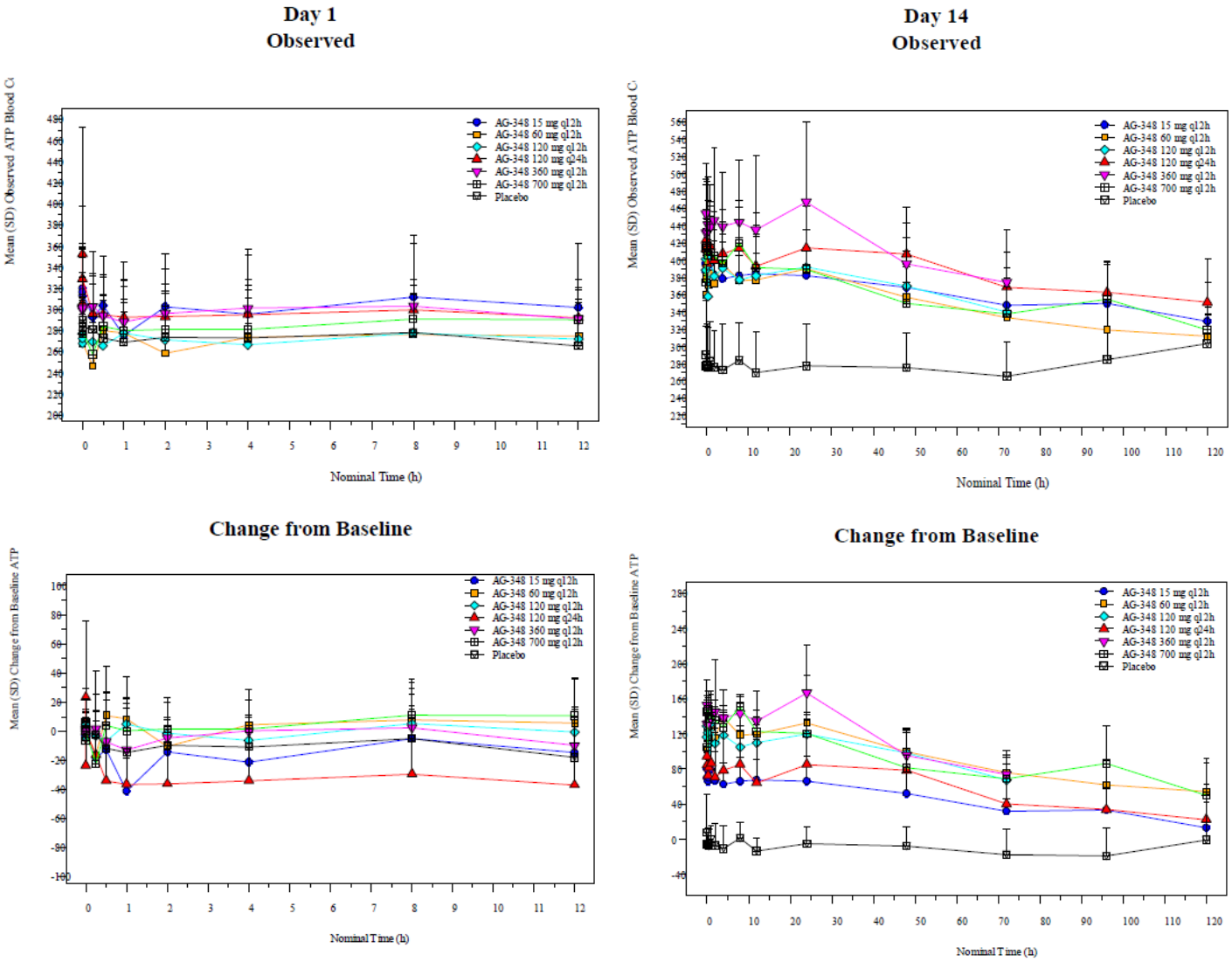
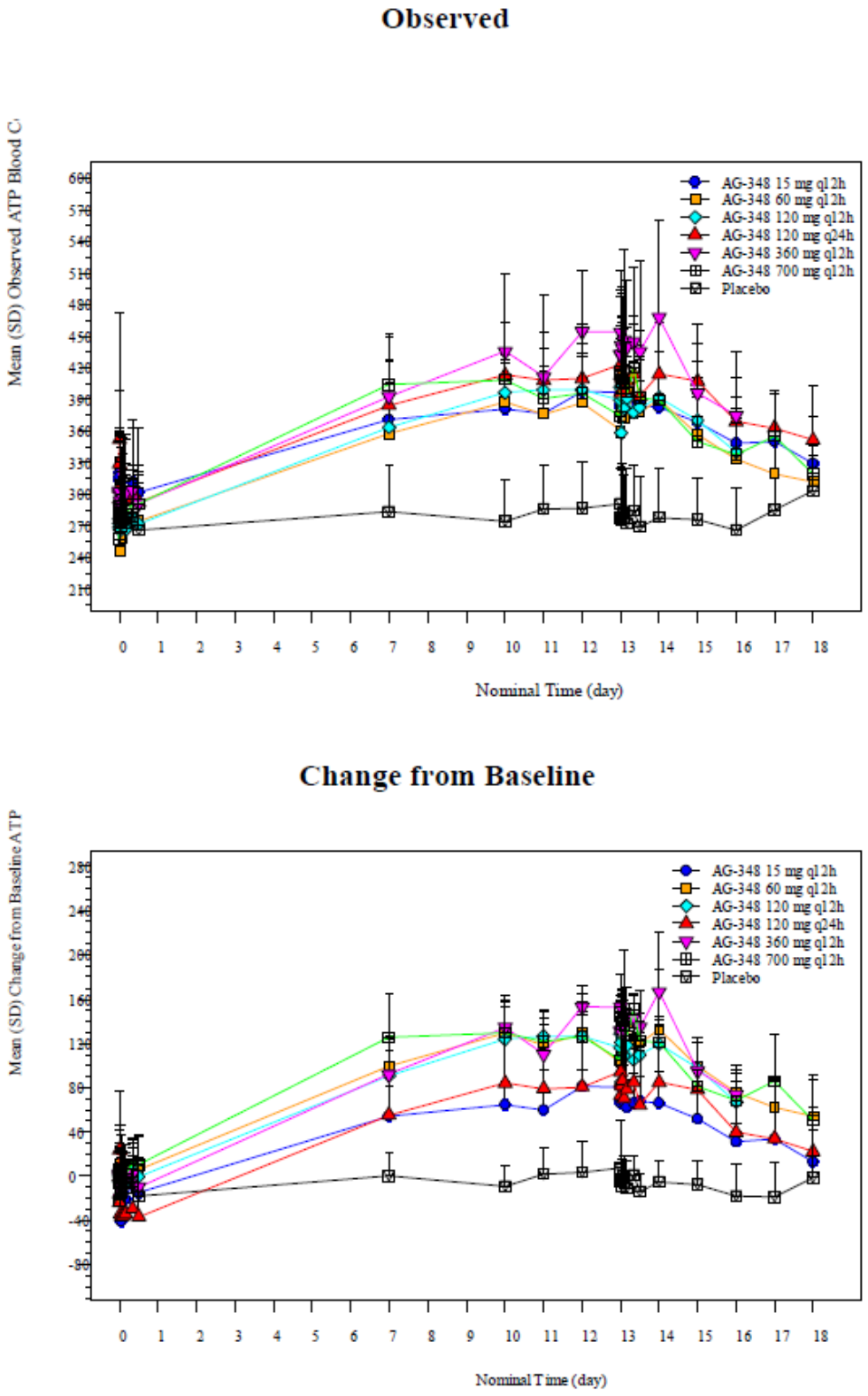


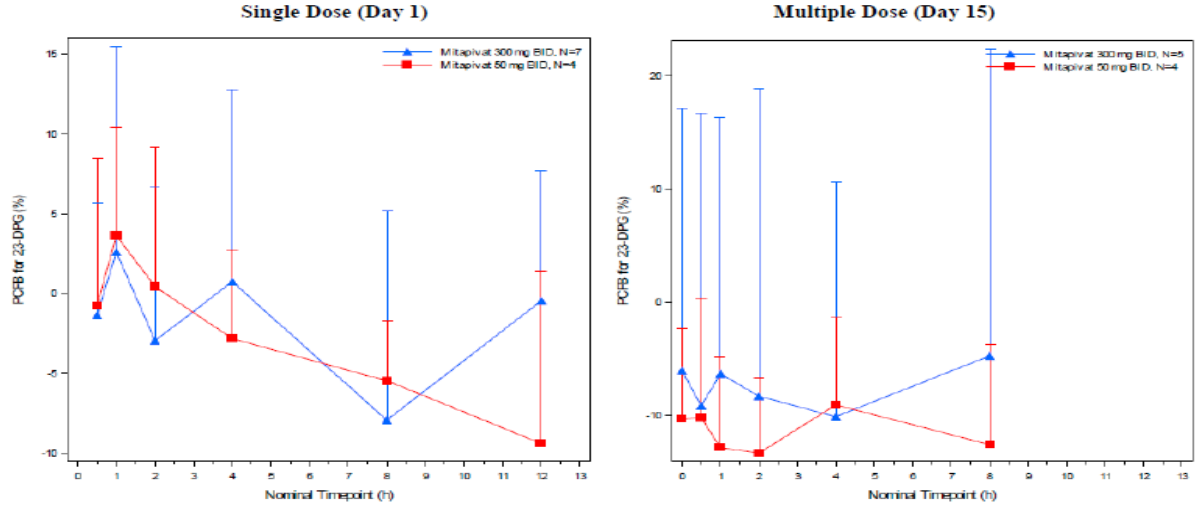
Figure 7 Mean (SD) Observed and Change From Baseline in Trough Blood Concentrations of ATP Versus Time After Multiple Oral Doses of Mitapivat (Study AG348-C-002)



Study AG348-C-003 (Phase 2 Dose Ranging)

2,3-Diphosphoglycerate Pharmacodynamic Results

Figure 8 Mean (SD) Percent Change From Baseline Blood Concentrations of 2,3-DPG Versus Time After Multiple (Day 15) Oral Doses of Mitapivat (Study AG348-C-003; Core Period)



Source: AG348-C-003-NCA, Figure 7.2.2-27 and Figure 7.2.2-28.
Abbreviations: 2,3-DPG = 2,3-diphosphoglycerate; AG-348 = mitapivat; BID = twice daily; PCFB = percent change from baseline.

Table 7 Summary of 2,3-DPG Pharmacodynamic Parameters After Single (Day 1) and Multiple (Day 15) Oral Doses of Mitapivat (Study AG348-C-003, Core Period).

Day	Pharmacodynamic Parameters	Mitapivat Dose and Regimen	
		50 mg BID	300 mg BID
1	n	4	7
	Baseline (µg/mL)	861.5 (8.0)	728.9 (22.0)
	AUC_Net_$B_{0\text{-}12hr}$ (hr·µg/mL)	-374.6 (147.6)	-71.70 (737.2)
	BR_{min} (µg/mL)	-94.00 (85.6)	-70.29 (85.6)
	$\%BR_{min}$ (%)	-10.59 (86.8)	-11.16 (105.8)
	t_{min} (h)	8.96 (0.50, 12.03)	8.00 (0.00, 11.93)
15	n	4	5
	Baseline (µg/mL)	861.5 (8.0)	763.4 (22.6)
	AUC_Net_$B_{0\text{-}8hr}$ (hr·µg /mL)	-788.5 (68.8)	-324.0 (415.9)
	BR_{min} (µg/mL)	-133.3 (58.3)	-92.60 (164.3)
	$\%BR_{min}$ (%)	-15.34 (55.8)	-14.89 (150.6)
	t_{min} (h)	5.00 (1.95, 8.08)	0.50 (0.00, 2.00)

Source: AG348-C-003-NCA, Table 5.2.2.3-1 and Table 5.2.2.3-2.
Abbreviations: $\%BR_{min}$ = maximum percent decrease from baseline response value; 2,3-DPG = 2,3-diphosphoglycerate; AUC_Net_$B_{0\text{-}8hr}$ = net area of the response curve above and below the baseline effect value from 0 to 8 hours; AUC_Net_$B_{0\text{-}12hr}$ = net area of the response curve above and below the baseline effect value from 0 to 12 hours; BID = twice daily; BR_{min} = maximum decrease from baseline response value; R_{min} = minimum observed response value; RSD = relative standard deviation; t_{min} = time of observed R_{min}.
Note: Values are arithmetic mean (RSD%), except for t_{min}, which is median (minimum, maximum).

Adenosine Triphosphate Pharmacodynamic Results

Figure 9 Mean (SD) Percent Change From Baseline Blood Concentrations of ATP Versus Time After Multiple (Day 15) Oral Doses of Mitapivat (Study AG348-C-003; Core Period)

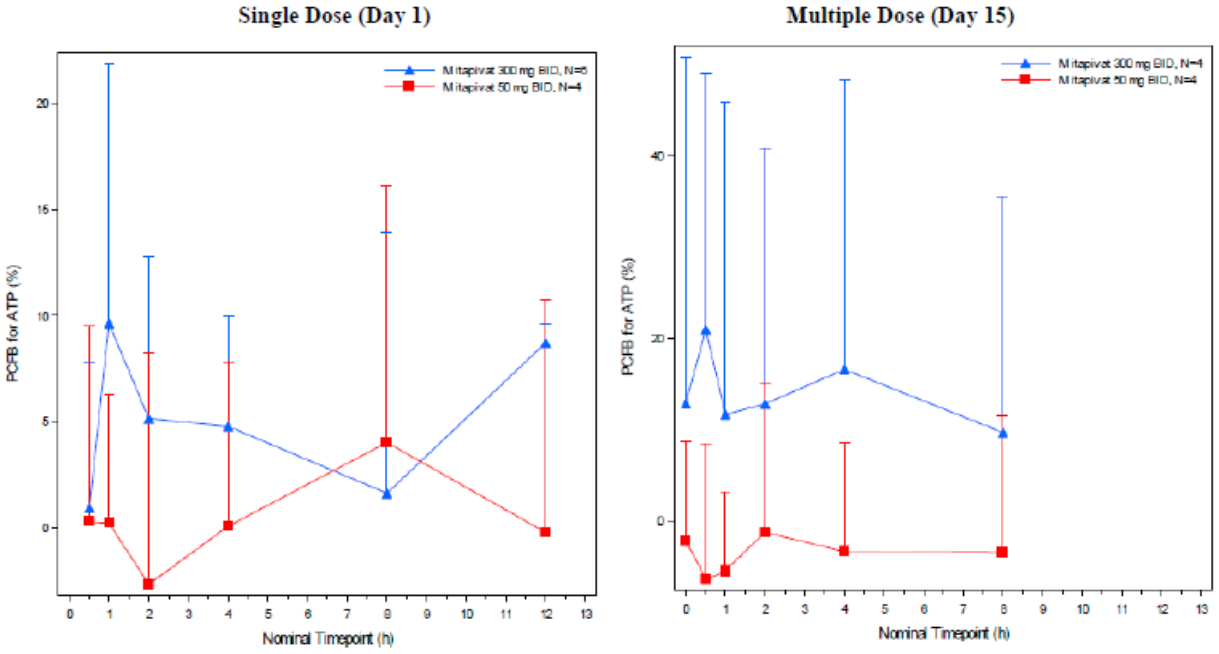


Table 8 Summary of ATP Pharmacodynamic Parameters After Single (Day 1) and Multiple (Day 15) Oral Doses of Mitapivat (Study AG348-C-003, Core Period)

Day	Pharmacodynamic Parameters	Mitapivat Dose and Regimen	
		50 mg BID	300 mg BID
1	n	4	6
	Baseline (µg/mL)	215.0 (16.1)	169.5 (21.6)
	AUC_Net_$B_{0\text{-}12hr}$ (hr·µg/mL)	13.92 (1,547.1)	82.78 (75.0)
	BR_{max} (µg/mL)	16.50 (71.5)	20.67 (33.3)
	$\%BR_{max}$ (%)	8.414 (73.5)	13.76 (65.8)
	t_{max} (h)	1.21 (0.00, 7.65)	5.08 (0.50, 12.03)
15	n	4	4
	Baseline (µg/mL)	215.0 (16.1)	179.5 (12.7)
	AUC_Net_$B_{0\text{-}8hr}$ (hr·µg/mL)	-79.81 (318.0)	226.6 (211.6)
	BR_{max} (µg/mL)	1.500 (2,068.8)	45.50 (134.1)
	$\%BR_{max}$ (%)	2.076 (647.6)	22.77 (126.2)
	t_{max} (h)	1.50 (1.00, 8.03)	2.00 (0.00, 8.00)

Source: AG348-C-003-NCA, Table 5.2.2.3-4 and Table 5.2.2.3-5.
Abbreviations: $\%BR_{max}$ = maximum percent increase from baseline response value; ATP = adenosine triphosphate; AUC_Net_$B_{0\text{-}8hr}$ = net area of the response curve above and below the baseline effect value from 0 to 8 hours; AUC_Net_$B_{0\text{-}12hr}$ = net area of the response curve above and below the baseline effect value from 0 to 12 hours; BID = twice daily; BR_{max} = maximum increase from baseline response value; NA = not available; R_{max} = maximum observed response value; RSD = relative standard deviation; t_{max} = time of observed R_{max}.
Note: Values are arithmetic mean (RSD%), except for t_{max}, which is median (minimum, maximum)

After single and multiple doses in healthy subjects, an increase in the mitapivat dose was associated with a decrease in 2,3-DPG concentrations. Minimal changes in ATP were observed after single doses of mitapivat, but ATP concentrations increased after multiple doses of mitapivat.

In subjects with PK deficiency, changes in these markers are minimal.

Relationship between plasma concentration and response

- Exposure-response (E-R) analysis

Exposure-response (E-R) analyses (report AG348-PMx-002) for safety and efficacy were conducted using data from AG348-C-003, AG348-C-006, AG348-C-007, and AG348-C-011 (safety) and studies AG348-C-006, AG348-C-007, and AG348-C-011 Cohort 1 (efficacy).

Evaluation of the relationship between mitapivat exposure and safety endpoints of interest included treatment-emergent adverse events (TEAEs; All Grade Insomnia and hot flush), laboratory abnormalities (alanine aminotransferase [ALT], aspartate aminotransferase [AST], total bilirubin, triglyceride), and changes in sex hormone levels (total and free testosterone, estrone, estradiol). Endpoints for efficacy were haemoglobin response and transfusion burden reduction.

Data were analysed graphically and by modelling the E-R relationship, testing different models, such as linear, saturable, sigmoidal, or Emax models. For the E-R relationship of efficacy only linear models were tested due to the number of limited available data. If deemed plausible, covariate analyses were conducted. Model selection was based on the OFV.

E-R for safety:

Data for all Grade ALT, AST, total bilirubin, triglycerides, All Grade of insomnia and hot flush elevation was available from 155 mitapivat- and 39 placebo-treated patients. For the 155 participants who received mitapivat treatment, exposure estimates could be estimated. The time-averaged mean AUC was 10.2 µg*h/mL (median=4.89 µg*h/mL, min=0.521 µg*h/mL, max= 62.7 µg*h/mL).

ALT and AST: For ALT, events were observed in 10 (25.7%) placebo-treated and 35 (22.6%) mitapivat-treated patients. For AST, in 7 (17.9%) placebo-treated subjects and 35 (22.6%) mitapivat-treated patients. Across exposure quartiles, no apparent trend in incidence was present. A logistic regression confirmed the absence of a statistically significant exposure effect across the observed exposure range.

Total bilirubin: Events were observed in 7 (17.9%) placebo-treated and 11 (7.1%) mitapivat-treated patients (**Table 9**). Across exposure quartiles, there was a tendency of lower incidence with higher exposures. Logistic regression was borderline statistically significant (P=0.048). The trend of decreasing total bilirubin with the increase in mitapivat exposure indicated an improvement in haemolysis. No covariate evaluation was performed due to borderline statistically significance and equally distributed covariates.

Table 9 Number and percent of patients with an all-grade total bilirubin event

New or Worsening All Grade Bilirubin	AUC (µg•h/mL)						Exposure Effect
	Placebo (N=39)	Mitapivat-Treated Subjects					
		Quartile 1 [0.63;4.04] (N=39)	Quartile 2 [0.63;4.04] (N=39)	Quartile 3 [5.22;9.25] (N=38)	Quartile 4 [9.25;46.2] (N=39)	Overall (N=155)	
No	32 (82.1%)	33 (84.6%)	38 (97.4%)	37 (97.4%)	36 (92.3%)	144 (92.9%)	*P*=0.048
Yes	7 (17.9%)	6 (15.4%)	1 (2.6%)	1 (2.6%)	3 (7.7%)	11 (7.1%)	

Source: ER-analysis.rmd.
Abbreviations: AUC = time-average area under the curve up to event; N = number of subjects.

Triglyceride: Events were observed in 21 (53.8%) placebo-treated and 62 (40.0%) mitapivat-treated patients. Across exposure quartiles, incidences were somewhat lower in the second to fourth exposure quartile compared with the first exposure quartile. A logistic regression indicated that exposure effects were not statistically significant across the observed exposure range.

All Grade insomnia events were observed in 7 (17.9%) placebo-treated and 46 (29.7%) mitapivat-treated patients (

Table 10). No new or no worsening of All Grade insomnia were observed in 82.1 % of the placebo treated volunteers and in 70.3 % of mitapivat treated patients. Overall, an upward trend of the incidence of All Grade insomnia with exposure appears present. A linear logistic regression showed a significant exposure effect and an increasing event occurrence with increasing exposure. No covariates were identified.

Table 10 Number and percent of patients with an all-grade insomnia event

New or Worsening All Grade Insomnia	Placebo (N=39)	Mitapivat-Treated Subjects - AUC (µg•h/mL)					Exposure Effect
		Quartile 1 [0.521;3.61] (N=39)	Quartile 2 [3.61;4.89] (N=39)	Quartile 3 [4.89;8.2] (N=38)	Quartile 4 [8.24;62.] (N=39)	Overall (N=155)	
No	32 (82.1%)	21 (53.8%)	36 (92.3%)	30 (78.9%)	22 (56.4%)	109 (70.3%)	$P<0.001$
Yes	7 (17.9%)	18 (46.2%)	3 (7.7%)	8 (21.1%)	17 (43.6%)	46 (29.7%)	

Source: ER-analysis.rmd.
Abbreviations: AUC = time-average area under the curve up to event; N = number of subjects.

All Grade hot flush events were observed in 0 (0%) placebo-treated and in 13 (8.4%) mitapivat-treated patients, with most events in the exposure quartile with the highest exposures (**Table 11**). No new or no worsening of All Grade hot flush were observed in 100 % of the placebo treated volunteers and in 91.6 % of mitapivat treated patients. Overall, an upward trend of the incidence of All Grade hot flush with exposure appears present. A linear logistic regression showed that exposure effect was significant and showed an increasing event occurrence with increasing exposure. No covariates were identified.

Table 11 Number and percent of patients with an all Grade hot flush event

New or Worsening All Grade Hot Flush	AUC (µg•h/mL)						Exposure Effect
	Placebo (N=39)	Mitapivat-Treated Subjects					
		Quartile 1 [0.701;4.04] (N=39)	Quartile 2 [4.04;5.22] (N=39)	Quartile 3 [5.22;9.05] (N=38)	Quartile 4 [9.05;67.1] (N=39)	Overall (N=155)	
No	39 (100%)	36 (92.3%)	38 (97.4%)	36 (94.7%)	32 (82.1%)	142 (91.6%)	$P\leq0.001$
Yes	0 (0%)	3 (7.7%)	1 (2.6%)	2 (5.3%)	7 (17.9%)	13 (8.4%)	

Source: ER-analysis.rmd.
Abbreviations: AUC = time-average area under the curve up to event; N = number of subjects.

Sex hormones were only evaluated in male participants. Total and free testosterone, estrone, and estradiol data were available for all 83 male patients (n=15 placebo, n=68 mitapivat-treated). Exposure estimates could be estimated in all 68 mitapivat-treated male subjects. The mean time-averaged AUC in male patients was 11.9 µg*h/mL (median=8.5 µg*h/mL, min=0.695 µg*h/mL, max=46.1 µg*h/mL). The average total and free testosterone level through the Core Period (AG348-C-003) or the Fixed-Dose Period (studies AG348-C-006, AG348-C-007, and AG348-C-011) was stratified by exposure quartiles. A weak trend of increased total and free testosterone with increasing mitapivat exposure was observed and modelled by a linear model with baseline total and free testosterone as covariates on the intercept and the slope. Median total testosterone increased from 26.6 nmol/L (min=13.3, max=42.4) in the 1st quartile to 27.9 nmol/L (min=14.3, max=42.8) in the 4th quartile (

Table 12). Total testosterone was higher in mitapivat treated patients compared to placebo (median 25.5 nmol/L vs. 18.5 nmol/L). Median free testosterone increased from 579 pmol/L (min=226, max=999) in the 1st quartile to 947 pmol/L (min=373, max=1830) in the 4th quartile (**Table 13**). Total testosterone was higher in mitapivat treated patients compared to placebo (median 612 pmol/L vs 374 pmol/L).

Table 12 Summary statistic of <u>total</u> testosterone by exposure group

Total Testosterone (nmol/L)	AUC (µg•h/mL)						Exposure Effect
	Placebo (N=15)	Mitapivat-Treated Subjects					
		Quartile 1 [0.695;6.66] (N=17)	Quartile 2 [6.66;8.5] (N=17)	Quartile 3 [8.5;12.7] (N=17)	Quartile 4 [12.7;46.1] (N=17)	Overall (N=68)	
Mean (SD)	18.7 (6.70)	25.7 (7.38)	24.6 (11.4)	22.0 (10.4)	28.5 (6.78)	25.2 (9.31)	*P*<0.001
Median [Min, Max]	18.5 [5.88, 29.5]	26.5 [13.3, 42.4]	22.5 [11.5, 56.4]	24.6 [0.746, 37.0]	27.9 [14.3, 42.8]	25.5 [0.746, 56.4]	

Source: ER-analysis.rmd.
Abbreviations: AUC = time-average area under the curve across Fixed-Dose Period; Max = maximum; Min = minimum; N = number of subjects; SD = standard deviation.

Table 13 Summary statistic of <u>free</u> testosterone by exposure group

Free Testosterone (pmol/L)	AUC (µg•h/mL)						Exposure Effect
	Placebo (N=15)	Mitapivat-Treated Subjects					
		Quartile 1 [0.695;6.66] (N=17)	Quartile 2 [6.66;8.5] (N=17)	Quartile 3 [8.5;12.7] (N=17)	Quartile 4 [12.7;46.1] (N=17)	Overall (N=68)	
Mean (SD)	345 (108)	588 (217)	516 (152)	920 (688)	1,030 (437)	763 (472)	*P*=0.0117
Median [Min, Max]	374 [139, 519]	579 [226, 999]	517 [289, 867]	951 [12.8, 2,440]	947 [373, 1,830]	612 [12.8, 2,440]	

Source: ER-analysis.rmd.
Abbreviations: AUC = time-average area under the curve across Fixed-Dose Period; Max = maximum; Min = minimum; N = number of subjects; SD = standard deviation.

A trend of decreased estrone level with increasing exposure was observed (Table 14). The magnitude of the effect reached a plateau at the high end of the exposure range. A linear regression applied to the individual data confirmed the presence of a statistically significant exposure effect across the observed exposure range. An Emax model resulted in a significant improvement of the fit and described best the data. Covariate effects were evaluated on the intercept and the maximum effect of the exposure effect. Baseline estrone was the only covariate that was retained in the model, both on the intercept and the maximum effect.

Table 14 Summary statistic of estrone by exposure group

Estrone (pmol/L)	AUC (µg•h/mL)						Exposure Effect
	Placebo (N=15)	Mitapivat-Treated Subjects					
		Quartile 1 [0.695;6.66] (N=17)	Quartile 2 [6.66;8.5] (N=16)	Quartile 3 [8.5;12.7] (N=17)	Quartile 4 [12.7;46.1] (N=17)	Overall (N=67)	
Mean (SD)	132 (50.1)	59.7 (31.6)	48.0 (39.0)	38.7 (20.6)	33.2 (16.1)	44.9 (29.3)	Emax *P*<0.001 EC50 *P*=0.0161
Median [Min, Max]	133 [49.8, 235]	47.0 [27.4, 128]	42.3 [16.6, 187]	37.5 [6.10, 88.8]	29.0 [13.9, 77.9]	38.8 [6.10, 187]	

Source: ER-analysis.rmd.
Abbreviations: AUC = time-average area under the curve across Fixed-Dose Period; Max = maximum; Min = minimum; N = number of subjects; SD = standard deviation.

Estradiol levels were lower in mitapivat-treated patients compared with placebo, but levels were similar across the exposure quartiles. The treatment effect model best described the data. Median estradiol was 88.1 pmol/L (min=48.8, max=119) in the 1st quartile and 69.7 pmol/L (min=22.2, max=167) in the 4th quartile. For participants who received placebo values were: median=118 pmol/L with min=45.3 pmol/L, max=161 pmol/L.

Simulations for the proposed recommended doses showed that the predicted probability of All Grade insomnia and All Grade hot flush slightly increased with the dose increase from 5 to 50 mg BID (

Table 15).

Table 15 Predicted probability of All Grade insomnia and hot flush

Dose (mg BID)	Probability of All Grade Insomnia (%) (Mean [95% CI])	Probability of All Grade Hot Flush (%) (Mean [95% CI])
5	19.9 [13.3, 28.0]	3.37 [1.22, 7.35]
20	22.1 [15.5, 29.9]	4.03 [1.61, 8.36]
50	26.0 [19.3, 33.9]	5.5 [2.48, 10.5]

Source: ER-analysis.rmd.
Abbreviation: BID = twice a day.
Notes: Results based on 1,000 replicate simulations of 155 subjects for which post hoc pharmacokinetic data were available.

For 50 mg mitapivat BID, estrone levels were predicted to decrease compared to placebo from a value of 137 to 43.4 pmol/L (

Table 16).

Table 16 Predicted estrone levels

Dose (mg BID)	Absolute Value (pmol/L) (Mean [95%CI])	% Change Compared to Placebo (Mean [95%CI])	Percentage (Below, Within, Above Normal Range)
5	93.7 [68.2, 109]	-31.5 [-51, -21.1]	0.154, 94.5, 5.39
20	59.4 [46.5, 70.0]	-56.5 [-67.3, -48.4]	1.92, 98.1, 0.0149
50	43.4 [36.5, 50.4]	-68.2 [-74.5, -62.6]	11.6, 88.4, 0

Source: ER-analysis.rmd.
Abbreviation: BID = twice a day.
Notes: Results based on 1,000 replicate simulations of 67 male subjects for which post hoc pharmacokinetic and baseline estrone data were available. Normal range defined as 33-133 pmol/L.

Dose-response for the total testosterone (**Table 17**) and free testosterone (

Table 18) in male subjects showed an upward trend.Mean change compared to placebo was <30% with mean = 26 % (min= 7.14 %, max=55.1 %) for free testosterone and 7.59 % (min=3.29 %, max = 13.7 %) for total testosterone at 50 mg BID.

Table 17 Predicted <u>total</u> testosterone levels

Dose (mg BID)	Absolute Value (nmol/L) (Mean [95%CI])	% Change Compared to Placebo (Mean [95%CI])
5	21.8 [20.1, 23.3]	0.877 [0.41, 1.43]
20	22.3 [20.7, 23.6]	3.18 [1.49, 5.4]
50	23.2 [21.9, 24.4]	7.59 [3.29, 13.7]

Source: ER-analysis.rmd.
Abbreviation: BID=twice a day.
Notes: Results based on 1000 replicate simulations of 68 male subjects for which post hoc pharmacokinetic and total testosterone baseline data was available.

Table 18 Predicted free testosterone levels

Dose (mg BID)	Absolute Value (pmol/L) (Mean [95%CI])	% Change Compared to Placebo (Mean [95%CI])
5	561 [456, 658]	6.01 [1.66, 13.2]
20	603 [509, 688]	14.1 [4, 27.5]
50	663 [577, 753]	26.0 [7.14, 55.1]

Source: ER-analysis.rmd.
Abbreviation: BID = twice a day.
Notes: Results based on 1,000 replicate simulations of 68 male subjects for which post hoc pharmacokinetic and baseline free testosterone data were available.

E-R for efficacy:

Data from 57 mitapivat-treated patients, and 39 placebo-treated patients were included in the efficacy analyses. Of note, 17 participants who first received placebo treatment in Study AG348-C-006 and then rolled over to mitapivat treatment in Study AG348-C-011 were counted in both the placebo and mitapivat treatment groups. A total of 26 patients treated with mitapivat (titrated 5-20-50 mg) were available for analysis in study AG348-C-007. The mean time-averaged AUC in studies AG348-C-006 and AG348-C-011 was 6.78 µg*h/mL (median=6,69 µg*h/mL, min=0.695 µg*h/mL, max=12.4 µg*h/mL). Exposure tertiles were used in the exploratory E-R plot. All 26 efficacy evaluable patients in study AG348-C-007 had mitapivat exposure estimates. The mean time-averaged AUC was 6.51 µg*h/mL (median=6.09 µg*h/mL, min=2.91 µg*h/mL, max= 16.7 µg*h/mL). Bitiles were used in the exploratory E-R plots.

The number of haemoglobin responders, defined as a ≥1.5 g/dL increase in haemoglobin concentration from baseline assessed at Weeks 16, 20, and 24 during the Fixed-Dose Period, was stratified by exposure tertiles. After mitapivat treatment 38.6% of the patients were haemoglobin responders (**Table 19**). No significant trends with exposure were apparent (responder 1st tertile 31.6 %, 2nd tertile 36.8 %, and 3rd tertile 47.4 %; placebo 0 %). A treatment effect was observed, but a statistically significant E-R relationship was not identified across the observed exposure range. Overall, 61.4 % were not haemoglobin responders. A logistic regression confirmed the absence of a statistically significant exposure effect across the observed exposure range.

Table 19 Number and exposure of haemoglobin responders studies AG348-C-006 & AG348-C-011

Hemoglobin Responder	AUC (µg•h/mL)					Exposure Effect
	Placebo (N=39)	Mitapivat-Treated Subjects				
		Tertile 1 [0.695;6.08] (N=19)	Tertile 2 [6.08;7.57] (N=19)	Tertile 3 [7.57;12.4] (N=19)	Overall (N=57)	
No	39 (100%)	13 (68.4%)	12 (63.2%)	10 (52.6%)	35 (61.4%)	*P*=0.624
Yes	0 (0%)	6 (31.6%)	7 (36.8%)	9 (47.4%)	22 (38.6%)	

Source: ER-analysis.rmd.
Abbreviations: AUC = time-average area under the curve across the Fixed-Dose Period; N = number of subjects.

Indirect bilirubin, LDH, haptoglobin, reticulocytes, and ferritin was stratified by exposure tertiles. Data is characterised by a large amount of heterogeneity. Differences in responses among the exposure tertiles are little with no evidence for an exposure effect across the observed exposure range.

Data for transfusion burden reduction, defined as a ≥33% reduction in the number of RBC units transfused through the 24 weeks of the Fixed-Dose Period compared with the historical transfusion

burden standardised to 24 weeks, and transfusion free, defined as 0 transfusion administered through the 24 weeks of the Fixed-Dose Period, was available from 26 mitapivat-treated patients. The number and percentage of responders (**Table 20**) were lower in the higher exposure bitile for both endpoints (transfusion-burden-responder reduction 1st bitile 53.8 %, 2nd bitile 23.1 %, transfusion-free-responder 1st bitile 38.5 %, 2nd bitile 7.7 %) which may be confounded by prior splenectomy status. Overall, 61.5 % did not experience transfusion-burden reduction and 76.9 % were not transfusion-free-responders.

Table 20 Number and percent of transfusion burden reduction responders and transfusion-free responders (AG348-C-007)

Parameter	AUC (µg•h/mL)			Exposure Effect
	Bitile 1 [2.91;6.09] (N=13)	Bitile 2 [6.09;16.7] (N=13)	Overall (N=26)	
Transfusion burden reduction responder				
No	6 (46.2%)	10 (76.9%)	16 (61.5%)	*P*=0.659
Yes	7 (53.8%)	3 (23.1%)	10 (38.5%)	
Transfusion-free responders				
No	8 (61.5%)	12 (92.3%)	20 (76.9%)	*P*=0.110
Yes	5 (38.5%)	1 (7.7%)	6 (23.1%)	

Source: ER-analysis.rmd.
Abbreviations: AUC = time-average area under the curve across Fixed-Dose Period; N = number of subjects.

Data from Study AG348-C-003 were initially not included in the exposure-response analysis for efficacy because haemoglobin (Hb) response was a secondary endpoint in the study. An updated exposure-response analysis for Hb response including the data from Study AG348-C-003 has been performed. Results and conclusions from this updated analysis are consistent with those described in the original analysis (Report AG348-PMX-002) where a non-statistically significant relationship was observed between mitapivat exposure (with doses up to 300 mg) and Hb response.

The updated exposure-response analysis of haemoglobin response included patients from Studies AG348-C-003, AG348-C-006, and AG348-C-011 Cohort 1 for whom mitapivat AUC could be estimated, as well as the placebo-treated participants in Study AG348-C-006. The exposure metric was the time-averaged AUC across the Core Period (Study AG348-C-003) or Fixed-Dose Period (Studies AG348-C-006 and AG348-C-011). Data from 99 mitapivat-treated and 39 placebo-treated patients were included. The mean time-averaged AUC for the 99 patients was 10.3 µg*h/mL (standard deviation = 8.05 µg*h/mL). The median time-averaged AUC was 7.62 µg*h/mL (min= 0.695 µg*h/mL, max=46.2 µg*h/mL). Covariate distributions for continuous and categorical covariates based on the exposure tertile were summarised (not shown here). There were no Hb responders with placebo treatment, while 41.4% of the patients achieved Hb response after mitapivat treatment. No apparent exposure-response relationship was observed.

A sensitivity analysis was conducted, including 10 participants with the nonmissense/nonmissense mutations from Study AG348-C-003; all 10 participants were Hb nonresponders. Thus, in the sensitivity analysis dataset, a total of 148 patients were included: 39 who received placebo with no Hb response and 109 patients who received mitapivat with 37.6% (41 out of 109 subjects) achieving Hb response (

Table 21).

Table 21 Number and percent of haemoglobin responders by exposure group in sensitivity analysis using the updated model

Haemoglobin responder	Time-averaged AUC (µg*h/mL)				
	Placebo (N=39)	Mitapivat-treated patients			
		Tertile 1 [0.695;6.56) (N=37)	Tertile 2 [6.56;9.75) (N=36)	Tertile 3 [9.75;46.2] (N=36)	Overall (N=109)
No	39 (100%)	23 (62.2%)	21 (58.3%)	24 (66.7%)	68 (62.4%)
Yes	0 (0%)	14 (37.8%)	15 (41.7%)	12 (33.3%)	41 (37.6%)

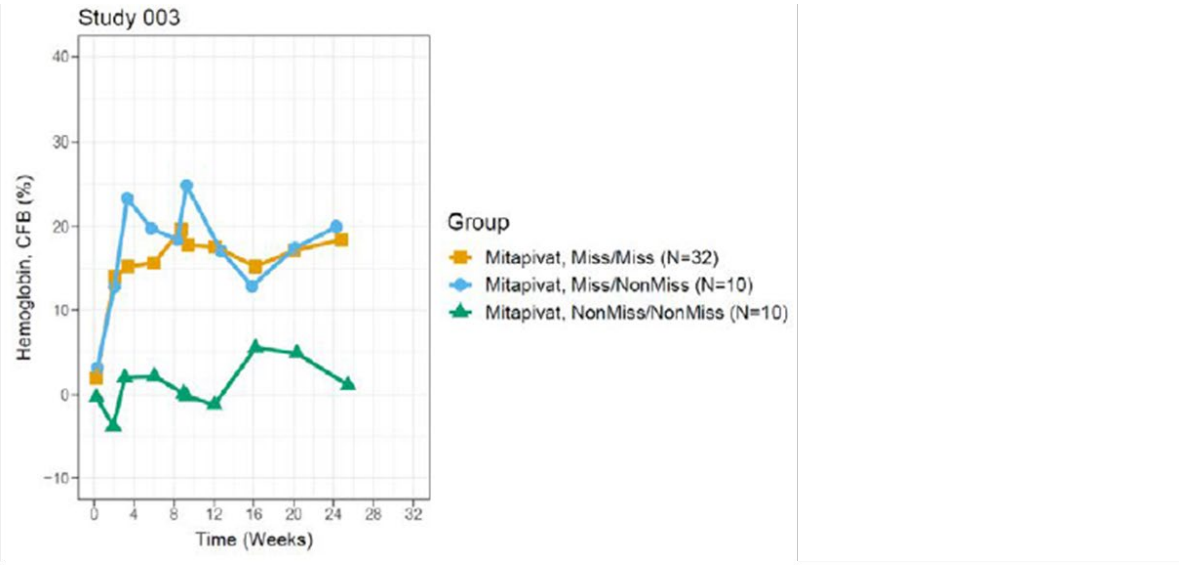
- Pharmacokinetic-pharmacodynamic (PK-PD) model

A pharmacokinetic-pharmacodynamic (PK-PD) model was developed (report AG348-PMx-002). Data from 168 patients and finally 1447 samples taken in studies AG348-C-003, AG348-C-006, and AG348-C-011 (Cohort 1) were used. Because the 10 patients with non-missense/non-missense PKLR alleles did not respond to treatment with mitapivat in Study AG348-C-003, and were not included in Study AG348-C-006 and Study AG348-C-011. Inititially they were also excluded from formal PK-PD modelling analysis. Likewise, data from placebo treated participants were not included in the PKPD modelling analysis. The mean haemoglobin change from baseline stratified by PKLR mutation status in study AG348-C-003 is presented in

Figure 9.

An updated PKPD model was developed including the 10 patients with non-missense/non-missense PKLR alleles from Study AG348-C-003. Placebo data from Study AG348-C-006 were not included because there was no change in the haemoglobin (Hb) concentration over time in participants who received placebo for 24 weeks; therefore, placebo data were not deemed to contribute to the primary purpose of understanding the dynamic relationship between mitapivat concentration and Hb concentration and were deemed unnecessary for inclusion in the analysis.

Figure 10 Observed mean haemoglobin change-from-baseline time profiles stratified by PKLR mutation status in Study AG348-C-003



Source: 2021-02-25-pkpd-eda-v2.r.

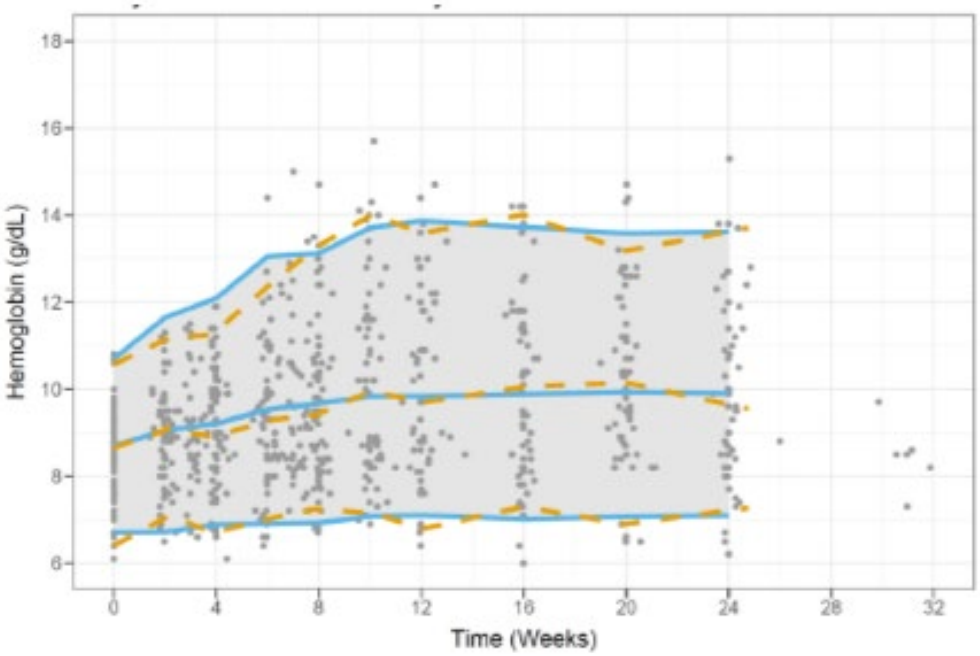
Abbreviations: CFB = change from baseline; Hgb = hemoglobin; Miss/Miss = missense/missense mutation; Miss/NonMiss = missense/non-missense mutation; NonMiss/NonMiss = non-missense/non-missense mutation; PKLR = liver-specific and red blood cell-specific forms of pyruvate kinase.

The final PK-PD model for haemoglobin and mitapivat concentrations was an indirect-effect model with zero-order production and first-order degradation for haemoglobin (turnover model). Mitapivat plasma concentration (central compartment) inhibiting the degradation rate constant of haemoglobin in a saturable fashion was modelled using an Emax model, leading to an increase in haemoglobin levels (i.e. stabilisation in haemoglobin levels; Emax = 29 %, EC50 = 73.2 ng/mL). IIV were found on baseline haemoglobin half-life (population mean 6.8 days, 54.8 %CV) and on Emax for patients without splenectomy (72.7 %CV). A decreased of haemoglobin stabilisation was estimated for patients with splenectomy and an increased for patients with higher indirect bilirubin levels. Covariates on haemoglobin half-life revealed an increase with increasing body weight and decrease with increasing baseline ferritin. In the updated PKPD model, a large covariate effect of non-missense/non-missense PKLR alleles on E_{max} (mitapivat maximum effect) was identified. The E_{max} estimate was 28.6% in a typical patient with a missense/missense or missense/non-missense PKLR allele type, and 5.2% in a typical patient with a non-missense/non-missense PKLR allele type. All other parameter estimates were similar between the updated and original models. The VPC of the final updated model is presented in

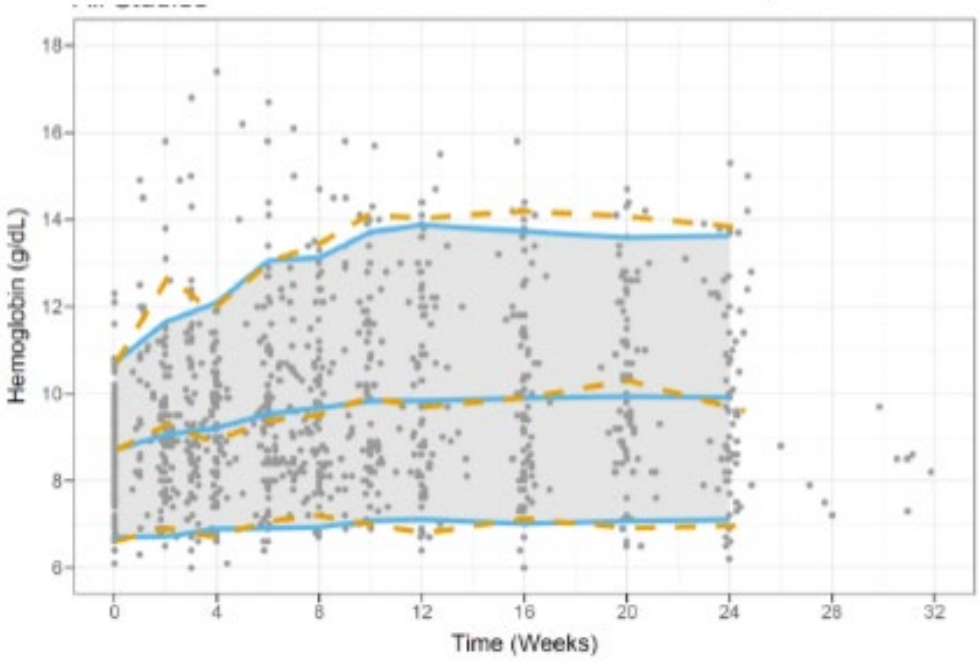
Figure 10.

Figure 11 VPCs for the Final updated PKPD model

(a) Studies AG348-C-006 and AG348-C-011



(b) All 3 Studies (AG348-C-003, AG348-C-006, and AG348-C-011)



Source: 2021-11-22PKPD rerun-tables-figures.

Note: The blue lines represent the 5th, median, and 95th percentile of simulated data; there were 10 simulations per subject with post hoc estimates. The shaded region includes the 5th to 95th percentile range. Each simulation included the titration algorithm. The yellow lines represent the 5th, median, and 95th percentile of the observed data, which are represented with solid dots.

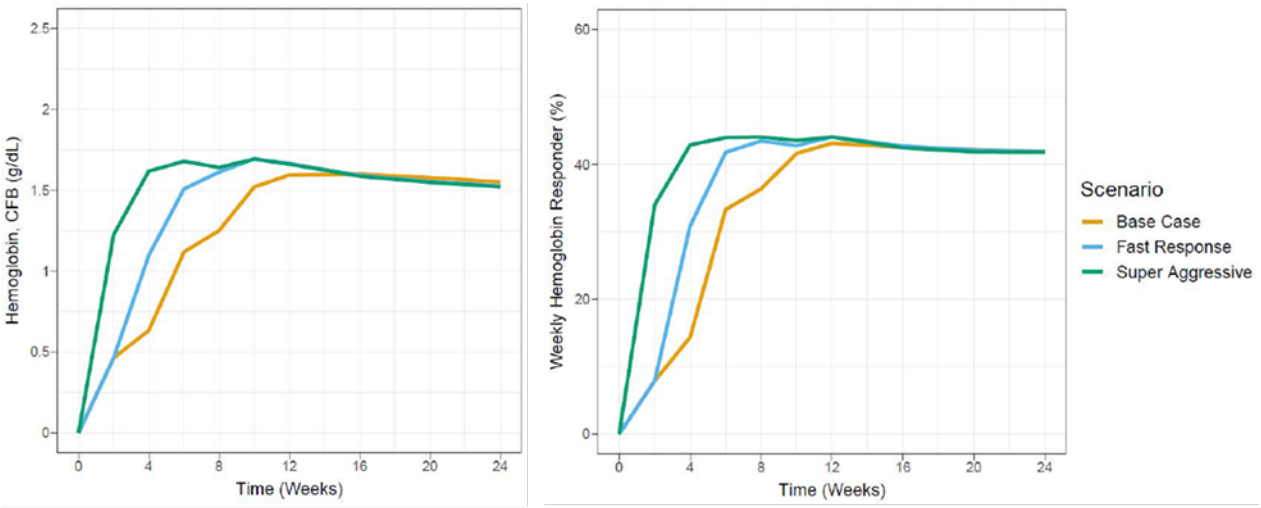
The saturable decrease of haemolysis was estimated with an EC50 of 73.2 ng/mL (updated model 72.7 ng/mL) and a median maximum decrease of about 29 % in patients without splenectomy, and about 9.3 % in patients with prior splenectomy. At maximal mitapivat concentrations, this translates to a 40.8 % increase in haemoglobin levels in patients without splenectomy and a 10.2 % increase in patients with prior splenectomy. The baseline haemoglobin half-life was estimated to be 6.8 days (a patient with a

body weight of 70 kg and ferritin level of 560 µg/L) with a range of 2.4 to 25.1 days. After maximal mitapivat treatment, the haemoglobin half-life changes to a median of 8.1 days with a range of 3.7 to 63.3 days.

Different up-titration dosing scenarios were simulated using the final PK-PD model. Results for simulations of study AG348-C-006 revealed that using a faster up-titration regimen (after 2 and 4 weeks) or a higher starting dose (50 mg instead of 5 mg) did not results in a higher probability of response compared to the proposed recommended dosing regimen (response 41.4%, 95% CI of 33% to 49.8%, down titration margin 2 g/dL below ULN, i.e. if haemoglobin values were above the down-titration margin at any visit, the dose was reduced to the next lower dose). Time profiles for haemoglobin mean change from baseline and for weekly haemoglobin responder percentage for the three dosing scenarios with down-titration margin 2 g/dL below ULN (scenarios 1 to 3) are shown in **Figure 11**. Mean change from baseline in haemoglobin during the Fixed-Dose Period was 1.58 g/dL (95% CI of 1.31 to 1.81 g/dL) with a probability of dose reduction of 3.9% (95% CI of 1.5% to 6.8%) (**Table 22**).

For patients with non-missense/non-missense PKLR alleles, the simulation results suggested that increasing the dose from 50 mg BID to 300 mg BID or 500 mg BID would result in a small numerical increase in Hb. This is consistent with the non-responder status of these patients in Study AG348-C-003, in which none of the 10 patients with non-missense/non-missense PKLR alleles reached the threshold of the Hb response criterion defined as change from baseline in Hb ≥1.5 g/dL at >50% assessments in the Core Period, excluding those within 2 months (61 days) of transfusion.

Figure 12 Simulations haemoglobin-time profiles down-titration margin 2 g/mL below ULN



Abbreviations: CFB = change from baseline; Hgb = hemoglobin; PK = pharmacokinetic; PK-PD = pharmacokinetic-pharmacodynamic; ULN = upper limit of normal.
Notes: Titration simulations including dose adjustments are performed for 118 subjects, each repeated 10 times, using post hoc PK parameters, post hoc PK-PD parameters, and covariates.

Table 22 Haemoglobin response metrics down-titration margin of 2 g/dL below ULN based on updated PKPD model

Scenario	Probability of response during FDP (%)	Probability of dose reduction (%)	Mean CFB in FDP (g/dL)	Mean CFB at 4 weeks (g/dL)	Probability of CFB ≥1.5 g/dL at 4 weeks (%)
Base case	41.4 [33.0, 49.8]	3.9 [1.5, 6.9]	1.58 [1.31, 1.81]	0.63 [0.52, 0.75]	14.3 [9.8, 18.7]
Fast response	41.5 [32.8, 50.2]	6.8 [3.0, 10.8]	1.56 [1.31, 1.83]	1.10 [0.92, 1.30]	30.8 [24.5, 38.0]

Super aggressive	41.2 [32.1, 49.6]	8.9 [4.7, 13.8]	1.55 [1.29, 1.81]	1.62 [1.33, 1.90]	42.7 [34.7, 51.1]

Source: 2021-11-22PKPD rerun-tables-figures.
Abbreviations: CFB=change from baseline; FDP=fixed-dose period; PK=pharmacokinetic; PD=pharmacodynamic.
Notes: Data are shown as the estimated probability [95% CI]. Titration simulations including dose adjustments are performed for 118 subjects, each repeated 10 times, using post hoc PK parameters, post hoc PK/PD parameters, and covariates. Additionally, 95% CIs are obtained by resampling subjects 1,000 times, and repeating simulations.

Simulations were conducted for 10 patients with non-missense/non-missense *PKLR* alleles, each repeated 10 times, using post hoc PK parameters, post hoc PKPD parameters, and covariates. Dose regimens evaluated were continuous dosing of 50 mg, 300 mg, or 500 mg BID for 24 weeks. Results suggested that at 50 mg BID, there is a mean increase of 0.16 g/dL (range, 0.02 to 0.54 g/dL) in Hb from baseline to Week 24. Increasing the dose to 300 mg BID or 500 mg BID resulted in small numerical increases in Hb **Table 23**.

Table 23 Simulation results in patients with non-missense/non-missense PKLR alleles

Scenario (fixed dosing)	Mean (range) change from baseline at 24 weeks (g/dL)
50 mg BID	0.16 (0.02, 0.50)
300 mg BID	0.18 (0.02, 0.65)
500 mg BID	0.19 (0.02, 0.66)

Source: 2021-11-22PKPD rerun-tables-figures.

- QTc analyses

Report AG348-C-004-QTC: Two linear mixed-effects models were developed using Phase 1 data from study AG348-C-004 (doses of 5, 50, and 200 mg, no placebo), describing concentration – QTc effects for mitapivat and its metabolite AGI-8702. Predicted mean increase in QTcF at geometric mean Cmax for 200 mg dose were 3.19 ms (0.79 to 5.65 ms) for AGI-8702 and 1.53 ms (-1.27 to 4.32 ms) for mitapivat. For the highest dose of 200 mg and considering mean QTcF baselines of ≤403 ms, maximal effects of up to 7.41 ms (AGI-8702) and 5.96 ms (mitapivat) can be expected. The results for the 5 mg dose group were not presented. The effects of mitapivat and metabolite AGI-8702 on QTcF change from baseline fall below the 10 msec threshold.

Report AG348-C-014-QTc cardiac safety: A linear mixed-effects model was developed using Phase 1 data from the fasted arms (i.e. Treatments A, B, and D) in study AG348-C-014 (doses of 100 and 300 mg or placebo), describing concentration – QTc effects for mitapivat and its metabolite AGI-8702. Serial ECGs were extracted from continuous ECG recordings (Holters) and time matched to the pharmacokinetic samples. A combined model for both analytes was chosen. Results reveal that QTc effects are not expected to exceed 10 ms. Highest changes were observed after about 4 h. An increase in heart rate could be observed after administration of 100 mg (up to 3.3 bpm) and 300 mg (up to 6.5 bpm) between 0.5 and 4 h post-dose.

2.5.3. Discussion on clinical pharmacology

Pharmacometrics

Data from 186 healthy volunteers and 155 patients with PK deficiency obtained in five Phase 1, one Phase 2, and three Phase 3 studies were analysed developing a population pharmacokinetic model for mitapivat. The nonlinear pharmacokinetics (exposure increases less than dose proportional) of mitapivat was described by a 3-compartment model that included a first-order absorption and a lag-time on

absorption. Clearance was modeled a function of time and dose and increased exponentially to steady state, thus accounting for the auto-induction observed after multiple doses. Shrinkages for the central and a peripheral volume of distribution are considered too high. However, overall the parameter estimates were generally precise (RSE between 3.18 and 34.4 %). Statistically significant covariates were found for dose and haematocrit on CL (increasing with dose and haematocrit), body weight on all three volumes of distribution (increasing with body weight), and gender on volume of distribution of the central compartment (decreasing for female). Model-based simulations were performed in order to evaluate the expected exposures (AUC, C_{max}, and C_{trough}) for different subpopulations. Overall, results reveal that exposures after 50 mg BID dosing are expected in the order of magnitude of the exposures for the reference patients. In addition, expected exposure over a broader weight range up to 140 kg were presented. $C_{max,ss}$ is expected to be about 27 % higher in patients with lower body weight (40 kg) and 24 % lower in patients with higher body weight (140 kg). C_{trough} is expected to be about 26 % and 22 % lower in patients with lower body weight (40 kg and 45 kg, respectively) and 49 % higher in patients with higher body weight (140 kg). Overall, C_{max} can be expected to be slightly too high for lighter patients and slightly too low for heavier patients. The expected C_{trough} values for patients with a higher body weight are considered too high from the PK perspective (+ 49 % compared to the reference). But $AUC_{0\text{-}24hr,ss}$, which was found as predictor for safety, seems comparable for the different body weights between 40 and 140 kg. Model-based expected exposures for a broader range of haematocrit levels of 19 to 52 % were provided. AUC (predictor for safety measures) is expected to decrease by about 20 % with increasing haematocrit (of up to 52 % haematocrit). The decrease in exposure with increasing haematocrit is more pronounced for C_{trough}, which is expected to decrease by 41 % for patients reaching 52 % haematocrit levels, and by 24 % for patients reaching 39 % haematocrit levels. Among the 155 patients included in the population PK analysis, only five patients were older than 65 years (n=4 [2.6] % aged 65 to 74 years and one patient [0.6 %] in the age group 75 – 84 years). The low number of older patients have been adequately addressed in the SmPC. However, results with regard to older age (65 years and onwards) require cautious interpretation. Pyruvate kinase deficiency mutation type was not statistically significantly covariate on the pharmacokinetics of mitapivat and seem not have an impact on mitapivat exposure. In summary, the developed population pharmacokinetic model might be sufficient for the intended purpose to characterise the pharmacokinetic behaviour and evaluate effects of covariates.

Special populations

Renal impairment

The ADME study showed that the renal excretion of mitapivat is negligible (< 3% of the dose). Based on PopPK analysis, steady-state AUC values from subjects with moderate renal impairment were within the range for subjects with normal renal function, and a comment has been added to the SmPC, regarding the limited available data.

Hepatic impairment

The ADME study showed that approximately half of the administered dose was metabolised. Therefore, there is the potential for hepatic impairment to affect mitapivat exposure. There is too limited data to allow drawing any conclusion. The lack of information is correctly reflected in the SmPC sections 4.2 and 5.2. The applicant has committed to undertake a dedicated study aiming to assess the influence of hepatic impairment on exposure, and to provide the results as a post-approval measure.

Pharmacokinetic interactions

Mitapivat interactions with other drugs is based on one interaction study where mitapivat was co-administered with a strong CYP3A4 inhibitor and a strong CYP3A4 inducer, and PBPK modeling.

Although mitapivat PBPK was demonstrated to be able to accurately describe mitapivat in certain conditions with metabolism-related parameters estimated using adequate clinical data, there are still concerns regarding the model's capability to accurately estimate both auto-induction and auto-inhibition due to the lack of qualification dataset and some remaining questions on misprediction following multiple dose administration at high dose (i.e. 120 mg QD and 360 mg BID). A combined mitapivat PBPK model

with the PD model on gastric pH was also presented to predict mitapivat interactions with acid reducing agent (ARA). The latter model is is not considered qualified to predict mitapivat interaction with gastric acid reducing agent. The qualification dataset was not considered robust and importantly, the model verification overpredicted the interaction between ranitidine and ketoconazole by ten- to four- fold, when considering geometric and arithmetic means respectively. Consequently, decreases in mitapivat absorption in case of such concomitant treatment cannot be excluded. This is appropriately reflected in the SmPC.

Mitapivat interactions with CYP3A4 substrate are still uncertain given mitapivat's inhibition and induction potentials. This is, however, reflected in the SmPC and caution of use should be applied in case of co-administration with CYP3A4 substrate. Close monitoring should be performed in case of NTI drugs. Given the complex metabolism of mitapivat being CYP3A4 substrate, inhibitor and inducer, and the large fraction of drugs being metabolised by CYP3A4, especially those with narrow therapeutic index, a DDI study with midazolam to assess the magnitude of interaction between mitapivat at the highest dose with midazolam will be conducted by the applicant as a post-approval measure.

The clinical relevance of potential interaction with P-gp substrates is thus unknown. The caution of use in case of concomitant treatment with P-gp substrates is recommended.

As inducer of CYP2C8, 2C9, 2C19, 2B6, and UGT1A1 mitapivat may decrease drug substrate of those enzymes and caution should be used in case of concomitant treatment with these drug substrates with close monitoring in case of NTI drugs.

Mitapivat exposure may be decreased in case of co-administrations of ARAs, which has been reflected in the SmPC together with appropriate recommendations.

Relation between plasma concentration and response

Different E-R models for efficacy and safety endpoints were developed using logistic regression, linear, and Emax models. Overall, results reveal that All Grade insomnia, hot flush, and total and free testosterone increase with increasing mitapivat exposure. Estrone levels decreased with increasing mitapivat exposure although no consistent trend with exposure was observed across the exposure quartiles. Logistic regression or linear regression models were applied for efficacy endpoints. Exposure-efficacy for haemoglobin response, indirect bilirubin, lactate dehydrogenase, haptoglobin, reticulocytes, and ferritin (studies AG348-C-006 and AG348-C-011), as well as transfusion burden reduction and transfusion-free response (study AG348-C-007) were investigated. For none of the investigated endpoints a clear exposure-response trend was found. For haemoglobin a treatment effect was observed, but a statistically significant E-R relationship was not identified across the observed exposure range.

Initially, data from Study AG348-C-003 were not included in the exposure-response analysis for efficacy because haemoglobin response was a secondary endpoint in this study. However, the exposure-response analysis was rerun and these data (each n=21 treated with 50 and 300 mg BID, respectively) were included in the exposure-response analysis for efficacy. In addition a sensitivity analysis was conducted, including 10 participants with the non-missense/non-missense mutations from Study AG348-C-003 (all non-responder). No clear relationship between exposure (AUC) and haemoglobin response can be observed.

The PK-PD relationship between mitapivat plasma concentration and increase in haemoglobin levels was analysed by modelling a reduction of the degradation rate constant of haemoglobin with increasing mitapivat doses. An Emax function was used to model the saturable effect of mitapivat on haemoglobin increase. Stabilisation of haemoglobin turnover during mitapivat treatment was less in patients with prior splenectomy and increased in patients with higher indirect bilirubin. Haemoglobin half-life increased with increasing baseline body weight and decreased with increased baseline ferritin. Model-based simulations reveal that the proposed every 4-weeks up-titration dosing scheme of 5 mg to 20 mg up to 50 mg BID result in a similar response (41.4%, 95% CI of 33.4% to 49.5%, down titration margin 2 g/dL below ULN) compared to faster up-titration or using a higher starting dose of 50 mg, but resulting in less dose reductions during the treatment. Dosing schemes designed to achieve faster haemoglobin response could enable 31 or 43% of participants to achieve a haemoglobin change $\geq$ 1.5 g/dL at 4 weeks but had no

effect on haemoglobin response during the Fixed-Dose Period. Such schemes did not allow sufficient time for the individual Hgb level to achieve a plateau prior to up-titrating to the next dose level, and therefore were predicted to increase dose reductions by approximately 2-fold. Based on these results, the proposed titration and dosing scheme is acceptable.

While the dosing titration scheme is well justified in case Hb level is below normal range or within normal range, it was not clear how to manage patients with Hb above normal range. Therefore, the applicant updated the text in the SmPC section 4.2). All patients are to be titrated through sequential doses of 5 mg BID and 20 mg BID to a maximum of 50 mg BID, with dose increases occurring after 4 weeks at each dose level, after assessing haemoglobin (Hb) levels and transfusion requirements. Some patients may respond at lower dose levels (5 or 20 mg BID). If Hb levels is within normal range and patient has not required a transfusion within the last 8 weeks, patients can maintain on 5 or 20 mg BID. If Hb levels decrease, patients who are at one of the lower maintenance dose levels can be up titrated to a maximum dose of 50 mg BID. The dose can be reduced to the next lower dose level for adverse event management and/or tolerability reasons.

In Study AG348-C-003, 10 patients with non-missense/non-missense PKLR alleles did not respond to treatment with mitapivat, and were not included in Study AG348-C-006 and Study AG348-C-011. Therefore, they were excluded from formal PK-PD modelling analysis.

The applicant provided results for an updated PKPD model that included, in addition to the previously used data, data from 10 patients with non-missense/non-missense PKLR alleles. Placebo data were not included. The applicant's argumentation that inclusion of placebo data are unnecessary is not agreed, as they would help to directly quantify the difference between placebo and mitapivat treatment, in particular for patient populations who experience very little or no response only (e.g. patients with missense/non-missense PKLR alleles).

Overall, results between participants of studies AG348-C-003, AG348-C-006 and AG348-C-011, mitapivat and placebo treated, and the three PKLR mutation types, are difficult to compare, because they were presented in different ways and simulations were performed for different dosing scenarios. Moreover, placebo data should have been assessed along with the mitapivat treated participants to better quantify the differences in response. Nonetheless, results suggest, that patients with non-missense/non-missense PKLR mutation do not response (markedly) to the treatment with mitapivat, independent of the dose administered. Please refer also to the section on clinical efficacy below.

All taken together it can be expected, that based on the results of the population pharmacokinetic, PK-PD and the E-R modelling analyses, and due to the nonlinear pharmacokinetics, higher doses of mitapivat (above 50 mg BID) would not lead to proportional higher exposure and are not expected to translate to a higher efficacy. However, an increase in exposure may results in an increased safety risk with respect to insomnia, hot flush and testosterone levels. The SmPC provides guidance to prescribers for situations when a patient may need a maintenance dose lower than 50 mg BID. Two QTc analyses were conducted using Phase 1 data were the effect of mitapivat and its metabolite AGI-8702 on QT intervals and heart rate were investigated after administration of mitapivat doses of 5, 50, 100, 200, and 300 mg. Linear mixed effects models were developed. Overall, results reveal that QTc effects can be expected to be below 10 ms.

Pharmacodynamics

A dose-exposure-response relationship between mitapivat and 2 PD endpoints (ATP and 2,3-DPG) was shown in healthy subjects, consistent with the hypothesised mechanism of action (PKR activation). Dose-dependency decrease in 2,3-DPG concentrations was observed up to 360 mg BID mitapivat. No dose-dependent increases in ATP were observed above mitapivat doses of 60 mg BID.

Significant changes in these markers were not observed in subjects with pyruvate kinase deficiency. The reason for this difference is not clear but may be related to the composition of blood in subjects with PK deficiency, who often have a considerable degree of reticulocytosis and a severely curtailed RBC life span. The assessment of activity must imperatively focus on clinical parameters.

2.5.4. Conclusions on clinical pharmacology

Overall the PK of mitapivat has been sufficiently characterised in healthy subjects and in the target population. Results of the ongoing hepatic impairment study are awaited and will be submitted by the applicant post-approval, which is acceptable.

2.5.5. Clinical efficacy

- ***Dose-response study***

Study AG348-C-003 is a Phase 2, open-label, 2-arm, multicentre, randomised, dose-ranging study in adult subjects with PK deficiency. This study consists of a 24-week core period and an 8-year extension period.

The choice of dose and schedule of administration of mitapivat for arms 1 and 2 of the study 003 was based on PK/PD data from AG348-C-002 multiple ascending dose study in healthy adult subjects. The study 002 demonstrated that the exposures produced by mitapivat doses from 60 mg BID to 360 mg BID resulted in maximal changes from baseline for the PD markers 2,3-DPG (reduction from baseline) and ATP (increase from baseline). The exposures resulting from doses less than 60 mg BID were of lesser magnitude and the exposures resulting from doses greater than 360 mg BID were of no greater magnitude than the aforementioned range. Therefore, the starting doses for this first dose ranging study in subjects with PK deficiency were selected to be 300 mg BID (Arm 1) and 50 mg BID (Arm 2).

Subjects remaining on study received mitapivat across a wide range of doses (from 300 mg BID to 5 mg QD). These observations indicate that the optimal maintenance dose of AG-348 is likely to vary by subject and cannot be determined in advance.

Based on simulations from the population PK-efficacy-safety analyses, doses of 5 mg BID, 20 mg BID, and 50 mg BID were selected for study 006 and study 007. These same doses were also used in the dose optimisation period for subjects in Cohort 1 of the extension study (Study 011).

- ***Main studies***

Study AG348-C-006

Study AG348-C-006 was a Phase 3, multicentre, randomised, double-blind, placebo-controlled efficacy and safety study of orally administered mitapivat compared with placebo in subjects with PK deficiency who were not regularly receiving blood transfusions. The study consisted of a dose optimisation period (Part 1) followed by a fixed dose period (Part 2).

Methods

Study Participants

Inclusion Criteria

- Documented clinical laboratory confirmation of PK deficiency, defined as documented presence of at least 2 mutant alleles in the PKLR gene, of which at least 1 was a missense mutation, as determined per the genotyping performed by the central genotyping laboratory.

- An Hb concentration less than or equal to 100 g/L (10.0 g/dL; 6.21 mmol/L) regardless of sex (average of at least 2 Hb measurements [separated by a minimum of 7 days] during the Screening Period).

- Not regularly transfused, defined as having had no more than 4 transfusion episodes in the 12-month period up to the first day of study treatment and no transfusions in the 3 months before the first day of study treatment.

- Received at least 0.8 mg oral folic acid daily for at least 21 days before the first dose of study treatment and continued daily during study participation.

- Adequate organ function, as defined by:

  a. Serum aspartate aminotransferase (AST) ≤2.5×ULN (unless the increased AST was assessed by the Investigator as due to haemolysis and/or hepatic iron deposition) and alanine aminotransferase (ALT) ≤2.5×ULN (unless the increased ALT was assessed by the Investigator as due to hepatic iron deposition).

  b. Normal or elevated levels of serum bilirubin. In subjects with serum bilirubin >ULN, the elevation was not associated with choledocholithiasis, cholecystitis, biliary obstruction, or hepatocellular disease. Elevated bilirubin attributed to haemolysis with or without Gilbert's syndrome was not exclusionary.

  c. Estimated glomerular filtration rate (GFR) ≥60 mL/min/1.73 m2, measured GFR ≥60 mL/min, or calculated creatinine clearance (Cockcroft-Gault) ≥60 mL/min.

  d. Absolute neutrophil count ≥1.0×109/L (based on an average of at least 2 measurements [separated by a minimum of 7 days] during the Screening Period).

  e. Platelet count ≥100×109/L in the absence of a spleen, or platelet count ≥50×109/L in the presence of a spleen and in the absence of any other cause of thrombocytopenia (based on an average of at least 2 measurements [separated by a minimum of 7 days] during the Screening Period).

  f. Activated partial thromboplastin time and international normalised ratio ≤1.25×ULN, unless the subject received therapeutic anticoagulants.

Exclusion Criteria

1. Homozygous for the R479H mutation or had 2 nonmissense mutations, without the presence of another missense mutation, in the PKLR gene as determined per the genotyping performed by the central genotyping laboratory.

2. Significant medical condition that conferred an unacceptable risk to participating in the study, and/or that could have confounded the interpretation of the study data.

3. Splenectomy scheduled during the study treatment period or had undergone splenectomy within 12 months before signing informed consent.

4. Prior bone marrow or stem cell transplant.

5. A history of major surgery within 6 months of signing informed consent. Note that procedures such as laparoscopic gallbladder surgery are not considered major in this context.

6. Currently receiving medications that are strong inhibitors of cytochrome P450 34A (CYP)3A4, strong inducers of CYP3A4, strong inhibitors of P-glycoprotein (P-gp), or digoxin (a P-gp sensitive substrate medication) that had not been stopped for a duration of at least 5 days or a time frame equivalent to 5 half-lives (whichever was longer) before the first dose of study treatment.

7. Currently receiving haematopoietic stimulating agents (eg, erythropoietins, granulocyte colony stimulating factors, thrombopoietins) that had not been stopped for a duration of at least 28 days before the first dose of study treatment.

8. A history of allergy to sulfonamides if characterised by acute haemolytic anaemia, drug-induced liver injury, and anaphylaxis, rash of erythema multiforme type or Stevens - Johnson syndrome, cholestatic hepatitis, or other serious clinical manifestations.

9. Currently receiving anabolic steroids, including testosterone preparations, within 28 days before the first dose of study treatment.

Treatments

All subjects should receive an initial dose of 5 mg BID of study treatment with 2 potential sequential steps for dose level increase (ie, from 5 to 20 mg BID and from 20 to 50 mg BID; no increases beyond 50 mg BID will be allowed).

Objectives

The primary objective of the study was to evaluate the efficacy of treatment with mitapivat compared with placebo in increasing haemoglobin (Hb) concentrations.

The secondary objectives of the study were to evaluate the safety of mitapivat, to determine the effect of the study treatment regimens on markers of haemolysis, haematopoietic activity, and other indicators of clinical activity, and to determine the effect of the study treatment regimens on health-related quality of life (HRQOL).

The exploratory objectives of the study were to determine the effect of the study treatment regimens on number of transfusion events and number of red blood cell (RBC) units transfused and to evaluate markers of iron metabolism and indicators of iron overload.

Outcomes/endpoints

Primary endpoint

The primary endpoint was the Hb response, defined as a ≥15 g/L (1.5 g/dL; 0.93 mmol/L) increase in Hb concentration from baseline that is sustained at 2 or more scheduled assessments at Weeks 16, 20, and 24 during the Fixed Dose Period.

Key Secondary Endpoint

The key secondary endpoint was the average change from baseline in Hb concentration at Weeks 16, 20, and 24.

Other Secondary Endpoints

• Maximal Hb concentration increase from baseline

• Time to first achieve an increase in Hb concentration of 15 g/L (1.5 g/dL; 0.93 mmol/L) or more from baseline.

• Average change from baseline at Weeks 16, 20, and 24 in markers of haemolysis: bilirubin, lactate dehydrogenase (LDH), and haptoglobin levels

• Average change from baseline at Weeks 16, 20, and 24 in markers of haematopoietic activity: reticulocyte percentages

• Change from baseline in HRQOL Patient-Reported Outcome (PRO) scores: Pyruvate Kinase Deficiency Diary (PKDD) and Pyruvate Kinase Deficiency Impact Assessment (PKDIA)

Exploratory Endpoints

• Change from baseline in additional markers of haematopoietic activity.

• Change from baseline in markers of iron metabolism and indicators of iron overload.

• Change from baseline in HRQOL PRO scores: European quality of life 5-dimensional descriptive system (EQ-5D-5L).

• Proportion of subjects requiring transfusions and the total number of RBC units transfused.

Sample size

Approximately 76 subjects were to be randomised in the study in a 1:1 ratio to mitapivat or matched placebo. Assuming a response rate of 35% in the mitapivat arm and 5% in the placebo arm, 76 subjects (38 per arm) were needed to have 90% power to reject the null hypothesis based on a 2-sided Fisher's exact test with 0.05 significance level.

The sample size of 76 subjects (38 per arm) also provided >80% power to detect a difference of 1.4 g/dL between mitapivat and placebo in the average of mean change from baseline in Hb at Weeks 16, 20, and 24, based on a 2-sample t-test, assuming a standard deviation (SD) of 1.5 g/dL (98% power) or 2.1 g/dL (81% power).

To control the overall type I error in the study at the 2-sided 5% level, a fixed sequence testing procedure (Mauer, 1995) was used to adjust for multiple statistical testing of the primary and secondary efficacy endpoints. This is detailed in the statistical method section.

No formal interim analysis was planned or performed.

Randomisation and blinding (masking)

Eligible subjects were randomised in a 1:1 ratio to mitapivat or matching placebo.

Randomisation assignment was implemented by an interactive response system and stratified by:

- The average of screening Hb concentrations (<8.5, ≥8.5 g/dL [5.28 mmol/L])
- The PKLR gene mutation category (missense/missense, missense/nonmissense). In rare instances where PKLR gene mutation category could not be made definitively (eg, if the subject harbored 3 mutant PKLR alleles), the subject was assigned to the missense/nonmissense category.

Statistical methods

Analysis populations

The **Full Analysis Set (FAS)** included all subjects who were randomised. Subjects were classified according to the randomised treatment arm.

The **Safety Analysis Set** included all subjects who received at least 1 dose of study treatment. Subjects were classified according to the treatment actually received. If a subject randomised to placebo received at least 1 dose of mitapivat then the subject was classified to the mitapivat arm.

The **Per-Protocol Set (PPS)** was a subset of the FAS. Subjects who met any of the following criteria were excluded from the PPS:

– Did not receive at least 1 dose of the randomised treatment

– Did not have Hb assessments at Weeks 16, 20, and 24 during the Fixed Dose Period

Results

Participant flow

Table 24 Subject disposition (full analysis set)

	Placebo N=40 n (%)	Mitapivat N=40 n (%)	Total N=80 n (%)
Disposition: End of randomization			
Discontinued	1 (2.5)	0	1 (1.3)
Reason for discontinuation			
Lost to follow-up	1 (2.5)	0	1 (1.3)
Completed	39 (97.5)	40 (100)	79 (98.8)
Ongoing	0	0	0
Disposition: End of treatment			
Discontinued	0	0	0
Completed	39 (97.5)	40 (100)	79 (98.8)
Ongoing	0	0	0
Disposition: End of study			
Discontinued	1 (2.5)	0	1 (1.3)
Reason for discontinuation			
Lost to follow-up	1 (2.5)	0	1 (1.3)
Completed	39 (97.5)	40 (100)	79 (98.8)
Ongoing	0	0	0

Source: Table 14.1-2.2.
Notes: The denominator used to calculate percentages is N, the number of subjects in the Full Analysis Set within each treatment arm. Disposition for end of randomization reflects the disposition after randomization but before start of study treatment.

Recruitment

The first subject was enrolled on 01-Oct-2018 and the last subject completed on 09-Oct-2020. A total of 46 study sites participated in this study. All subjects who remain on study during Part 2 through the Week 24 Visit may be eligible for an open-label extension study (011), in which all subjects will receive AG-348.

Conduct of the study

Protocol Amendments

The protocol was amended 3 times.

Protocol Deviations

The following were subject-level major protocol deviations considered to have the potential to impact study conclusions (table below).

Table 25 Summary of Major Protocol Deviations (Full Analysis Set)

Deviations	Placebo N=40 n (%)	Mitapivat N=40 n (%)	Total N=80 n (%)
Subjects with major deviations	11 (27.5)	10 (25.0)	21 (26.3)
ICH/GCP Deviation	7 (17.5)	6 (15.0)	13 (16.3)
GCP-Other Deviations	1 (2.5)	0	1 (1.3)
Informed Consent	3 (7.5)	3 (7.5)	6 (7.5)
Source Documentation	3 (7.5)	3 (7.5)	6 (7.5)
Protocol Deviation	4 (10.0)	5 (12.5)	9 (11.3)
SAE-Related Deviations	0	1 (2.5)	1 (1.3)
Study Treatment Deviation	1 (2.5)	2 (5.0)	3 (3.8)
Unblinding Deviation	3 (7.5)	2 (5.0)	5 (6.3)

Source: Table 14.1-4.1.
Abbreviations: FAS = Full Analysis Set; GCP = Good Clinical Practice; ICH = International Council for Harmonisation; SAE = serious adverse event.
Note: The denominator used to calculate percentages is N, the number of subjects in the FAS within each treatment arm.

Baseline data

Demographic Characteristics

Table 26 Summary of Demographic Characteristics and Physical Measurements (Full Analysis Set)

	Placebo N=40	Mitapivat N=40	Total N=80
Age (yr)			
n	40	40	80
Mean (SD)	37.2 (15.92)	36.0 (15.18)	36.6 (15.47)
Median (Q1, Q3)	35.5 (22.5, 49.5)	31.5 (23.5, 49.0)	32.5 (23.0, 49.5)
Min, Max	19, 78	18, 70	18, 78
Age category (yr), n (%)			
<65	38 (95.0)	38 (95.0)	76 (95.0)
≥65	2 (5.0)	2 (5.0)	4 (5.0)
Age category (yr), n (%)			
<35	20 (50.0)	22 (55.0)	42 (52.5)
≥35	20 (50.0)	18 (45.0)	38 (47.5)
Sex, n (%)			
Male	16 (40.0)	16 (40.0)	32 (40.0)
Female	24 (60.0)	24 (60.0)	48 (60.0)
Childbearing Potential [1]			
Yes	20 (83.3)	17 (70.8)	37 (77.1)
No	4 (16.7)	7 (29.2)	11 (22.9)
Ethnicity, n (%)			
Hispanic or Latino	1 (2.5)	2 (5.0)	3 (3.8)
Not Hispanic or Latino	34 (85.0)	28 (70.0)	62 (77.5)
Not reported	5 (12.5)	10 (25.0)	15 (18.8)

	Placebo N=40	Mitapivat N=40	Total N=80
Race, n (%)			
White	32 (80.0)	28 (70.0)	60 (75.0)
Asian	3 (7.5)	5 (12.5)	8 (10.0)
Native Hawaiian or Other Pacific Islander	0	1 (2.5)	1 (1.3)
American Indian or Alaska Native	0	0	0
Black or African American	0	0	0
Other	1 (2.5)	0	1 (1.3)
Not reported	4 (10.0)	6 (15.0)	10 (12.5)
Height (cm)			
n	40	40	80
Mean (SD)	169.72 (9.489)	168.64 (7.751)	169.18 (8.626)
Median (Q1, Q3)	169.50 (162.70, 175.65)	167.80 (162.80, 175.00)	168.10 (162.70, 175.15)
Min, Max	152.8, 195.6	153.7, 188.0	152.8, 195.6
Weight (kg)			
n	40	40	80
Mean (SD)	69.66 (16.643)	67.25 (13.619)	68.45 (15.159)
Median (Q1, Q3)	67.00 (58.95, 76.60)	64.25 (58.75, 77.05)	65.85 (58.75, 76.85)
Min, Max	40.0, 132.9	41.4, 106.3	40.0, 132.9
BMI (kg/m^2)			
n	40	40	80
Mean (SD)	24.20 (6.035)	23.72 (5.026)	23.96 (5.523)
Median (Q1, Q3)	23.70 (20.89, 26.06)	22.50 (19.86, 26.46)	23.24 (20.28, 26.23)
Min, Max	15.6, 53.6	14.7, 37.6	14.7, 53.6

Source: Table 14.1-5.1.
Abbreviations: BMI = body mass index; max = maximum; min = minimum; Q1 = first quartile; Q3 = third quartile.
Notes: The denominator to calculate percentages is N, the number of subjects in the Full Analysis Set within each treatment arm.
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 arm.

Baseline Disease Characteristics

Table 27 Summary of Baseline Disease Characteristics (Full Analysis Set)

	Placebo N=40	Mitapivat N=40	Total N=80
Baseline Hb (g/L)			
n	40	40	80
Mean (SD)	85.36 (8.478)	85.71 (9.901)	85.54 (9.160)
Median (Q1, Q3)	84.50 (79.83, 91.67)	86.83 (79.33, 92.33)	85.08 (79.83, 92.17)
Min, Max	64.0, 99.7	64.3, 102.3	64.0, 102.3
Baseline Hb Category, n (%)			
<85 g/L (8.5 g/dL)	21 (52.5)	19 (47.5)	40 (50.0)
≥85 g/L (8.5 g/dL)	19 (47.5)	21 (52.5)	40 (50.0)
Baseline Ferritin (ug/L)			
n	38	39	77
Mean (SD)	688.047 (605.2494)	747.862 (1116.1796)	718.343 (895.6438)
Median (Q1, Q3)	510.730 (293.180, 854.960)	382.805 (222.355, 843.710)	479.420 (252.685, 843.710)
Min, Max	76.37, 2356.95	21.36, 5890.25	21.36, 5890.25
Prior Transfusions, n (%)[1]			
0	30 (75.0)	29 (72.5)	59 (73.8)
1	7 (17.5)	8 (20.0)	15 (18.8)
2	1 (2.5)	0	1 (1.3)
3	1 (2.5)	3 (7.5)	4 (5.0)
≥4	1 (2.5)	0	1 (1.3)

	Placebo N=40	Mitapivat N=40	Total N=80
Prior Splenectomy Status, n (%)[2]			
Yes	30 (75.0)	28 (70.0)	58 (72.5)
Age at Splenectomy (yr)			
N	30	28	58
Mean (SD)	7.7 (7.48)	8.0 (6.43)	7.8 (6.93)
Median (Q1, Q3)	5.0 (3.0, 9.0)	5.0 (3.0, 12.5)	5.0 (3.0, 11.0)
Min, Max	1, 29	1, 23	1, 29
No	10 (25.0)	12 (30.0)	22 (27.5)
Prior Cholecystectomy Status, n (%)[2]			
Yes	30 (75.0)	28 (70.0)	58 (72.5)
Age at Cholecystectomy (yr)			
n	30	28	58
Mean (SD)	21.1 (15.25)	16.0 (11.42)	18.6 (13.67)
Median (Q1, Q3)	17.0 (12.0, 25.0)	14.0 (8.0, 21.5)	17.0 (9.0, 23.0)
Min, Max	4, 69	0, 56	0, 69
No	10 (25.0)	12 (30.0)	22 (27.5)
Prior Chelation Status, n (%)[3]			
Yes	10 (25.0)	5 (12.5)	15 (18.8)
No	30 (75.0)	35 (87.5)	65 (81.3)

Source: Table 14.1-6.1.
Abbreviations: eCRF = electronic case report form; Hb = hemoglobin; max = maximum; min = minimum; Q1 = first quartile; Q3 = third quartile.
Notes: The denominator used to calculate percentages is N, the number of subjects in the Full Analysis Set within each treatment arm.
For laboratory parameters, baseline is defined as the average of all screening assessments within 45 (42+3) days before randomization for subjects randomized and not dosed or before start of study treatment for subjects randomized and dosed excluding assessments collected within 61 days after a transfusion.
[1] As recorded in transfusion history eCRF.
[2] As recorded in medical/surgical history eCRF.
[3] "Yes" if a subject has received chelation therapy within 52 weeks (364 days) before first dose of study treatment.

Table 28 Summary of Bone Baseline Characteristics (Full Analysis Set)

	Placebo N=40	Mitapivat N=40	Total N=80
Dual-energy X-ray Absorptiometry (DXA) Scan			
Femoral Total			
Bone Mineral Density (g/cm^2)			
N	40	39	79
Mean (SD)	0.9026 (0.15975)	0.8639 (0.15481)	0.8835 (0.15753)
Median (Q1, Q3)	0.8780 (0.8090, 1.0075)	0.8230 (0.7650, 1.0270)	0.8500 (0.7790, 1.0140)
Min, Max	0.609, 1.264	0.601, 1.170	0.601, 1.264
T-Score			
N	40	39	79
Mean (SD)	-0.792 (1.0982)	-1.120 (1.0809)	-0.954 (1.0952)
Median (Q1, Q3)	-1.000 (-1.520, -0.375)	-1.100 (-1.910, -0.500)	-1.020 (-1.640, -0.450)
Min, Max	-3.00, 2.10	-3.43, 1.29	-3.43, 2.10
T-Score Category, n (%)			
≤-2.5	2 (5.0)	3 (7.5)	5 (6.3)
>-2.5 - <-1.0	18 (45.0)	18 (45.0)	36 (45.0)
≥-1.0	20 (50.0)	18 (45.0)	38 (47.5)
Missing	0	1 (2.5)	1 (1.3)
Z-Score			
N	40	39	79
Mean (SD)	-0.527 (1.1414)	-0.914 (1.0451)	-0.718 (1.1052)
Median (Q1, Q3)	-0.590 (-1.200, -0.280)	-0.930 (-1.770, -0.370)	-0.680 (-1.380, -0.330)
Min, Max	-2.58, 2.22	-3.04, 1.29	-3.04, 2.22
Adjusted Spine			
Bone Mineral Density (g/cm^2)			
n	40	39	79
Mean (SD)	0.9861 (0.16219)	0.9232 (0.14698)	0.9550 (0.15710)
Median (Q1, Q3)	0.9630 (0.8490, 1.0840)	0.9170 (0.8060, 1.0260)	0.9420 (0.8390, 1.0350)
Min, Max	0.779, 1.413	0.649, 1.328	0.649, 1.413

	Placebo N=40	Mitapivat N=40	Total N=80
T-Score			
n	40	39	79
Mean (SD)	-1.135 (1.1528)	-1.776 (1.1036)	-1.451 (1.1670)
Median (Q1, Q3)	-1.530 (-2.015, -0.215)	-1.690 (-2.420, -1.180)	-1.670 (-2.190, -0.710)
Min, Max	-3.00, 1.94	-3.78, 1.23	-3.78, 1.94
T-Score Category, n (%)			
≤-2.5	2 (5.0)	9 (22.5)	11 (13.8)
>-2.5 - <-1.0	23 (57.5)	23 (57.5)	46 (57.5)
≥-1.0	15 (37.5)	7 (17.5)	22 (27.5)
Missing	0	1 (2.5)	1 (1.3)
Z-Score			
n	40	39	79
Mean (SD)	-0.820 (1.3161)	-1.532 (1.1817)	-1.171 (1.2941)
Median (Q1, Q3)	-1.090 (-1.790, 0.010)	-1.610 (-2.310, -0.730)	-1.430 (-2.060, -0.450)
Min, Max	-3.00, 2.58	-3.78, 1.23	-3.78, 2.58

Source: Table 14.1-6.1.
Abbreviations: max = maximum; min = minimum; Q1 = first quartile; Q3 = third quartile; SD = standard deviation
Notes: The denominator used to calculate percentages is N, the number of subjects in the Full Analysis Set within each treatment arm.
For DXA, baseline is defined as the last assessment before randomization for subjects randomized and not dosed or the last assessment before start of study treatment for subjects randomized and dosed.

Table 29 Subject Disposition – Stratification Factors at Randomisation (Full Analysis Set)

	Placebo N=40 n (%)	Mitapivat N=40 n (%)	Total N=80 n (%)
Source: IXRS			
Average of screening Hb concentrations			
Hb <85 g/L (8.5 g/dL)	18 (45.0)	17 (42.5)	35 (43.8)
Hb ≥85 g/L (8.5 g/dL)	22 (55.0)	23 (57.5)	45 (56.3)
PKLR gene mutation category			
Missense/Missense	27 (67.5)	28 (70.0)	55 (68.8)
Missense/Non-missense	13 (32.5)	12 (30.0)	25 (31.3)
Average of screening Hb concentrations and *PKLR* gene mutation category			
Hb <85 g/L (8.5 g/dL) & Missense/Missense	12 (30.0)	12 (30.0)	24 (30.0)
Hb <85 g/L (8.5 g/dL) & Missense/Non-missense	6 (15.0)	5 (12.5)	11 (13.8)
Hb ≥85 g/L (8.5 g/dL) & Missense/Missense	15 (37.5)	16 (40.0)	31 (38.8)
Hb ≥85 g/L (8.5 g/dL) & Missense/Non-missense	7 (17.5)	7 (17.5)	14 (17.5)

Source: Table 14.1-3.2.
Abbreviations: Hb = hemoglobin; IXRS = interactive response technology.
Notes: The denominator used to calculate percentages is N, the number of subjects in the Full Analysis Set within each treatment arm.
The randomization was stratified by the average of screening Hb concentrations (<85 g/L [8.5g/dL] vs ≥85 g/L [8.5 g/dL]) and the *PKLR* gene mutation category (missense/missense vs missense/nonmissense).

Numbers analysed

A total of 80 subjects were randomised in the study and included in the FAS, with 40 in the mitapivat arm and 40 in the placebo arm. Of the subjects randomised, 62 (77.5%) were included in the PPS (82.5% in the mitapivat arm and 72.5% in the placebo arm). One subject who was randomised to the placebo

arm did not receive treatment and was lost to follow-up and therefore not included in the Safety Analysis Set (N=79).

Outcomes and estimation

Primary Endpoint: Haemoglobin Response

Table 30 Sensitivity Analysis of Haemoglobin Response - Mantel-Haenszel Stratum Weighted Method (Full Analysis Set)

	Placebo N=40	Mitapivat N=40
Hb responders, n(%)	0	16 (40.0)
Adjusted difference in response rate (Mitapivat vs Placebo), %		39.3
95% CI		(24.1, 54.6)
2-sided p-value		<0.0001

Source: Table 14.2-1.1.1.
Abbreviations: Hb = hemoglobin.
Notes: Hb responders: subjects who obtained ≥15 g/L (1.5 g/dL) increase in Hb concentration from baseline at 2 or more scheduled assessments at Weeks 16, 20, and 24 during the Fixed Dose Period.
The estimated adjusted difference in response rate, 95% CI, and p-value are based on the Mantel-Haenszel stratum weighted method adjusting for the randomization stratification factors.

Table 14.2-1.4
Sensitivity Analysis of Hemoglobin Response - Mantel-Haenszel Stratum Weighted Method
Per-Protocol Set

	Placebo N=29	Mitapivat N=33
Hb responders, n(%)	0	16 (48.5)
Adjusted difference in response rate (Mitapivat vs Placebo), %		47.7
95% CI		(30.1, 65.3)
2-sided p-value		<0.0001

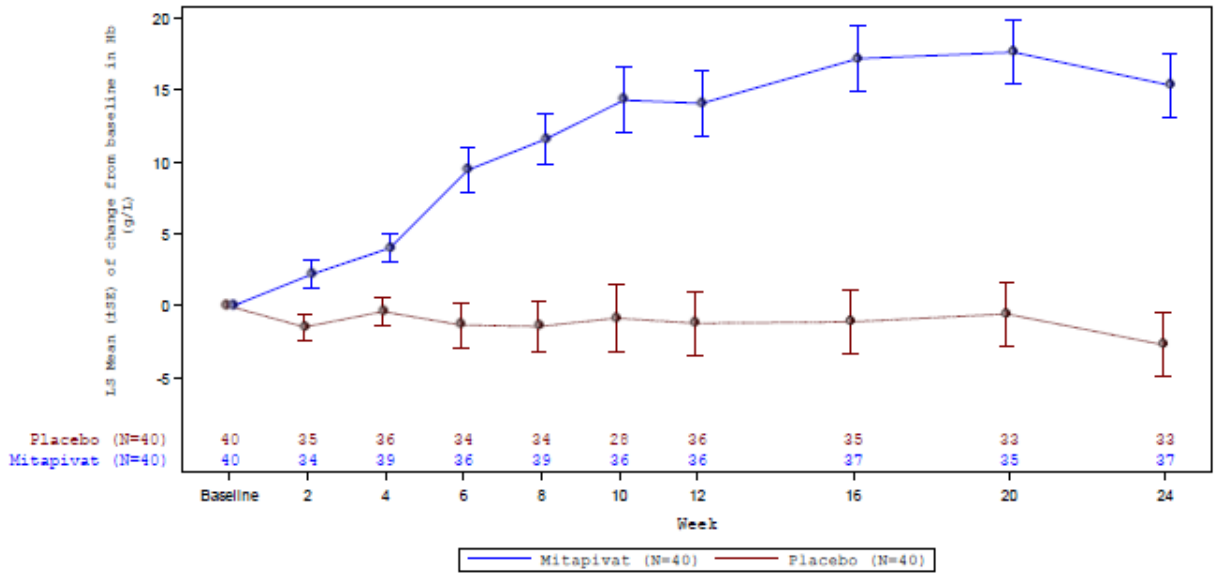
Key Secondary Endpoint: Average Haemoglobin Change From Baseline at Weeks 16, 20, and 24

Table 31 Analysis of Average Change from Baseline in Haemoglobin at Weeks 16, 20, and 24 by MMRM (Full Analysis Set)

Hemoglobin (g/L)		
Visit	Placebo N=40	Mitapivat N=40
Baseline		
n	40	40
Mean (SD)	85.36 (8.478)	85.71 (9.901)
Average of Weeks 16, 20, and 24		
Change from baseline		
LS Mean (SE)	-1.48 (2.082)	16.73 (2.075)
95% CI	(-5.63, 2.67)	(12.60, 20.86)
Difference in LS mean (SE) (Mitapivat-Placebo)		18.21 (2.913)
95% CI		(12.41, 24.01)
2-sided p-value		<0.0001

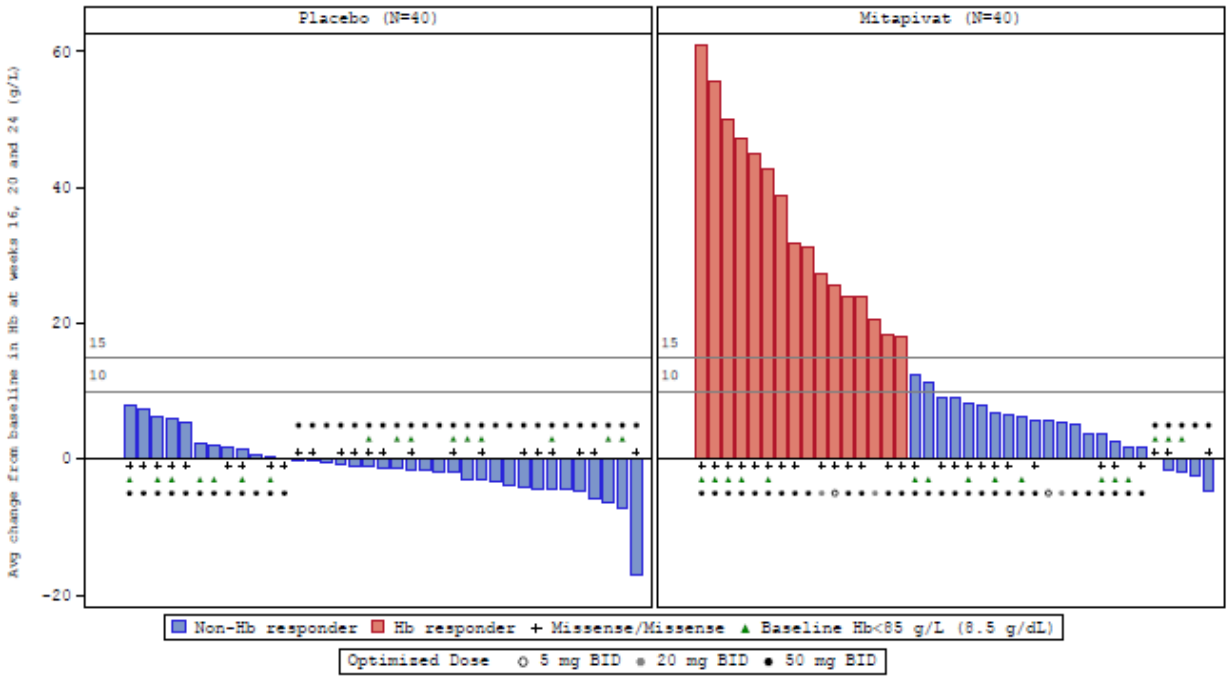
Source: Table 14.2-2.1.
Abbreviations: LS = least square; MMRM = mixed-effect model repeated measure.
Notes: The estimates, 95% CIs, and p-value were based on the MMRM method, which included change from baseline as the dependent variable, baseline as a covariate, and treatment arm, visit, treatment-by-visit interaction, and the randomization stratification factors as fixed factors and subject as the random effect. All scheduled visits were included in the model.
Baseline was defined as the average of all screening assessments within 45 (42+3) days before randomization for subjects randomized and not dosed or before start of study treatment for subjects randomized and dosed.
Assessments collected within 61 days after a transfusion were excluded from the baseline derivation.

Figure 13 Least Square Mean (±SE) of Change From Baseline in Haemoglobin Over Time (Full Analysis Set)



Source: Figure 14.2-1.1.
Abbreviations: Hb = hemoglobin; LS = least square.
Notes: Baseline was defined as the average of all screening assessments within 45 (42+3) days before randomization for subjects randomized and not dosed or before start of study treatment for subjects randomized and dosed.
Assessments collected within 61 days after a transfusion were excluded from the baseline derivation.

Figure 4: Waterfall Plot of Average Change From Baseline in Hemoglobin at Weeks 16, 20, and 24 (Full Analysis Set)



Source: Figure 14.2-2.1.
Abbreviations: Avg = average; BID = twice daily; Hb = hemoglobin.
Notes: Baseline was defined as the average of all screening assessments within 45 (42+3) days before randomization for subjects randomized and not dosed or before start of study treatment for subjects randomized and dosed.
Assessments collected within 61 days after a transfusion were excluded from the baseline derivation.

Haemolysis Markers - Average Change from Baseline

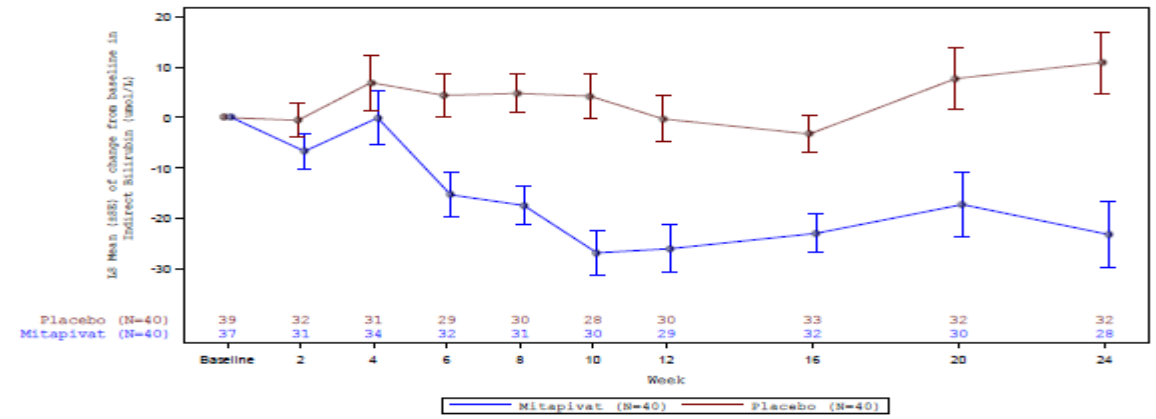
Table 32 Analysis of Average Change from Baseline in Haemolysis Markers at Weeks 16, 20, and 24 by MMRM (Full Analysis Set)

Visit	Placebo N=40	Mitapivat N=40
Indirect Bilirubin (µmol/L)		
Baseline		
n	39	37
Mean (SD)	89.14 (61.793)	81.81 (61.317)
Average of weeks 16, 20 and 24		
Change from baseline		
LS Mean (SE)	5.10 (4.061)	-21.16 (4.228)
95% CI	(-3.00, 13.21)	(-29.59, -12.72)
Difference in LS mean (SE) (Mitapivat-Placebo)		-26.26 (5.788)
95% CI		(-37.82, -14.70)
2-sided p-value		<0.0001
Lactate Dehydrogenase (U/L)		
Baseline		
n	40	39
Mean (SD)	260.01 (140.232)	347.56 (276.029)
Average of Weeks 16, 20 and 24		
Change from baseline		
LS Mean (SE)	-21.18 (16.040)	-91.99 (16.222)
95% CI	(-53.30, 10.94)	(-124.47, -59.50)
Difference in LS mean (SE) (Mitapivat-Placebo)		-70.81 (22.488)
95% CI		(-115.88, -25.74)
2-sided p-value		0.0027
Haptoglobin (g/L)		
Baseline		
N	40	40
Mean (SD)	0.083 (0.1375)	0.082 (0.1072)
Average of Weeks 16, 20 and 24		
Change from baseline		
LS Mean (SE)	0.012 (0.0412)	0.169 (0.0408)
95% CI	(-0.070, 0.094)	(0.088, 0.251)
Difference in LS mean (SE) (Mitapivat-Placebo)		0.158 (0.0578)
95% CI		(0.043, 0.273)
2-sided p-value		0.0079

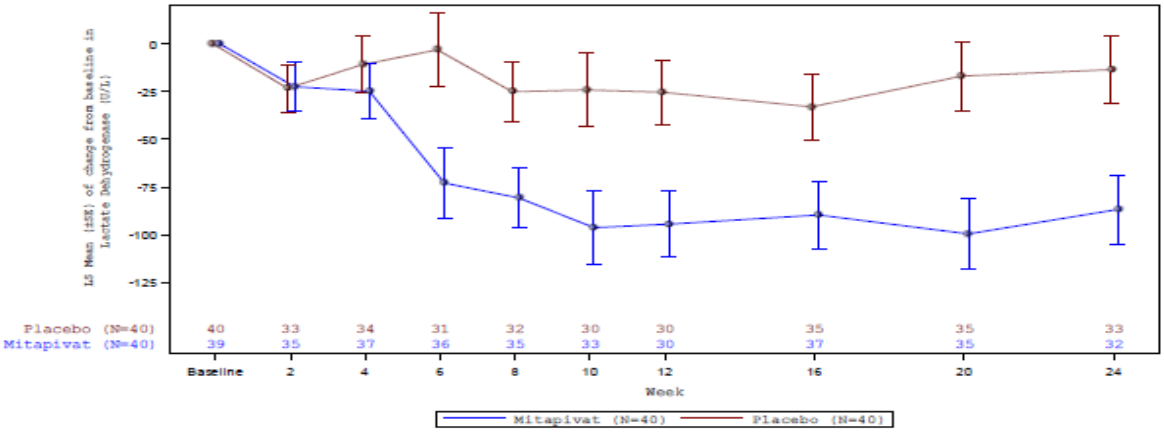
Source: Table 14.2-5.1.
Abbreviations: LS = least square; MMRM = mixed-effect model repeated measure.
Notes: The estimates, 95% CIs, and p-values were based on the MMRM method, which included change from baseline as the dependent variable, baseline as a covariate, and treatment arm, visit, treatment-by-visit interaction, and the randomization stratification factors as fixed factors and subject as the random effect. All scheduled visits were included in the model. Baseline was defined as the average of all screening assessments within 45 (42+3) days before randomization for subjects randomized and not dosed or before start of study treatment for subjects randomized and dosed. Assessments collected within 61 days after a transfusion are excluded from the baseline derivation.

Figure 14 Least Square Mean (±SE) Change from Baseline in Haemolysis Markers over Time (Full Analysis Set)

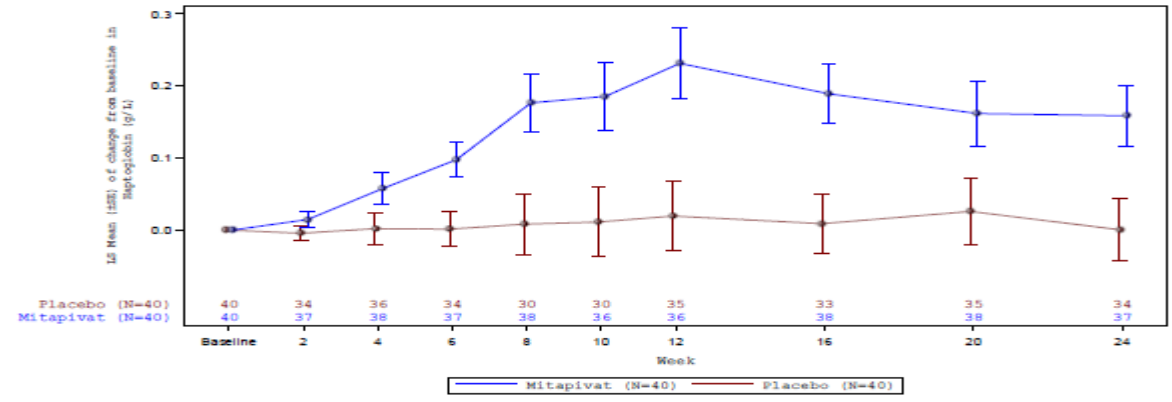
Indirect Bilirubin (µmol/L)



Lactate Dehydrogenase (U/L)



Haptoglobin (g/L)



Source: Figure 14.2-4.1.
Abbreviations: LS = least square.
Notes: Baseline was defined as the average of all screening assessments within 45 (42+3) days before randomization for subjects randomized and not dosed or before start of study treatment for subjects randomized and dosed.
Assessments collected within 61 days after a transfusion were excluded from the baseline derivation.

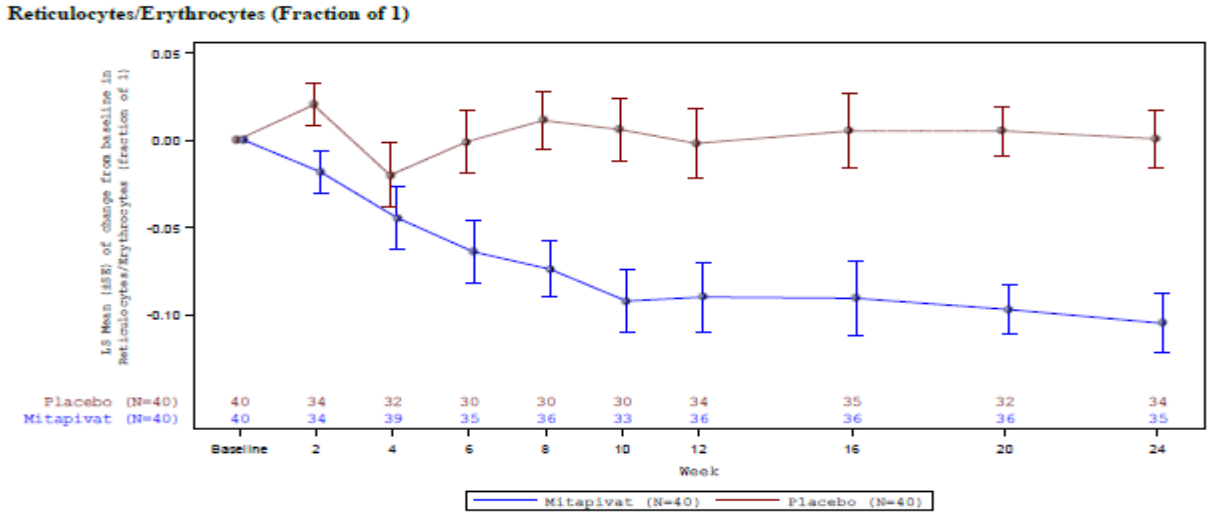
Haematopoietic Markers - Average Change from Baseline

Table 33 Analysis of Average Change From Baseline Reticulocyte Percentage at Weeks 16, 20, and 24 by MMRM (Full Analysis Set)

Reticulocytes/Erythrocytes (Fraction of 1)		
Visit	**Placebo N=40**	**Mitapivat N=40**
Baseline		
n	40	40
Mean (SD)	0.4007 (0.22202)	0.3706 (0.24085)
Average of Weeks 16, 20, and 24		
Change from baseline		
LS Mean (SE)	0.0038 (0.01390)	-0.0973 (0.01401)
95% CI	(-0.0239, 0.0315)	(-0.1252, -0.0694)
Difference in LS mean (SE) (Mitapivat-Placebo)		-0.1011 (0.01904)
95% CI		(-0.1391, -0.0632)
2-sided p-value		<0.0001

Source: Table 14.2-6.1.
Abbreviations: LS = least square; SD = standard deviation; SE = standard error; MMRM = mixed-effect model repeated measure.
Notes: The estimates, 95% CIs, and p-value were based on the MMRM method, which included change from baseline as the dependent variable, baseline as a covariate, and treatment arm, visit, treatment-by-visit interaction, and the randomization stratification factors as fixed factors and subject as the random effect. All scheduled visits were included in the model. Baseline was defined as the average of all screening assessments within 45 (42+3) days before randomization for subjects randomized and not dosed or before start of study treatment for subjects randomized and dosed. Assessments collected within 61 days after a transfusion are excluded from the baseline derivation.

Figure 15 Least Square Mean (±SE) of Change from Baseline in Reticulocyte Percentage over Time (Full Analysis Set)



Source: Figure 14.2-5.1.
Abbreviations: LS = least square.
Notes: Baseline was defined as the average of all screening assessments within 45 (42+3) days before randomization for subjects randomized and not dosed or before start of study treatment for subjects randomized and dosed.
Assessments collected within 61 days after a transfusion were excluded from the baseline derivation.

Efficacy Analysis of PKDD and PKDIA

Table 34 MMRM Analysis of Change from Baseline in Pyruvate Kinase Deficiency Diary Weekly Mean Score at Week 24 (Full Analysis Set)

Visit	Placebo N=40	Mitapivat N=40
Baseline		
n	36	37
Mean (SD)	47.04 (8.103)	50.45 (7.315)
Week 24		
Change from baseline		
LS mean (SE)	-2.05 (0.976)	-5.16 (0.955)
95% CI	(-4.00, -0.11)	(-7.06, -3.26)
Difference in LS mean (SE) (Mitapivat-Placebo)		-3.11 (1.352)
95% CI		(-5.80, -0.41)
2-sided p-value		0.0247

Source: Table 14.2-7.1.
Abbreviations: LS = least square; MMRM = mixed-effect model repeated measure.
Notes: The estimates, 95% CIs, and p-values are based on the MMRM method, which includes change from baseline as the dependent variable, baseline as a covariate, and treatment arm, visit, treatment-by-visit interaction, and the randomization stratification factors as fixed factors and subject as the random effect.
Baseline of weekly mean score is defined as the average of daily scores collected within 7 days before randomization for subjects randomized and not dosed or before start of study treatment for subjects randomized and dosed.

Table 35 MMRM Analysis of Change from Baseline in Pyruvate Kinase Deficiency Impact Assessment Score at Week 24 (Full Analysis Set)

Visit	Placebo N=40	Mitapivat N=40
Baseline		
n	39	39
Mean (SD)	48.5 (9.15)	49.2 (9.00)
Week 24		
Change from baseline		
LS mean (SE)	-1.39 (1.157)	-4.65 (1.123)
95% CI	(-3.70, 0.91)	(-6.88, -2.41)
Difference in LS mean (SE) (Mitapivat-Placebo)		-3.25 (1.574)
95% CI		(-6.39, -0.12)
2-sided p-value		0.0421

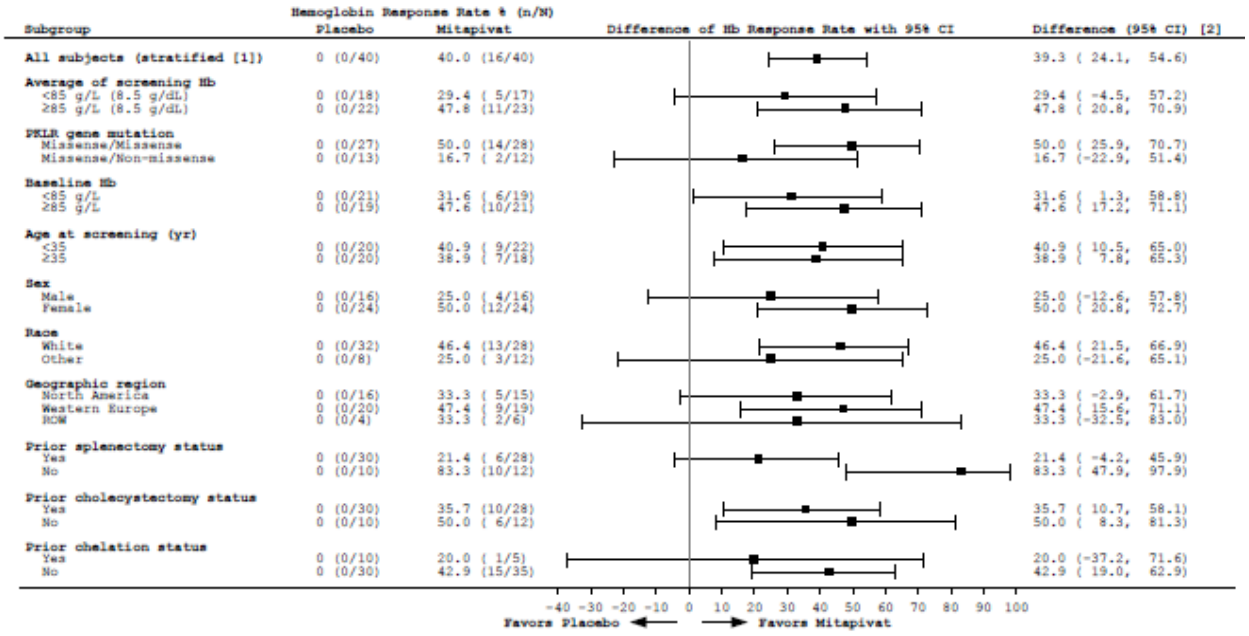
Source: Table 14.2-8.1.
Abbreviations: LS = least square; MMRM = mixed-effect model repeated measure.
Notes: The estimates, 95% CIs, and p-values are based on the MMRM method, which includes change from baseline as the dependent variable, baseline as a covariate, and treatment arm, visit, treatment-by-visit interaction, and the randomization stratification factors as fixed factors and subject as the random effect.
Baseline of weekly mean score is defined as the average of daily scores collected within 7 days before randomization for subjects randomized and not dosed or before start of study treatment for subjects randomized and dosed.

Table 36 Summary of On-Treatment Transfusion Episodes

	Placebo N=39	Mitapivat N=40
Subjects with transfusion episodes, n (%)		
0	32 (82.1)	38 (95.0)
1	5 (12.8)	1 (2.5)
2	1 (2.6)	0
≥3	1 (2.6)	1 (2.5)
Number of transfusion episodes		
n	7	2
Mean (SD)	1.4 (0.79)	2.5 (2.12)
Median (Q1, Q3)	1.0 (1.0, 2.0)	2.5 (1.0, 4.0)
Min, Max	1, 3	1, 4
Reason for transfusion, n (%)		
Adverse Event	4 (10.3)	0
Clinically significant or poorly tolerated anemia	5 (12.8)	2 (5.0)

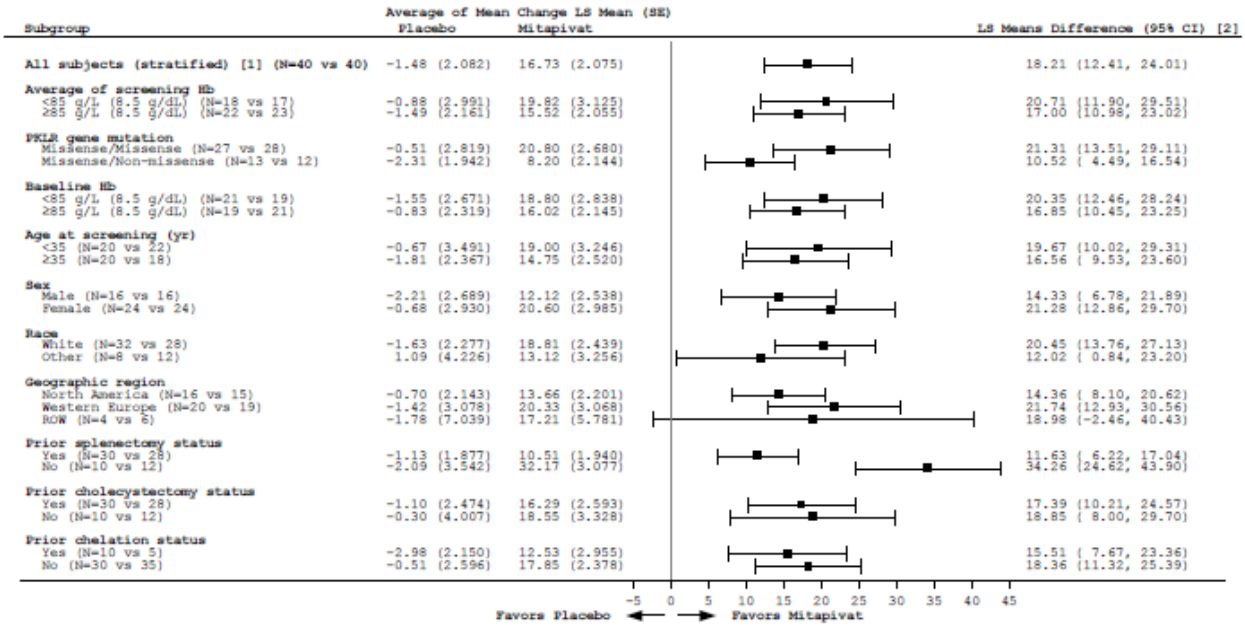
Ancillary analyses

Figure 16 Forest Plot for Difference in Haemoglobin Response Rates (Full Analysis Set)



Source: Figure 14.2-3.1.
Abbreviations: Hb = hemoglobin; ROW = rest of world; IXRS = interactive response technology.
Notes: N is the number of subjects in the Full Analysis Set within each subgroup category and treatment arm.
Prespecified subgroups with ≤10% of the subjects in the FAS were pooled (race: Asian and Other were pooled).
[1] Stratified by the average of screening Hb concentrations (<85 g/L vs ≥85 g/L) and *PKLR* gene mutation category (missense/missense vs missense/nonmissense), per IXRS.
[2] For "All Subjects," the estimate for difference and the 95% CI are based on the Mantel-Haenszel stratum weighted method adjusting for the randomization stratification factors. For subgroups, the estimates for difference and the exact 95% CIs are based on unstratified analyses.

Figure 17 Forest Plot for Average Change from Baseline in Haemoglobin at Weeks 16, 20 and 24 Full Analysis Set



N is the number of subjects in the full analysis set within each subgroup category and treatment group.
[1] Stratified by the Average of Screening Hb concentrations and PKLR gene mutation category, per IXRS
[2] For "All Subjects", the estimate for difference and the 95% CI are based on the mixed-effect model repeated measure (MMRM) method adjusting for the randomization stratification factors. For subgroups, the estimate for difference and the 95% CIs are based on unstratified analyses.
Pre-specified subgroups with ≤10% of the subjects in the full analysis set were pooled (Race: Asian and Other were pooled).

AG348-C-007

This was a Phase 3, multicentre, single-arm, open-label efficacy and safety study of orally administered mitapivat in subjects with PK deficiency who were regularly receiving blood transfusions. The study consisted of a dose optimisation period (Part 1) followed by a Fixed-Dose Period (Part 2).

Methods

Study participants

Inclusion Criteria

1. Aged 18 years or older

2. Documented clinical laboratory confirmation of PK deficiency, defined as documented presence of at least 2 mutant alleles in the PKLR gene, of which at least 1 was a missense mutation, as determined per the genotyping performed by the study central genotyping laboratory.

3. A history of a minimum of 6 transfusion episodes in the 52-week period before the date of informed consent as documented in the transfusion history of the subject, which reflected the subject's typical transfusion burden.

4. Complete records of transfusion history, defined as having the following available for the 52 weeks before the date of informed consent: (1) all the transfusion dates, (2) the number of blood units transfused for all the transfusions, and (3) Hb concentrations within 1 week before transfusion for at least 80% of the transfusions.

5. Received at least 0.8 mg of oral folic acid daily for at least 21 days before the first dose of study treatment, to be continued daily during study participation

6. Adequate organ function, as defined by:

a. Serum aspartate aminotransferase (AST) ≤2.5× the upper limit of normal (ULN) (unless the increased AST was assessed by the Investigator as due to haemolysis and/or hepatic iron deposition) and alanine aminotransferase (ALT) ≤2.5×ULN (unless the increased ALT was assessed by the Investigator as due to hepatic iron deposition).

b. Normal or elevated levels of serum bilirubin. In subjects with serum bilirubin >ULN, the elevation was not to be associated with choledocholithiasis, cholecystitis, biliary obstruction, or hepatocellular disease. Elevated bilirubin attributed to haemolysis with or without Gilbert syndrome was not exclusionary.

c. Estimated glomerular filtration rate (GFR) ≥60 mL/min/1.73 m2, measured GFR ≥60 mL/min, or calculated creatinine clearance (Cockcroft-Gault) ≥60 mL/min

d. Absolute neutrophil count ≥1.0 × 109/L

e. Platelet count ≥100 × 109/L in the absence of a spleen, or platelet count ≥50 × 109/L in the presence of a spleen and in the absence of any other cause of thrombocytopenia.

f. Activated partial thromboplastin time and international normalised ratio ≤1.25 ×ULN, unless the subject was receiving therapeutic anticoagulants.

Exclusion Criteria

1. Homozygous for the R479H mutation or have 2 non-missense mutations, without the presence of another missense mutation, in the PKLR gene as determined per the genotyping performed by the study central genotyping laboratory.

2. A significant medical condition that confers an unacceptable risk to participating in the study and/or that could confound the interpretation of the study data.

3.- A history of transfusions occurring on average more frequently than once every 3 weeks during the 52 weeks prior to signing informed consent.

4. A splenectomy scheduled during the study drug period or have undergone splenectomy within 12 months prior to signing informed consent.

5. Prior bone marrow or stem cell transplant.

Treatments

Part 1: Dose Optimisation Period of the Study

During Part 1 of the study, all enrolled subjects will begin treatment with AG-348 at a dose level of 5 mg BID on Day 1, which is scheduled to occur 2 to 7 days after a subject's Transfusion 0. Each subject will undergo individual dose optimisation to identify the highest appropriate dose for the subject with 2 potential sequential dose level increases (ie, from 5 to 20 mg BID and from 20 to 50 mg BID).

Part 2: Fixed-Dose Period of the Study

In Part 2 of the study, subjects will receive their optimised dose of AG-348 for 24 weeks. No dose adjustment should be performed during Part 2 of the study, unless a safety event experienced by a subject requires a dose reduction, in the opinion of the Investigator.

Objectives

The primary objective of the study was to evaluate the efficacy of treatment with mitapivat, as assessed by the reduction in transfusion burden.

The secondary objective of this study was to evaluate the safety of treatment with mitapivat.

The exploratory objectives of the study were the following:

• To determine the effect of mitapivat on markers of haemolysis, erythropoietic activity, and other indicators of clinical activity

• To determine the effect of mitapivat on markers of iron metabolism and indicators of iron overload

• To determine the effect of mitapivat on health-related quality of life (HRQOL), as determined using patient-reported outcomes (PROs)

Outcomes/endpoints

Primary Endpoint

The primary endpoint of this study was the proportion of subjects who achieved a reduction in transfusion burden, defined as a ≥33% reduction in the number of RBC units transfused during the 24 weeks of Part 2 compared with the historical transfusion burden standardised to 24 weeks (Standardised Control Period).

Secondary endpoints

• Annualised total number of RBC units transfused during the study (both Part 1 and Part 2) compared with the historical transfusion burden.

• Number of transfusion episodes during Part 2 compared with the Standardised Control Period.

• Proportion of subjects who became transfusion-free, defined as 0 transfusions administered during Part 2.

• Proportion of subjects who achieved Hb concentrations in the normal range at least once, 8 weeks or more after a transfusion in Part 2.

Exploratory endpoints

• Change from baseline in the following markers of haemolysis: bilirubin, lactate dehydrogenase (LDH), and haptoglobin levels, as measured by safety laboratory assessments.

• Change from baseline in markers of erythropoietic activity.

• Change from baseline in markers of iron metabolism and indicators of iron overload.

• Change from baseline over time in HRQOL scores (ie, Pyruvate Kinase Deficiency Impact Assessment [PKDIA], Pyruvate Kinase Deficiency Diary [PKDD], European Quality of Life Five-Dimensional Descriptive System [EQ-5D-5L]).

Sample size

Due to the rarity of the disease and the small patient population, the sample size was largely driven by feasibility, and the study was planned to enrol a minimum of 20, with up to 40, subjects. The power to reject H0 at a 1-sided α=0.025 for different sample sizes and under different assumptions for the true TRR rate is shown in the following table.

Power according to true TRR rate and sample size:

Sample Size	Target Response Rate (null=10%)		
	25%	30%	35%
20	0.38	0.58	0.75

30	0.48	0.71	0.87
40	0.71	0.90	0.97

There was no interim analysis planned for this study.

Randomisation and blinding (masking)

Not applicable, this was a single-arm open-label study.

Statistical methods

Analysis populations

The **Full Analysis Set (FAS)** included all subjects who received at least 1 dose of study treatment.

The **Safety Analysis Set** included all subjects who received at least 1 dose of study treatment. In this nonrandomised study, the FAS and the Safety Analysis Set were identical.

The **Per-Protocol Set (PPS)** was a subset of the FAS and included all subjects who completed 12 weeks of treatment in the Fixed-Dose Period (ie, end date of the Fixed-Dose Period – start date of the Fixed-Dose Period + 1 ≥84).

Results

Participant flow

Table 37 Subject Disposition (Full Analysis Set)

	Total N=27 n (%)
Disposition: end of treatment	
Discontinued	6 (22.2)
Reason for discontinuation	
Withdrawal by subject	6 (22.2)
Completed	21 (77.8)
Ongoing	0
Disposition: end of study	
Discontinued	7 (25.9)
Reason for discontinuation	
Withdrawal by subject	6 (22.2)
Lost to follow-up	1 (3.7)
Completed	20 (74.1)
Ongoing	0

Source: Table 14.1-2.2.
Note: The denominator used to calculate percentages is N, the number of subjects in the Full Analysis Set.

Recruitment

A total of 20 study sites participated in this study. The first subject was enrolled on 26 June 2018 and the last subject completed on 12 November 2020; all subjects who remain on study during Part 2 through the Week 40 Visit may be eligible for an open-label extension study, in which all subjects will receive mitapivat.

Conduct of the study

The original protocol (28 September 2017) was amended twice.

Baseline data

Demographic Characteristics

Table 38 Summary of Demographic Characteristics and Physical Measurements at Baseline (Full Analysis Set)

	Total N=27
Age (yr)	
n	27
Mean (SD)	36.6 (13.89)
Median (Q1, Q3)	36.0 (23.0, 47.0)
Min, max	18, 68
Age category 1 (yr), n (%)	
<65	26 (96.3)
≥65	1 (3.7)
Age category 2 (yr), n (%)	
<35	13 (48.1)
≥35	14 (51.9)
Sex, n (%)	
Male	7 (25.9)
Female	20 (74.1)
Childbearing potential[1]	
Yes	15 (75.0)
No	5 (25.0)
Ethnicity, n (%)	
Hispanic or Latino	0
Not Hispanic or Latino	20 (74.1)
Not reported	7 (25.9)
Race, n (%)	
White	20 (74.1)
Asian	3 (11.1)
American Indian or Alaska Native	0
Black or African American	0

	Total N=27
Native Hawaiian or other Pacific Islander	0
Other	0
Not reported	4 (14.8)
Height (cm)	
n	27
Mean (SD)	166.34 (10.886)
Median (Q1, Q3)	165.00 (159.00, 175.00)
Min, max	145.0, 185.0
Weight (kg)	
n	27
Mean (SD)	65.77 (13.430)
Median (Q1, Q3)	67.10 (51.50, 73.50)
Min, max	43.1, 91.8
BMI (kg/m^2)	
n	27
Mean (SD)	23.71 (4.048)
Median (Q1, Q3)	23.64 (20.15, 26.82)
Min, max	17.5, 32.4

Source: Table 14.1-5.1.
Abbreviations: BMI = body mass index; max = maximum; min = minimum; Q1 = first quartile; Q3 = third quartile; SD = standard deviation.
Notes: The denominator used to calculate percentages is N, the number of subjects in the Full Analysis Set.
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.

Baseline Disease Characteristics

Table 39 Summary of Baseline Disease Characteristics (Full Analysis Set)

	Total N=27
Baseline Hb (g/L)	
n	27
Mean (SD)	91.5 (9.84)
Median (Q1, Q3)	91.0 (83.0, 99.0)
Min, max	74, 109
Baseline ferritin (ug/L)	
n	18
Mean (SD)	1153.671 (1221.4081)
Median (Q1, Q3)	748.445 (447.900, 1323.620)
Min, max	163.42, 5357.04
Prior splenectomy status, n (%)[1]	
Yes	21 (77.8)
Age at splenectomy (yr)	
n	21
Mean (SD)	7.0 (4.40)
Median (Q1, Q3)	6.0 (4.0, 9.0)
Min, max	2, 16
No	6 (22.2)
Prior cholecystectomy status, n (%)[1]	
Yes	23 (85.2)
Age at cholecystectomy (yr)	
n	23
Mean (SD)	16.5 (9.22)
Median (Q1, Q3)	14.0 (10.0, 20.0)
Min, max	3, 41
No	4 (14.8)
Prior chelation status, n (%)[2]	
Yes	24 (88.9)
No	3 (11.1)
UGT1A1 genotype, n (%)	
TA6/TA6	15 (55.6)
TA6/TA7	5 (18.5)
TA7/TA7	5 (18.5)
Missing	2 (7.4)
PKR genotype, n(%)	
Missense/missense	20 (74.1)
Missense/nonmissense	7 (25.9)

Source: Table 14.1-6.1.
Abbreviations: Hb = hemoglobin; max = maximum; min = minimum; Q1 = first quartile; Q3 = third quartile; SD = standard deviation.
Notes: The denominator used to calculate percentages is N, the number of subjects in the Full Analysis Set.
Baseline for Hb and ferritin is the last nonmissing assessment before Transfusion 0 (last transfusion 2-7 days before start of study treatment), and the last nonmissing assessment before start of study treatment for other parameters.
One subject's Transfusion 0 (the most recent transfusion occurring in the Screening Period 2-7 days before the start of study treatment on Day 1) occurred 14 days before the start of study treatment, and baseline Hb and ferritin were the last assessment before Transfusion 0.
Ferritin values reported as >1,500 µg/L were excluded from the baseline summaries.
[1] As recorded in medical/surgical history electronic case report form.
[2] "Yes" if a subject has received chelation therapy within 52 weeks (364 days) before the first dose of study treatment.

Table 40 Summary of Bone Baseline Characteristics (Full Analysis Set)

	Total N=27
Dual-Energy X-ray Absorptiometry (DXA) Scan	
Femoral total	
Bone mineral density (g/cm^2)	
n	26
Mean (SD)	0.8694 (0.13994)
Median (Q1, Q3)	0.8645 (0.7780, 0.9570)
Min, max	0.528, 1.144
T-score	
n	26
Mean (SD)	-1.083 (0.8252)
Median (Q1, Q3)	-1.165 (-1.470, -0.410)
Min, max	-3.39, 0.30
T-score category, n (%)	
≤-2.5	1 (3.7)
>-2.5 to <-1.0	15 (55.6)
≥-1.0	10 (37.0)
Missing	1 (3.7)
Z-score	
n	26
Mean (SD)	-0.833 (0.8563)
Median (Q1, Q3)	-0.775 (-1.360, -0.280)
Min, max	-3.04, 1.05
Adjusted spine	
Bone mineral density (g/cm^2)	
n	26
Mean (SD)	0.9788 (0.17737)
Median (Q1, Q3)	0.9485 (0.8720, 1.1310)
Min, max	0.510, 1.293
T-score	
n	26
Mean (SD)	-1.371 (1.1658)
Median (Q1, Q3)	-1.305 (-1.860, -0.530)
Min, max	-4.88, 0.61
T-score category, n (%)	
≤-2.5	3 (11.1)
>-2.5 to <-1.0	14 (51.9)
≥-1.0	9 (33.3)
Missing	1 (3.7)
Z-score	
n	26
Mean (SD)	-1.080 (1.1496)
Median (Q1, Q3)	-1.025 (-1.300, -0.260)
Min, max	-4.34, 0.73

Source: Table 14.1-6.1.
Abbreviations: max = maximum; min = minimum; Q1 = first quartile; Q3 = third quartile; SD = standard deviation.
Note: The denominator used to calculate percentages is N, the number of subjects in the Full Analysis Set.

Table 41 Summary of Baseline Disease Characteristics-Prior Transfusion Full Analysis Set

	Total N=27
Transfusion history during the 52 weeks before Informed Consent	
Number of transfusion episodes	
n	27
Mean (SD)	9.7 (3.62)
Median (Q1, Q3)	9.0 (6.0, 11.0)
Min, Max	6, 17
Number of transfusion episodes standardized to 24 weeks [1]	
n	27
Mean (SD)	4.46 (1.669)
Median (Q1, Q3)	4.15 (2.77, 5.08)
Min, Max	2.8, 7.8
Number of transfusion episodes standardized to 24 weeks categories [1]	
≤6	22 (81.5)
>6	5 (18.5)
Number of RBC units transfused	
n	27
Mean (SD)	16.6 (8.63)
Median (Q1, Q3)	15.0 (11.0, 21.0)
Min, Max	6, 44
Number of RBC units transfused standardized to 24 weeks [1]	
n	27
Mean (SD)	7.68 (3.981)
Median (Q1, Q3)	6.92 (5.08, 9.69)
Min, Max	2.8, 20.3
Number of RBC units transfused standardized to 24 weeks categories [1]	
≤6	12 (44.4)
>6	15 (55.6)

	Total N=27
Transfusion history during the 52 weeks before Informed Consent	
Mean Transfusion Frequency (weeks) [2]	
n	27
Mean (SD)	6.06 (1.988)
Median (Q1, Q3)	5.78 (4.73, 8.67)
Min, Max	3.1, 8.7
Average duration between transfusions (days) [3]	
n	27
Mean (SD)	41.48 (14.712)
Median (Q1, Q3)	39.88 (32.30, 52.67)
Min, Max	19.5, 70.4
Average RBC units transfused (unit/transfusion)	
n	27
Mean (SD)	1.70 (0.488)
Median (Q1, Q3)	1.83 (1.22, 2.00)
Min, Max	1.0, 2.6
Individual transfusion trigger (Hemoglobin, g/L)	
n	27
Mean (SD)	85.5 (9.09)
Median (Q1, Q3)	85.0 (79.0, 92.0)
Min, Max	64, 101
Transfusions on or after Informed Consent and before start of study treatment	
Number of transfusion episodes	
n	27
Mean (SD)	1.6 (0.64)
Median (Q1, Q3)	1.0 (1.0, 2.0)
Min, Max	1, 3

	Total N=27
Transfusions on or after Informed Consent and before start of study treatment	
Time from last transfusion to start of study treatment (days)	
n	27
Mean (SD)	5.4 (2.31)
Median (Q1, Q3)	6.0 (4.0, 6.0)
Min, Max	2, 14
Number of RBC units transfused	
n	27
Mean (SD)	2.7 (1.86)
Median (Q1, Q3)	2.0 (1.0, 4.0)
Min, Max	1, 8
Average of RBC units transfused (unit/transfusion)	
n	27
Mean (SD)	1.60 (0.567)
Median (Q1, Q3)	2.00 (1.00, 2.00)
Min, Max	1.0, 2.7

The denominator used to calculate percentages is N, the number of subjects in the full analysis set.
Transfusions received over up to 3 consecutive days are counted as 1 episode.
[1] Value standardized to 24 weeks = value for the 52-week period×24/52.
[2] Mean transfusion frequency (weeks) = 52 weeks/number of transfusion episodes.
[3] Average duration between transfusions (days) = (last transfusion date – first transfusion date + 1 – number of transfusions) / (number of transfusions – 1).

Numbers analysed

A total of 27 subjects were treated in the study and included in the FAS and Safety Analysis Set; of these, 23 (85.2%) were included in the PPS (included all subjects who completed 12 weeks of treatment in the Fixed-Dose Period) for sensitivity analysis.

Outcomes and estimation

Primary Endpoint

Reduction in Transfusion Burden

Table 42 Summary of Transfusion Reduction Response (Full Analysis Set)

	Total N=27
Transfusion reduction responders, n (%)	10 (37.0)
95% CI	(19.4, 57.6)
1-Sided p-value	0.0002

Source: Table 14.2-1.1.1.
Abbreviations: BID = twice daily; RBC = red blood cell.
Notes: Transfusion reduction response is defined as a ≥33% reduction in total number of RBC units transfused during the Fixed-Dose Period (on-study transfusion burden) standardized to 24 weeks compared with the historical transfusion burden standardized to 24 weeks.
Transfusion reduction responders: subjects who had ≥33% reduction in the number of RBC units transfused during the Fixed Dose Period standardized to 24 weeks compared with the historical number of RBC units transfused standardized to 24 weeks.
CI is based on Clopper-Pearson method.
P-value associated with the test of H0: transfusion reduction response rate ≤10% vs H1: transfusion reduction response rate >10%.

Table 43 Summary of Transfusion Reduction Response Per-Protocol Set

	50 mg BID N=22	20 mg BID N=1	5 mg BID N=0	Total N=23
Transfusion reduction responders, n (%)	9 (40.9)	1 (100)	0	10 (43.5)
95% CI	(20.7, 63.6)	(2.5, 100)		(23.2, 65.5)

Table 44 Summary of RBC Units Transfused Standardised to 24 Weeks (Full Analysis Set)

	Total N=27
Historical RBC units transfused	
n	27
Mean (SD)	7.68 (3.981)
Median (Q1, Q3)	6.92 (5.08, 9.69)
Min, max	2.8, 20.3
RBC units transfused during Fixed-Dose Period	
n	26
Mean (SD)	5.40 (5.739)
Median (Q1, Q3)	4.49 (0.00, 7.81)
Min, max	0.0, 23.7
Reduction from historical RBC units transfused	
n	26
Mean (SD)	2.12 (3.260)
Median (Q1, Q3)	1.57 (0.11, 4.62)
Min, max	-3.5, 8.8
Percent reduction from historical RBC units transfused	
n	26
Mean (SD)	37.09 (46.804)
Median (Q1, Q3)	18.75 (2.10, 100.00)
Min, max	-46.8, 100.0
Percent reduction from historical RBC units category, n (%)	
<0	6 (22.2)
≥0 to <20%	8 (29.6)
≥20% to <33%	1 (3.7)
≥33% to <50%	1 (3.7)
≥50%	10 (37.0)
Not evaluable	1 (3.7)

Source: Table 14.2-1.3.

Table 45 Summary of Transfusion Compliance Full Analysis Set

	Total N=27
Subjects who missed at least one transfusion after reaching individual TT, n(%) [1]	21 (77.8)
≥2	18 (66.7)
≥3	11 (40.7)
≥4	10 (37.0)
Reason for not transfusing when reaching individual TT, n(%) [1]	
Clinically Asymptomatic	7 (25.9)
Scheduling Challenges	7 (25.9)
Patient Decision	5 (18.5)
Waiting for Additional Assessments	4 (14.8)
Other	6 (22.2)
Number of transfusions missed after reaching individual TT	
n	21
Mean (SD)	3.9 (2.48)
Median (Q1, Q3)	3.0 (2.0, 5.0)
Min, Max	1, 9
Subjects who received a transfusion without reaching individual TT, n(%)	4 (14.8)
Reason for transfusion without reaching individual TT	
Adverse Event	4 (14.8)

Secondary Endpoints

Subjects Who Became Transfusion-Free

Table 46 Summary of Transfusion-Free Responders (Full Analysis Set)

	Total N=27
Transfusion-free responders, n (%)	6 (22.2)
95% CI	(8.6, 42.3)

Source: Table 14.2-4.1.
Notes: Transfusion-free responders: subjects who are transfusion-free in the Fixed-Dose Period.
CI is based on Clopper-Pearson method.

Annualised Total Number of RBC Units Transfused Compared With Historical Transfusion Burden

Table 47 Summary of Annualised RBC Units Transfused (Full Analysis Set)

	Total N=27
Annualized historical RBC units transfused[1]	
n	27
Mean (SD)	16.63 (8.625)
Median (Q1, Q3)	15.00 (11.00, 21.00)
Min, max	6.0, 44.0
Annualized total number of RBC units transfused (up to EOS)[2]	
n	27
Mean (SD)	11.38 (10.779)
Median (Q1, Q3)	8.74 (2.52, 18.48)
Min, max	0.0, 45.0
Reduction from annualized historical RBC units transfused (up to EOS)[2]	
n	27
Mean (SD)	5.25 (5.652)
Median (Q1, Q3)	3.42 (1.55, 9.71)
Min, max	-2.5, 18.1
Percent reduction from annualized historical RBC units transfused (up to EOS)[2]	
n	27
Mean (SD)	38.56 (38.846)
Median (Q1, Q3)	26.46 (10.54, 78.54)
Min, max	-16.1, 100.0
Percent reduction from annualized historical RBC units transfused category (up to EOS)[2], n (%)	
<0	4 (14.8)
≥0 to <20%	9 (33.3)
≥20% to <33%	2 (7.4)
≥33% to <50%	2 (7.4)
≥50%	10 (37.0)

	Total N=27
Annualized total number of RBC units transfused (up to end of Fixed-Dose Period)[3]	
n	27
Mean (SD)	11.52 (10.543)
Median (Q1, Q3)	10.40 (2.59, 19.02)
Min, max	0.0, 45.0
Reduction from annualized historical RBC units transfused (up to end of Fixed-Dose Period)[3]	
n	27
Mean (SD)	5.11 (5.766)
Median (Q1, Q3)	3.38 (1.34, 9.71)
Min, max	-3.2, 18.1
Percent reduction from annualized historical RBC units transfused (up to end of Fixed-Dose Period)[3]	
n	27
Mean (SD)	36.60 (39.191)
Median (Q1, Q3)	20.57 (5.45, 78.54)
Min, max	-19.7, 100.0
Percent reduction from annualized historical RBC units transfused category (up to end of Fixed-Dose Period)[3], n (%)	
<0	4 (14.8)
≥0 to <20%	9 (33.3)
≥20% to <33%	3 (11.1)
≥33% to <50%	1 (3.7)
≥50%	10 (37.0)

Source: Table 14.2-2.1.
Abbreviations: EOS = end of study; max = maximum; min = minimum; Q1 = first quartile; Q3 = third quartile; RBC = red blood cells; SD = standard deviation.
[1] Annualized historical RBC units transfused is the total number of RBC units transfused in the 52 weeks before informed consent.
[2] Annualized total number of RBC units transfused (units/52 weeks) during the study, including data up to EOS, is the total number of RBC units transfused during the entire study × 52 / [(date of EOS – date of start of study treatment + 1) / 7].
[3] Annualized total number of RBC units transfused (units/52 weeks) during the study, including data up to the end of Fixed-Dose Period, is the total number of RBC units transfused during the Dose Optimization and Fixed-Dose Periods combined × 52 / [(end date of the Fixed-Dose Period – date of start of study treatment + 1) / 7].

Transfusion Episodes during the Fixed-Dose Period Compared with the Standardised Control Period

Table 48 Summary of Number of Transfusion Episodes Standardised to 24 Weeks (Full Analysis Set)

	Total N=27
Number of historical transfusion episodes	
n	27
Mean (SD)	4.46 (1.669)
Median (Q1, Q3)	4.15 (2.77, 5.08)
Min, max	2.8, 7.8
Number of transfusion episodes during the Fixed-Dose Period	
n	26
Mean (SD)	2.88 (2.694)
Median (Q1, Q3)	2.94 (0.00, 3.91)
Min, max	0.0, 9.0
Reduction from number of historical transfusion episodes	
n	26
Mean (SD)	1.58 (1.918)
Median (Q1, Q3)	1.17 (-0.06, 2.77)
Min, max	-1.6, 5.4
Percent reduction from number of historical transfusion episodes	
n	26
Mean (SD)	39.57 (44.424)
Median (Q1, Q3)	21.34 (-0.76, 100.00)
Min, max	-22.3, 100.0

Source: Table 14.2-3.1.
Abbreviations: max = maximum; min = minimum; Q1 = first quartile; Q3 = third quartile; SD = standard deviation.
Notes: Both number of historical transfusion episodes and number of transfusion episodes during the Fixed-Dose Period are standardized to 24 weeks.

Achievement of Normal Haemoglobin Concentrations

Table 49 Summary of Subjects Who Achieved Normal Hb Concentrations (Full Analysis Set)

	Total N=27
Subjects who achieved normal Hb concentrations, n (%)	3 (11.1)
95% CI	(2.4, 29.2)

Source: Table 14.2-5.1.
Abbreviation: Hb = hemoglobin.
Notes: Subjects who achieved Hb concentrations in the normal range at least once in the Fixed-Dose Period, 8 weeks or more after a transfusion.
CI is based on Clopper-Pearson method.

Exploratory Endpoints

Haemolysis Markers

For transfusion-free responders, there was a consistent and sustained improvement in haemolysis markers (indirect bilirubin, haptoglobin, and LDH) during both the Dose Optimisation Period and the Fixed-Dose Period (with most subjects reaching values within the normal range for these markers).

For non–transfusion-free responders, there were fluctuations in the levels of markers of haemolysis, but interpretation of these markers is limited by concomitant transfusions.

Markers of Iron Metabolism and Indicators of Iron Overload

Table 50 Summary of Liver Iron Concentration by Magnetic Resonance Imaging and Change from Baseline Full Analysis Set

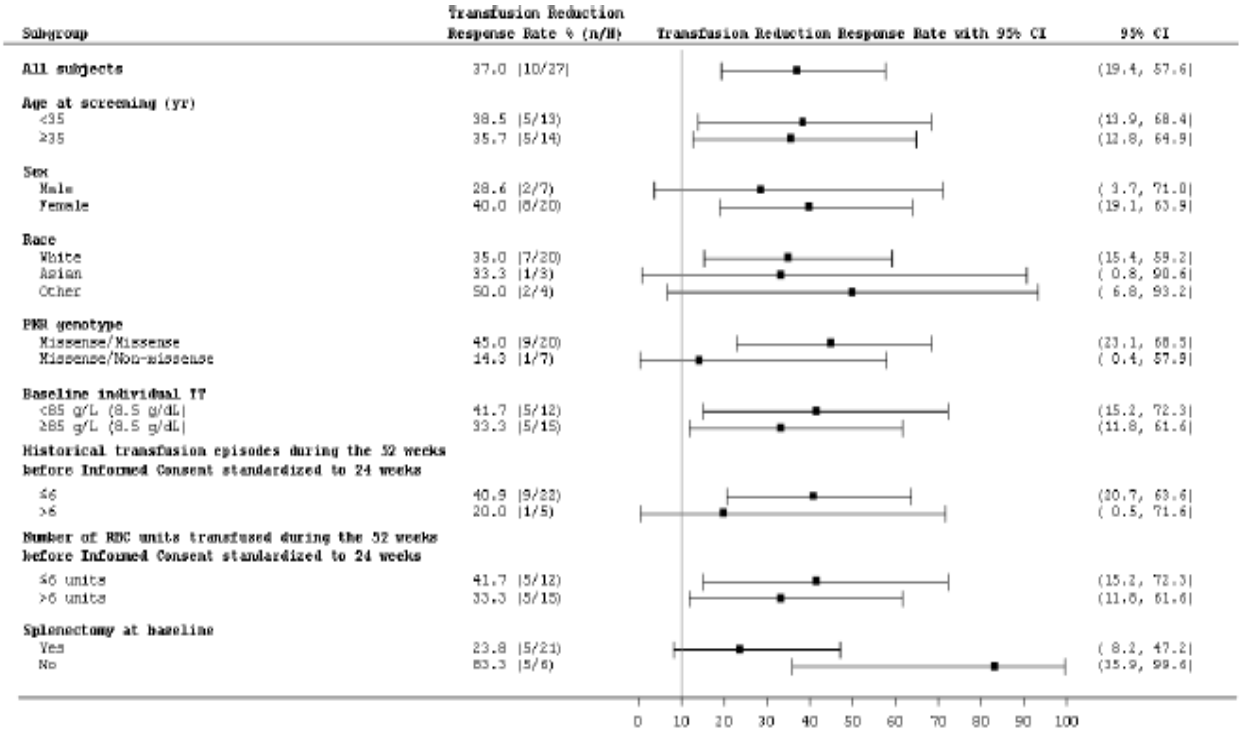
Average Liver Iron Concentration (mg Fe/g dw)

Visit	Total N=27
Baseline	
n	26
Mean (SD)	9.65 (11.122)
Median (Q1, Q3)	4.65 (2.80, 11.70)
Min, Max	1.2, 43.0
Part 2 Day 1	
n	22
Mean (SD)	8.13 (8.148)
Median (Q1, Q3)	5.60 (2.30, 9.60)
Min, Max	1.1, 29.2
Part 2 Day 1	
Change from baseline	
n	21
Mean (SD)	-0.20 (3.242)
Median (Q1, Q3)	0.30 (-0.80, 1.40)
Min, Max	-10.6, 6.9
Part 2 Week 24	
n	18
Mean (SD)	8.52 (8.690)
Median (Q1, Q3)	4.40 (2.60, 10.70)
Min, Max	1.1, 34.8
Part 2 Week 24	
Change from baseline	
n	17
Mean (SD)	1.44 (3.139)
Median (Q1, Q3)	0.40 (-0.40, 2.10)
Min, Max	-1.0, 9.2

n is the number of subjects in the full analysis set with an assessment at the visit or (for change from baseline summaries) with baseline and at least 1 post-baseline assessment at the visit.
Baseline is defined as the last assessment before start of study treatment.

Ancillary analyses

Figure 18 Forest Plot for Transfusion Reduction Response



Source: Figure 14.2-3.1.
Abbreviations: PKR = red blood cell–specific form of pyruvate kinase; RBC = red blood cell; TT = transfusion trigger.
Notes: N is the number of subjects in each subgroup.
Transfusion reduction responders: subjects who had ≥33% reduction in the number of RBC units transfused during the Fixed-Dose Period standardized to 24 weeks compared with the historical number of RBC units transfused standardized to 24 weeks.
The estimated 95% CI is based on the exact binomial distribution.

- ***Summary of main efficacy results***

The following tables summarise the efficacy results from the main studies supporting the present application. These summaries should be read in conjunction with the discussion on clinical efficacy as well as the benefit risk assessment (see later sections).

Table 51 Summary of efficacy for trial AG348 C 006 (ACTIVATE)

Title: A Phase 3, Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Efficacy and Safety of AG-348 in Not Regularly Transfused Adult Subjects with Pyruvate Kinase Deficiency		
Study identifier	Protocol code: AG348-C-006; EudraCT number: 2017-003823-31; US NCT number: NCT03548220	
Design	Phase 3, 2-part, multicentre, randomised, double-blind, placebo-controlled.	
	Duration of main phase:	24 weeks
Hypothesis	Superiority	

Title: A Phase 3, Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Efficacy and Safety of AG-348 in Not Regularly Transfused Adult Subjects with Pyruvate Kinase Deficiency			
Study identifier	Protocol code: AG348-C-006; EudraCT number: 2017-003823-31; US NCT number: NCT03548220		
Treatments groups	Mitapivat		BID oral tablet: Part 1: initial dose of 5 mg BID with potential sequential steps dose level increase (ie, from 5 to 20 mg BID and from 20 to 50 mg BID), depending on safety and efficacy. Part 2: Individually titrated dose from Part 1. 24 weeks, 40 randomised
	placebo		BID oral tablet, matched placebo, 24 weeks, 40 randomised
Endpoints and definitions	Primary endpoint	HbR	Hb response defined as a ≥15 g/L (≥1.5 g/dL; 0.93 mmol/L) increase in Hb concentration from baseline that is sustained at 2 or more scheduled assessments at Weeks 16, 20, and 24.
	Key secondary endpoint	Hb	Average change from baseline (BL) in Hb concentration at Weeks 16, 20, and 24.
Database lock	20 November 2020		

Results and Analysis

Analysis description	**Primary Analysis**		
Analysis population and time point description	Full analysis set (FAS): all subjects who were randomised.		
Descriptive statistics and estimate variability	Treatment group	Mitapivat	Placebo
	Number of subjects	40	40
	HbR, n (%)	16 (40)	0 (0)
	Hb (g/L), LS mean (SE)	16.73 (2.075)	-1.48 (2.082)
Effect estimate per comparison	HbR	Comparison groups	Mitapivat-placebo
		Adjusted difference in response rate, %	39.3
		95% CI	24.1, 54.6
		2-sided P-value (Mantel-Haenszel Stratum Weighted Method)	<0.0001

Title: A Phase 3, Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Efficacy and Safety of AG-348 in Not Regularly Transfused Adult Subjects with Pyruvate Kinase Deficiency			
Study identifier	Protocol code: AG348-C-006; EudraCT number: 2017-003823-31; US NCT number: NCT03548220		
	Hb (g/L)	Comparison groups	Mitapivat-placebo
		Difference in LS mean	18.21
		95% CI	12.41, 24.01
		2-sided p-value	<0.0001

Table 52 Summary of efficacy for trial AG348-C-007 (ACTIVATE-T)

Title: An Open-Label Study to Evaluate the Efficacy and Safety of Mitapivat in Regularly Transfused Adult Subjects with Pyruvate Kinase (PK) Deficiency			
Study identifier	Protocol code: AG348-C-007; EudraCT number: 2017-003803-22; US NCT number: NCT03559699		
Design	Phase 3, 2-part, multicentre, single-arm, open-label		
	Duration of main phase:		40 weeks
Hypothesis	Non-controlled: Mitapivat confers a significant reduction in transfusion burden		
Treatments groups	Mitapivat		BID oral tablet: Part 1: initial dose of 5 mg BID with 2 sequential steps for dose level increase (ie, from 5 to 20 mg BID and from 20 to 50 mg BID) depending on safety and efficacy. Part 2: Individually titrated dose BID from Part 1 Mitapivat. 40 weeks, 27 treated
Endpoints and definitions	Primary endpoint	TRR	Transfusion reduction response: defined as a ≥33% reduction in the number of red blood cell units transfused during the 24 weeks of Part 2 compared with the historical transfusion burden standardised to 24 weeks (Standardised Control Period).
	secondary endpoint	RBC	Annualised total number of red blood cell units transfused during the study (both Part 1 and Part 2) compared with the historical transfusion burden.
		Tn	Number of transfusion episodes during Part 2 compared with the Standardised Control Period.
		T0	Becoming transfusion-free, defined as 0 transfusions administered during Part 2.

Title: An Open-Label Study to Evaluate the Efficacy and Safety of Mitapivat in Regularly Transfused Adult Subjects with Pyruvate Kinase (PK) Deficiency		
Study identifier	Protocol code: AG348-C-007; EudraCT number: 2017-003803-22; US NCT number: NCT03559699	
Database lock	15 January 2021	
Results and Analysis		
Analysis description	**Primary Analysis**	
Analysis population and time point description	FAS: all subjects who received at least 1 dose of study treatment	
Descriptive statistics and estimate variability	Treatment group	Mitapivat
	Number of subjects	27
	TRR, n (%)	10 (37.0)
	95% CI	19.4, 57.6
	RBC, mean (SD)	5.25 (5.652)
	Tn, mean (SD)	1.58 (1.918)
	T0, n (%)	6 (22.2)
	95% CI	8.6, 42.3

- ***Clinical studies in special populations***

	Age 65-74 (Older subjects number /total number)	**Age 75-84 (Older subjects number /total number)**	**Age 85+ (Older subjects number /total number)**
Controlled Trials AG348-C-006	3/80 (3.75)	1/80 (1.25)	0
Non Controlled trials AG348-C-007	1/27 (3.70)	0	0

- ***In vitro biomarker test for patient selection for efficacy***

Enrolment in study 006, 007 was restricted to subjects with documented presence of at least 2 mutant alleles in the PKLR gene, of which at least 1 was a missense mutation, as determined per the genotyping performed by the study central genotyping laboratory.

For subjects with PK deficiency in the studies 006, 007 and 003, mutations were confirmed by central testing prior to enrollment.

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

Efficacy data from study 003 were not pooled with data from Studies 006 and 011 due to differences in key disease characteristics and dose levels of study treatment.

Table 53 Key Differences between Study AG348-C-003 and Study AG348-C-006

	Study AG348-C-003 (Phase 2)	Study AG348-C-006 (Phase 3)
Inclusion/Exclusion Criteria		
Hb-related	Hb ≤12.0 g/dL (if male) or ≤11.0 g/dL (if female)	Hb ≤10.0 g/dL regardless of sex
PKR genotype	• Have documented clinical laboratory confirmation of PK deficiency by RBC pyruvate kinase enzymatic assay performed at Screening, either by a designated central laboratory or by any participating investigative site's local hematology laboratory. Subjects with prior documentation of PK deficiency by RBC enzymatic assay must have reconfirmation of this result during Screening as a condition of enrollment • Have a blood sample for genotypic characterization of the mutant PKR gene performed by the designated central laboratory at Screening	• Have documented clinical laboratory confirmation of PK deficiency, defined as documented presence of at least 2 mutant alleles in the *PKLR* gene, of which at least 1 is a missense mutation, as determined per the genotyping performed by the central genotyping laboratory. • Excluding subjects who are homozygous for the R479H mutation, or have 2 nonmissense mutations without the presence of another missense mutation in the *PKLR* gene, as determined per the genotyping performed by the central genotyping laboratory.
Transfusion-related	Be considered transfusion independent, defined as having had ≤3 units of RBCs transfused in the 12-month period up to the first day of study drug dosing and no transfusions within 4 months of the first day of study dosing	Be considered not regularly transfused, defined as having had no more than 4 transfusion episodes in the 12-month period up to the first day of study treatment and no transfusions in the 3 months before the first day of study treatment
Dosing Schema		
Highest dose	300 mg BID	50 mg BID
Starting dose	50 mg BID or 300 mg BID	5 mg BID

Abbreviations: BID = twice daily; Hb = hemoglobin; PK deficiency = pyruvate kinase deficiency; PKR = red blood cell-specific form of pyruvate kinase; RBC = red blood cell.

For subjects who were not regularly transfused, efficacy data are summarised using 2 separate approaches:

1. Efficacy data from subjects randomised to placebo and mitapivat in Study 006 were longitudinally combined with data from Cohort 1 and Cohort 2 of Study 011, respectively.

2. Efficacy data from subjects randomised to mitapivat in Study 006 were pooled with data from subjects who were originally randomised to placebo in Study 006 and who later received mitapivat in Cohort 1 of Study 011.

Table 54 Data Integration for Efficacy Analyses (Layout 1) – Subjects Not Regularly Transfused

Study Drug	Placebo/Mitapivat	Mitapivat
Study	Study 006/ Study 011 Cohort 1[1]	Study 006/ Study 011 Cohort 2[2]
Number of Subjects in the Full Analysis Set	N=40	N=40
Data Cutoff or End of Study Date	On or after the latest of end of study date for Study 006 and Study 007	

[1] Includes all subjects randomized to placebo in Study 006.
[2] Includes all subjects randomized to mitapivat in Study 006.

Table 55 Data Integration for Efficacy Analyses (Layout 2) – Subjects Not Regularly Transfused

Study Drug	Placebo	Mitapivat		
Study	Study 006	Study 006	Study 011 Cohort 1	Total
Number of Subject in the Full Analysis Set	N=40	N=40	N=36	N=76
Data Cutoff or End of Study Date	End of study date	End of study date	On or after the latest of end of study date for Study 006 and Study 007	On or after the latest of end of study date for Study 006 and Study 007

Table 56 Data Integration for Efficacy Analyses – Subjects Regularly Transfused

Study Drug	Mitapivat	
Study	Study 007	Study 007/Study 011 Cohort 3
Number of Subject in the Full Analysis Set	N=27	N=27
Data Cutoff or End of Study Date	End of study date	On or after the latest of end of study date for Study 006 or Study 007

Subject Disposition

Table 57 Summary of Disposition of Subjects with Pyruvate Kinase Deficiency Who Are Not Regularly Transfused (Full Analysis Set)

	Study 006 Placebo/ Study 011 Cohort 1 Mitapivat N=40 n (%)	Study 006 Mitapivat/ Study 011 Cohort 2 Mitapivat N=40 n (%)	Total N=80 n (%)
Study 006 disposition: end of treatment			
Discontinued	0	0	0
Completed	39 (97.5)	40 (100)	79 (98.8)
Ongoing	0	0	0
Study 006 disposition: end of study			
Discontinued	1 (2.5)	0	1 (1.3)
Reason for discontinuation			
Lost to follow-up	1 (2.5)	0	1 (1.3)
Completed	39 (97.5)	40 (100)	79 (98.8)
Ongoing	0	0	0
Study 011 disposition: end of treatment			
Discontinued	5 (12.5)	2 (5.0)	7 (8.8)
Reason for discontinuation			
Adverse event	0	1 (2.5)	1 (1.3)
Withdrawal by subject	3 (7.5)	0	3 (3.8)
Lack of efficacy	2 (5.0)	0	2 (2.5)
Other	0	1 (2.5)	1 (1.3)
Completed	0	0	0
Ongoing	31 (77.5)	33 (82.5)	64 (80.0)
Study 011 disposition: end of study			
Discontinued	4 (10.0)	1 (2.5)	5 (6.3)
Reason for discontinuation			
Withdrawal by subject	2 (5.0)	1 (2.5)	3 (3.8)
Lack of efficacy	2 (5.0)	0	2 (2.5)
Completed	0	0	0
Ongoing	32 (80.0)	34 (85.0)	66 (82.5)

Source: Table 18.2.1-1.1 (Integrated Summary of Efficacy).
Note: The denominator used to calculate percentages is N, the number of subjects in the Full Analysis Set within each treatment

Table 58 Summary of Disposition of Subjects with Pyruvate Kinase Deficiency Who Are Regularly Transfused (Full Analysis Set)

	Study 007 Mitapivat/ Study 011 Cohort 3 Mitapivat N=27 n (%)
Study 007 disposition: end of treatment	
Discontinued	6 (22.2)
Reason for discontinuation	
Withdrawal by subject	6 (22.2)
Completed	21 (77.8)
Ongoing	0
Study 007 disposition: end of study	
Discontinued	7 (25.9)
Reason for discontinuation	
Withdrawal by subject	6 (22.2)
Lost to follow-up	1 (3.7)
Completed	20 (74.1)
Ongoing	0
Study 011 disposition: end of treatment	
Discontinued	3 (11.1)
Reason for discontinuation	
Withdrawal by subject	1 (3.7)
Lack of efficacy	1 (3.7)
Other	1 (3.7)
Completed	0
Ongoing	14 (51.9)
Study 011 disposition: end of study	
Discontinued	3 (11.1)
Reason for discontinuation	
Withdrawal by subject	2 (7.4)
Lack of efficacy	1 (3.7)

	Study 007 Mitapivat/ Study 011 Cohort 3 Mitapivat N=27 n (%)
Completed	0
Ongoing	14 (51.9)

Source: Table 18.2.2-1.1 (Integrated Summary of Efficacy).
Note: The denominator used to calculate percentages is N, the number of subjects in the Full Analysis Set.

Subjects with Pyruvate Kinase Deficiency Who Are Not Regularly Transfused

Haemoglobin Response

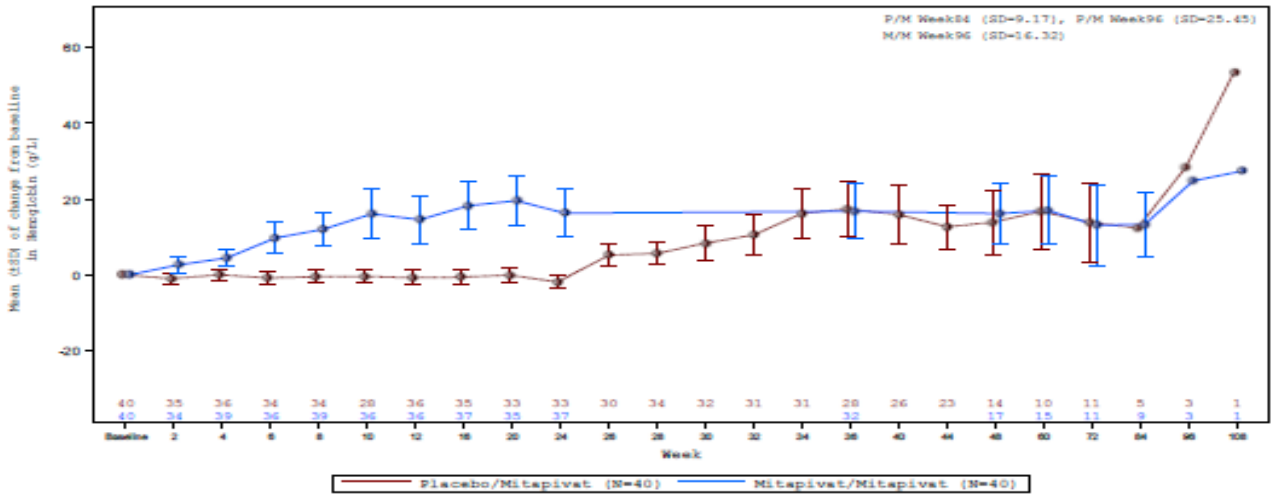
Table 59 Summary of Haemoglobin Response in Subjects with Pyruvate Kinase Deficiency Who Are Not Regularly Transfused (Full Analysis Set)

	Placebo	Mitapivat		
	Study 006 N=40	Study 006 N=40	Study 011 Cohort 1 N=17	Total N=57
Hb responders, n(%)	0	16 (40.0)	6 (35.3)	22 (38.6)
95% CI	(0.00, 8.81)	(24.86, 56.67)	(14.21, 61.67)	(26.00, 52.43)

Source: Table 18.2.1-2.1 (Integrated Summary of Efficacy).
Abbreviation: Hb = hemoglobin.
Notes: Hb responders: subjects who had a ≥15 g/L (1.5 g/dL) increase in Hb concentration from baseline that was sustained at 2 or more scheduled assessments at Weeks 16, 20 and 24 during the Fixed-Dose Period.
For Study 011 Cohort 1, only subjects who received the first dose of mitapivat more than 24 weeks (≥169 days) before the data cutoff date for Study 011 are included.
CI is based on Clopper-Pearson method.

Haemoglobin Concentrations

Figure 19 Mean (±SD) of Change from Baseline in Haemoglobin over Time – Subjects with Pyruvate Kinase Deficiency Who Are Not Regularly Transfused (Full Analysis Set)



Source: Figure 18.2.1-3.1 (Integrated Summary of Efficacy).
Abbreviations: M = mitapivat; P = placebo.
Notes: Baseline is defined as the average of all screening assessments within 45 (42+3) days before randomization for subjects randomized and not dosed or before start of study treatment for subjects randomized and dosed. Assessments collected within 61 days after a transfusion are excluded from the baseline derivation.

With the updated cutoff date of 12 September 2021, there were 31 Hb responders in Study AG348-C-006 or Study AG348-C-011, and for those subjects:

• Hb response was ongoing for 26 of the 31 Hb responders up to 29.1 months with continued mitapivat treatment.

• Of the 5 Hb responders who experienced loss of Hb, 3 subjects continued to have Hb concentration increased by ≥15 g/L with ongoing treatment with mitapivat after the initial loss of Hb response (ie, loss of Hb response was due to transient dip in Hb).

Table 60 Summary of Duration of Hb Response (Hb Responders in the Full Analysis Set, Not Regularly Transfused)

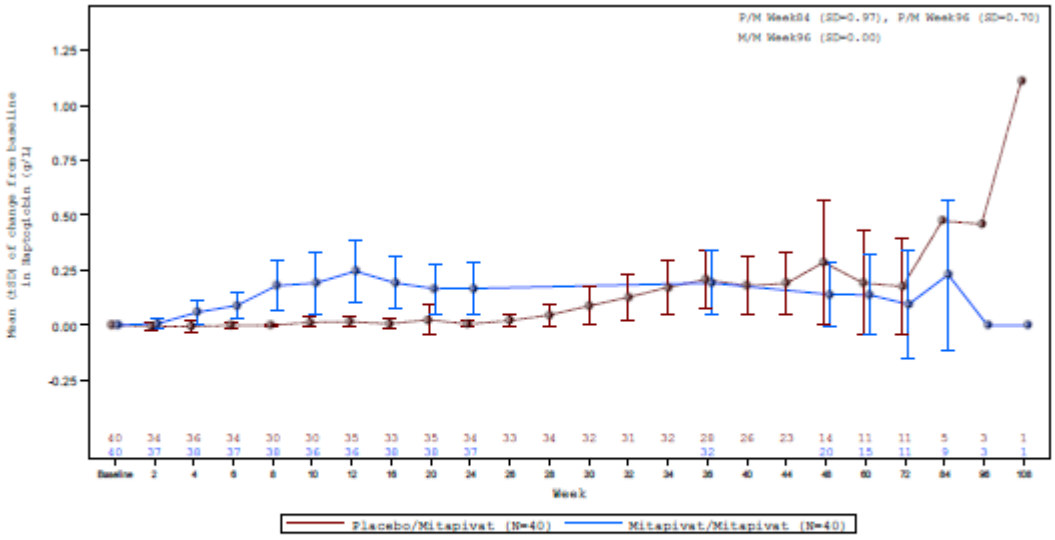
Summary	Study 011 Cohort 1 N=15	Study 006/ Study 011 Cohort 2 N=16	All Subjects N=31
Duration of Hb response (months)			
n	15	16	31
Mean (SD)	13.52 (5.743)	16.86 (7.172)	15.24 (6.633)
Median (Q1, Q3)	12.78 (10.15, 15.21)	16.79 (14.90, 21.68)	15.11 (10.84, 19.58)
Min, Max	4.9, 26.7 +	3.3, 29.1 +	3.3, 29.1 +

Source: AG348-C-006 AG348-C-011 MAA Day 80 Table 90-6.1 (Agios internal use only). Data cutoff date: 12SEP2021.
Abbreviations: Hb = hemoglobin, SD = standard deviation.
The denominator used to calculate percentages is N, the number of Hb responders in the full analysis set within each treatment group.
Hb response is defined as postbaseline change from baseline in Hb ≥15 g/L (1.5 g/dL) that is sustained at 2 or more assessments at Weeks 16, 20 and 24 in the Fixed Dose Period, excluding those within 61 days after a transfusion.
+ denotes ongoing Hb response.

Program Name: Z:\ag348\maa\Day 80 Response\final\programs\tables\t-hgb-dur-006-90.sas Run Date: 22NOV2021 (13:40)

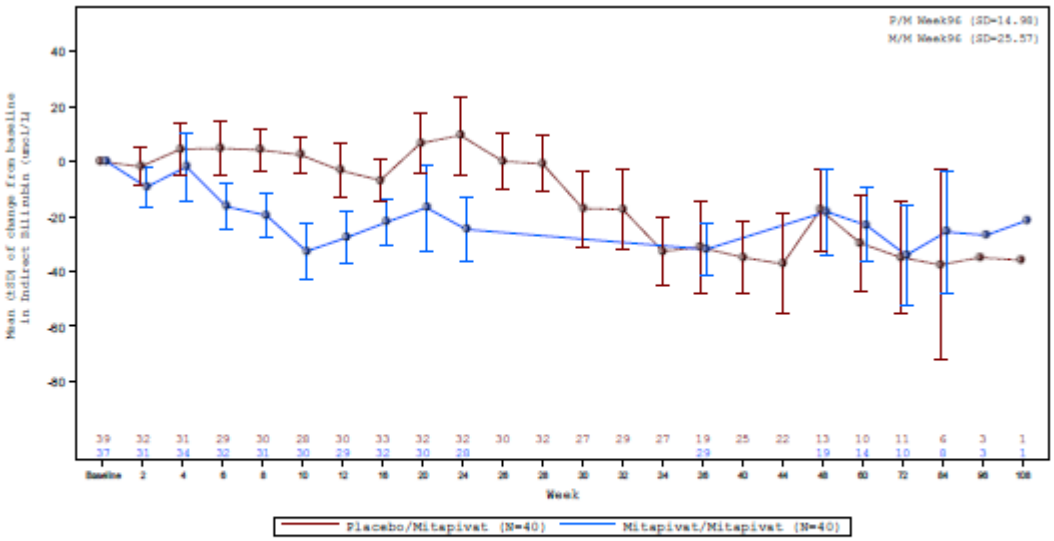
Haemolysis Markers

Figure 20 Mean (±SD) of Change from Baseline in Haptoglobin over Time in Subjects with Pyruvate Kinase Deficiency Who Are Not Regularly Transfused (Full Analysis Set)



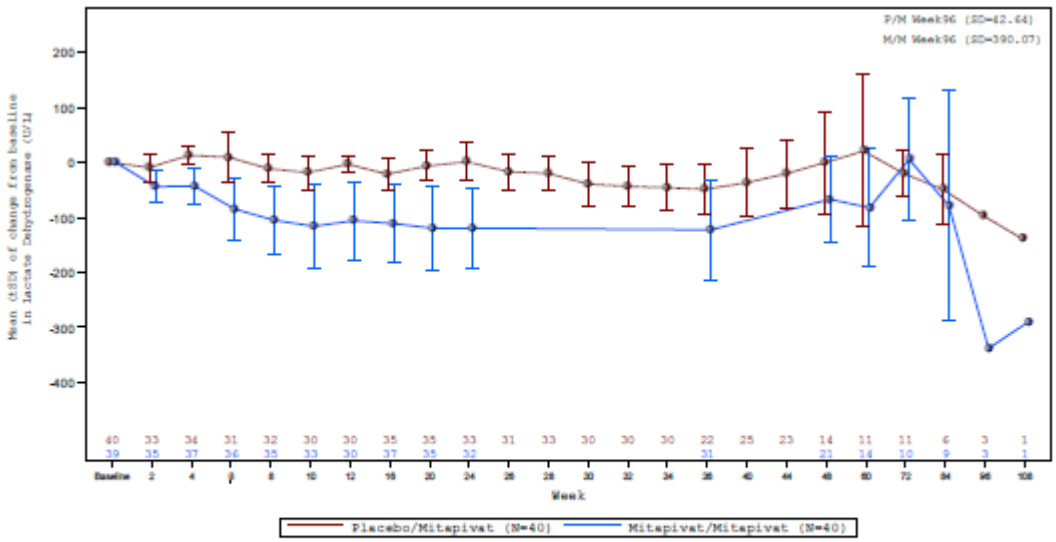
Source: Figure 18.2.1-4.1 (Integrated Summary of Efficacy).
Abbreviations: M = mitapivat; P = placebo.
Notes: Baseline is defined as the average of all screening assessments within 45 (42+3) days before randomization for subjects randomized and not dosed or before start of study treatment for subjects randomized and dosed. Assessments collected within 61 days after a transfusion are excluded from the baseline derivation.

Figure 21 Mean (±SD) of Change from Baseline in Indirect Bilirubin over Time in Subjects with Pyruvate Kinase Deficiency Who Are Not Regularly Transfused (Full Analysis Set)



Source: Figure 18.2.1-4.1 (Integrated Summary of Efficacy).
Abbreviations: M = mitapivat; P = placebo.
Notes: Baseline is defined as the average of all screening assessments within 45 (42+3) days before randomization for subjects randomized and not dosed or before start of study treatment for subjects randomized and dosed. Assessments collected within 61 days after a transfusion are excluded from the baseline derivation.

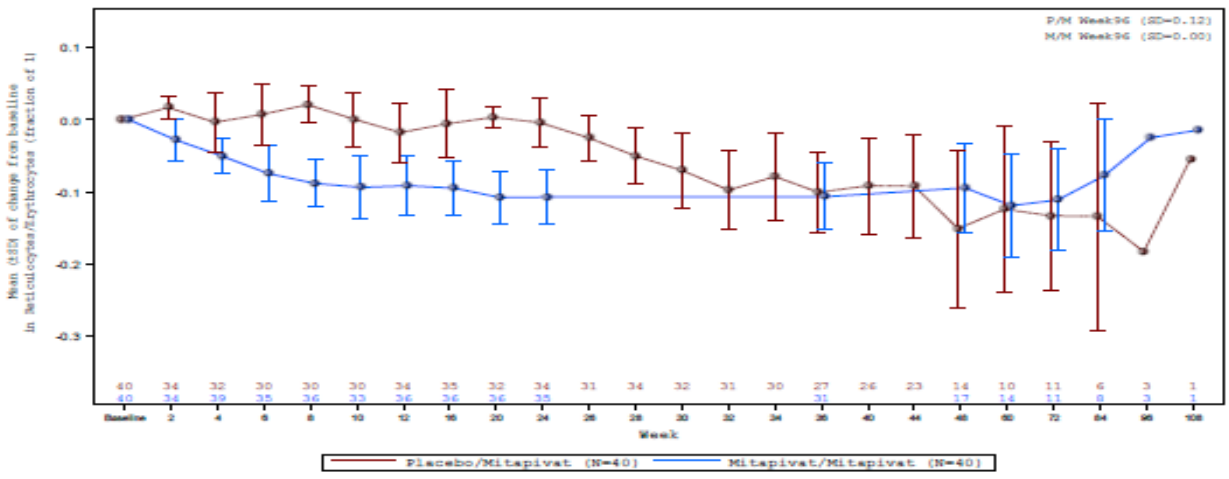
Figure 22 Mean (±SD) of Change from Baseline in Lactate Dehydrogenase over Time in Subjects with Pyruvate Kinase Deficiency Who Are Not Regularly Transfused (Full Analysis Set)



Source: Figure 18.2.1-4.1 (Integrated Summary of Efficacy).
Abbreviations: M = mitapivat; P = placebo.
Notes: Baseline is defined as the average of all screening assessments within 45 (42+3) days before randomization for subjects randomized and not dosed or before start of study treatment for subjects randomized and dosed. Assessments collected within 61 days after a transfusion are excluded from the baseline derivation.

Haematopoietic Activity Marker

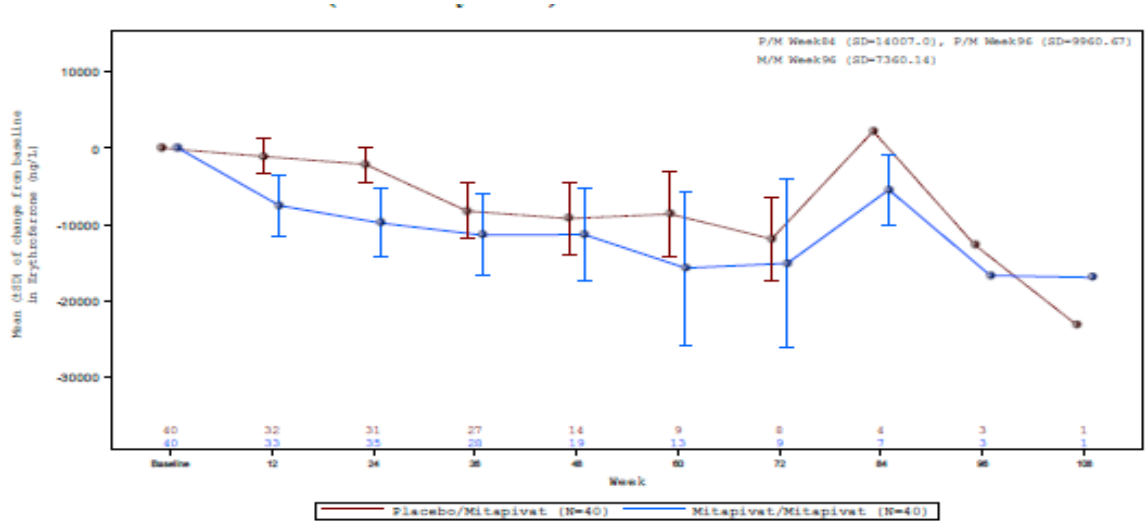
Figure 23 Mean (±SD) of Change from Baseline in Reticulocyte Percentage over Time in Subjects with Pyruvate Kinase Deficiency Who Are Not Regularly Transfused (Full Analysis Set)



Source: Figure 18.2.1-5.1 (Integrated Summary of Efficacy).
Abbreviations: M = mitapivat; P = placebo.
Notes: Baseline is defined as the average of all screening assessments within 45 (42+3) days before randomization for subjects randomized and not dosed or before start of study treatment for subjects randomized and dosed. Assessments collected within 61 days after a transfusion are excluded from the baseline derivation.

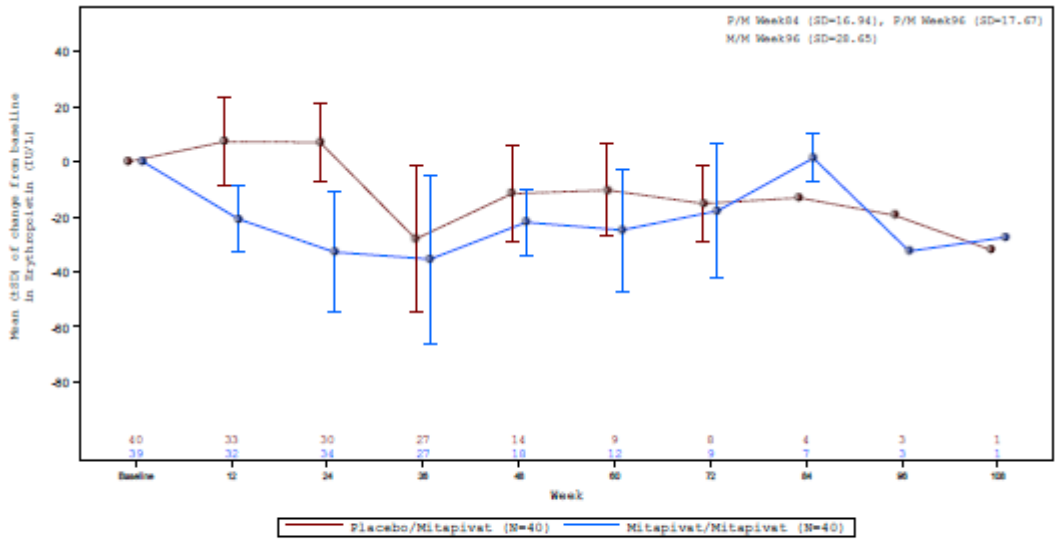
Erythropoietic Markers

Figure 24 Mean (±SD) of Change from Baseline in Erythroferrone over Time in Subjects with Pyruvate Kinase Deficiency Who Are Not Regularly Transfused (Full Analysis Set)



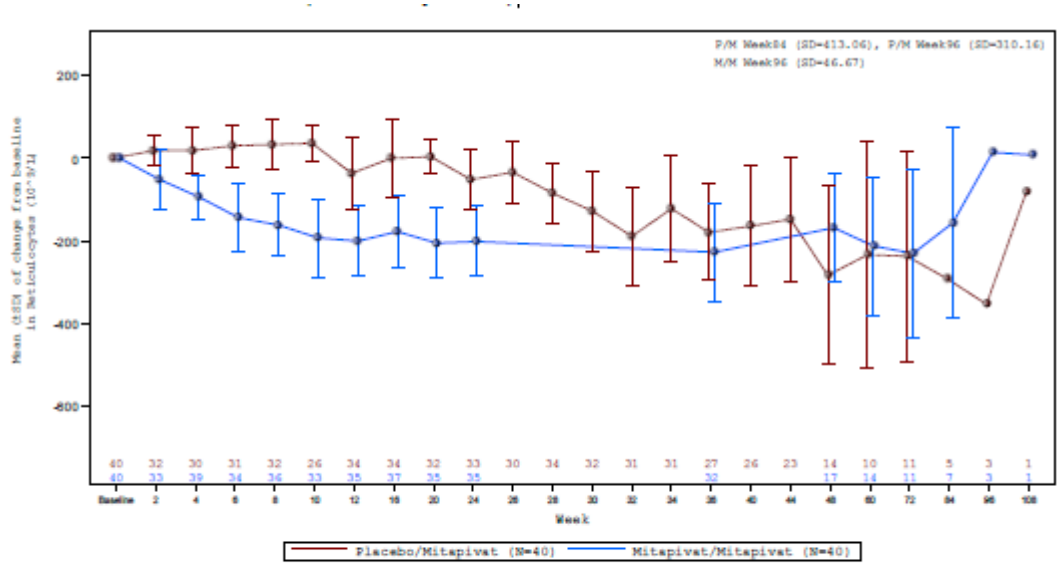
Source: Figure 18.2.1-7.1 (Integrated Summary of Efficacy).
Abbreviations: M = mitapivat; P = placebo.
Notes: Baseline is defined as the average of all screening assessments within 45 (42+3) days before randomization for subjects randomized and not dosed or before start of study treatment for subjects randomized and dosed. Assessments collected within 61 days after a transfusion are excluded from the baseline derivation.

Figure 25 Mean (±SD) of Change from Baseline in Erythropoietin over Time in Subjects with Pyruvate Kinase Deficiency Who Are Not Regularly Transfused (Full Analysis Set)



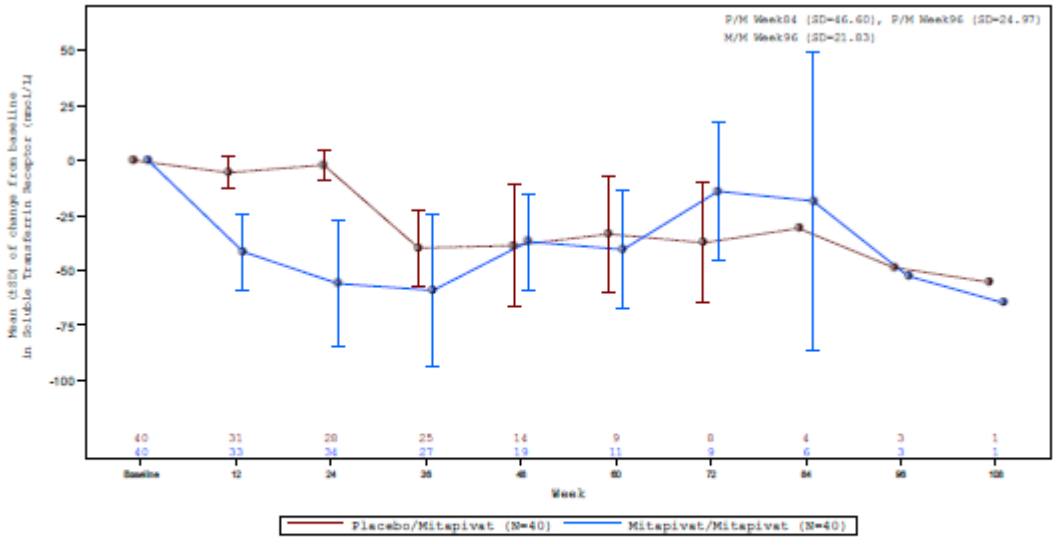
Source: Figure 18.2.1-7.1 (Integrated Summary of Efficacy).
Abbreviations: M = mitapivat; P = placebo.
Notes: Baseline is defined as the average of all screening assessments within 45 (42+3) days before randomization for subjects randomized and not dosed or before start of study treatment for subjects randomized and dosed. Assessments collected within 61 days after a transfusion are excluded from the baseline derivation.

Figure 26 Mean (±SD) of Change from Baseline in Reticulocytes over Time in Subjects with Pyruvate Kinase Deficiency Who Are Not Regularly Transfused (Full Analysis Set)



Source: Figure 18.2.1-7.1 (Integrated Summary of Efficacy).
Abbreviations: M = mitapivat; P = placebo.
Notes: Baseline is defined as the average of all screening assessments within 45 (42+3) days before randomization for subjects randomized and not dosed or before start of study treatment for subjects randomized and dosed. Assessments collected within 61 days after a transfusion are excluded from the baseline derivation.

Figure 27 Mean (±SD) of Change from Baseline in Transferrin Receptor over Time in Subjects with Pyruvate Kinase Deficiency Who Are Not Regularly Transfused (Full Analysis Set)



Source: Figure 18.2.1-7.1 (Integrated Summary of Efficacy).
Abbreviations: M = mitapivat; P = placebo.
Notes: Baseline is defined as the average of all screening assessments within 45 (42+3) days before randomization for subjects randomized and not dosed or before start of study treatment for subjects randomized and dosed. Assessments collected within 61 days after a transfusion are excluded from the baseline derivation.

Iron Markers (DCO date of 12 September 2021 for Study AG348-C-011)

Ferritin

Table 61 Summary of Ferritin Baseline and Change from Baseline by Haemoglobin Response Status (Subjects Not Regularly Transfused and Treated with Mitapivat)

Visit	Hb Responders N=31	Hb Non-Responders N=47
Baseline		
n	31	45
Mean (SD)	397.638 (558.7335)	928.591 (1006.0889)
Median (Q1, Q3)	267.480 (164.695, 494.930)	637.100 (359.640, 1055.260)
Min, Max	21.36, 3128.15	75.18, 5890.25
Week 12		
Change from baseline		
n	29	39
Mean (SD)	3.821 (152.7930)	10.153 (429.4764)
Median (Q1, Q3)	-17.760 (-44.850, 48.755)	-27.170 (-166.270, 55.495)
Min, Max	-280.62, 593.76	-822.35, 2173.43
Week 24		
Change from baseline		
n	29	37
Mean (SD)	64.769 (190.8339)	2.876 (270.4864)
Median (Q1, Q3)	25.610 (-22.550, 77.825)	-14.845 (-97.930, 43.100)
Min, Max	-160.89, 812.69	-508.09, 1291.86
Week 36		
Change from baseline		
n	28	29
Mean (SD)	100.573 (391.6455)	-30.699 (363.7017)
Median (Q1, Q3)	36.417 (-19.153, 95.090)	-52.760 (-234.920, 70.025)
Min, Max	-315.24, 2003.86	-1030.96, 817.58

Visit	Hb Responders N=31	Hb Non-Responders N=47
Week 48		
Change from baseline		
n	27	26
Mean (SD)	75.016 (238.9100)	-73.868 (260.9822)
Median (Q1, Q3)	26.730 (-29.570, 125.600)	-46.080 (-162.725, 75.085)
Min, Max	-300.11, 1072.97	-624.80, 678.26
Week 60		
Change from baseline		
n	23	26
Mean (SD)	120.559 (352.2058)	5.025 (242.8907)
Median (Q1, Q3)	29.670 (-29.395, 132.960)	33.245 (-94.990, 126.880)
Min, Max	-128.35, 1650.78	-596.43, 501.26
Week 72		
Change from baseline		
n	21	18
Mean (SD)	173.260 (511.8628)	-27.757 (394.0817)
Median (Q1, Q3)	35.840 (-19.430, 210.915)	-25.630 (-200.545, 138.680)
Min, Max	-133.29, 2326.12	-802.95, 1144.74
Week 84		
Change from baseline		
n	16	17
Mean (SD)	176.099 (599.6193)	5.380 (262.1599)
Median (Q1, Q3)	4.032 (-51.224, 168.013)	-10.500 (-105.240, 130.740)
Min, Max	-151.34, 2378.73	-613.42, 471.65

Source: AG348-C-006 MAA Day 80 Table 72-6.3 (Agios internal use only). Data cutoff date: 12SEP2021.
Abbreviations: Hb = hemoglobin, SD = standard derivation.
n is the number of subjects treated with mitapivat within each Hb response status category with an assessment at the visit or (for change from baseline summaries) with baseline and at least 1 postbaseline assessment at the visit.
Ferritin values reported as '>1,500 ug/L' due to a laboratory dilution error were excluded.
Baseline is the average of all assessments within 45 (42+3) before start of treatment with mitapivat.

Liver Iron Concentration by Magnetic Resonance Imaging

Table 62 Summary of Liver Iron Concentration by Magnetic Resonance Imaging at Baseline and Change from Baseline by Haemoglobin Response Status (Subjects Not Regularly Transfused and Treated with Mitapivat)

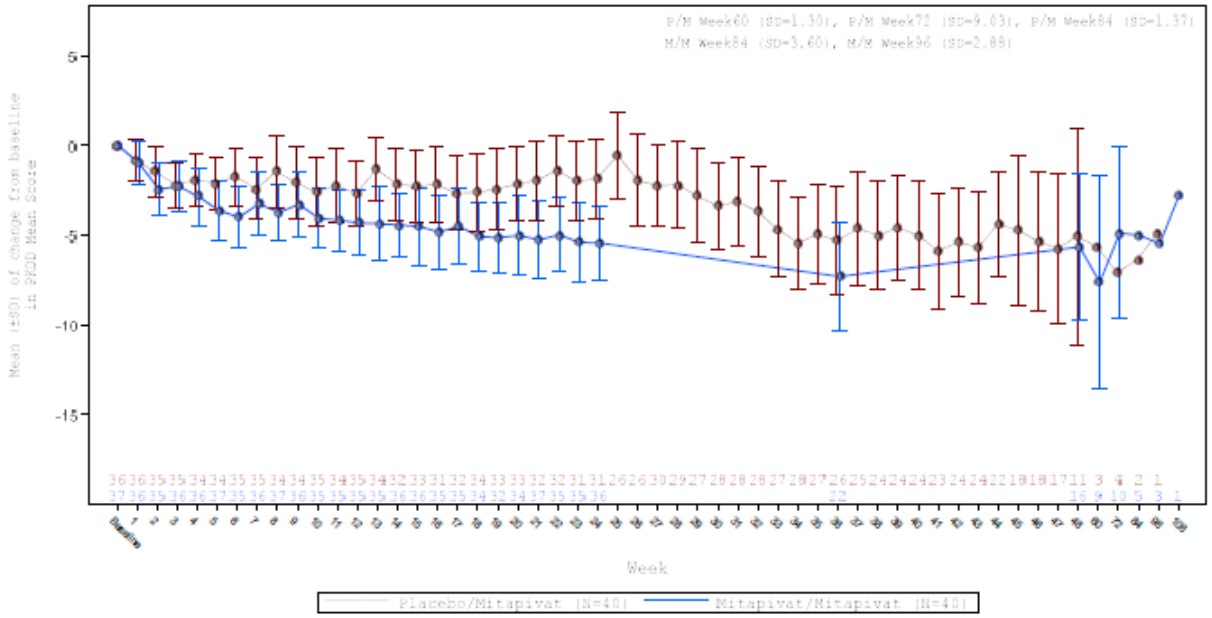
Visit	Hb Responders N=31	Hb Non-Responders N=47
Baseline		
n	30	46
Mean (SD)	4.23 (5.936)	9.53 (12.918)
Median (Q1, Q3)	2.45 (1.70, 5.00)	4.40 (2.50, 10.30)
Min, Max	0.6, 31.7	0.8, 67.8
Week 24		
Change from baseline		
n	23	37
Mean (SD)	-0.43 (1.641)	-0.56 (18.155)
Median (Q1, Q3)	-0.60 (-1.10, 0.30)	-0.50 (-1.70, 0.70)
Min, Max	-4.3, 5.2	-65.7, 83.8
Week 48		
Change from baseline		
n	21	25
Mean (SD)	-0.39 (2.468)	-3.66 (13.511)
Median (Q1, Q3)	-0.20 (-0.90, 0.70)	-0.70 (-2.10, 0.50)
Min, Max	-8.0, 5.0	-64.5, 11.8
Week 72		
Change from baseline		
n	17	18
Mean (SD)	0.25 (3.455)	-2.38 (4.527)
Median (Q1, Q3)	-0.60 (-1.10, 0.00)	-1.40 (-4.20, 0.10)
Min, Max	-3.1, 11.3	-13.6, 5.3
Week 96		
Change from baseline		
n	5	14
Mean (SD)	2.24 (5.291)	-2.64 (5.617)
Median (Q1, Q3)	-0.10 (-0.30, 2.30)	-1.15 (-2.70, -0.40)
Min, Max	-2.0, 11.3	-21.3, 1.2
Week 120		
Change from baseline		
n	2	7
Mean (SD)	-1.45 (1.626)	-3.81 (6.193)
Median (Q1, Q3)	-1.45 (-2.60, -0.30)	-1.20 (-4.50, -0.10)
Min, Max	-2.6, -0.3	-17.1, 1.2

Source: AG348-C-006 MAA Day 80 Table 72-6.4 (Agios internal use only). Data cutoff date: 12SEP2021.
Abbreviations: Hb = hemoglobin, LIC = liver iron concentration, SD = standard derivation.

Health-Related Quality of Life

Pyruvate Kinase Deficiency Diary

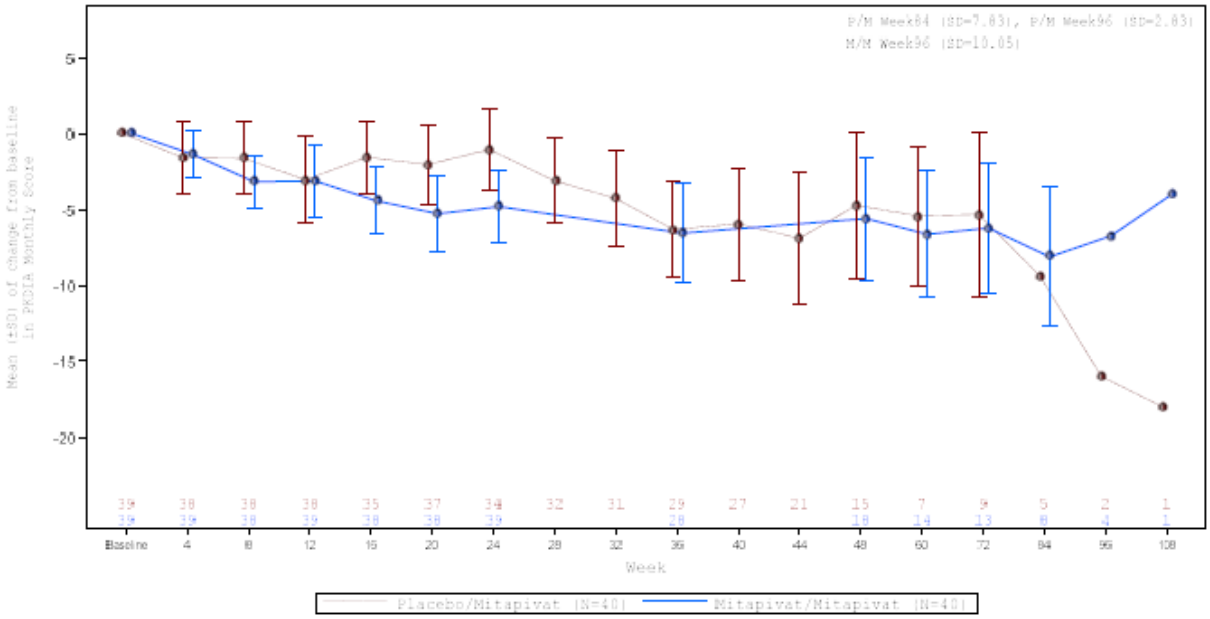
Figure 28 Mean (+/-SD) of Change from Baseline in Pyruvate Kinase Deficiency Diary (PKDD) Weekly Mean Score Over Time - NTD Subjects Full Analysis Set



Baseline of weekly mean score is defined as the average of daily scores collected within 7 days before randomization for subjects randomized and not dosed or before start of study treatment for subjects randomized and dosed.

Pyruvate Kinase Deficiency Impact Assessment

Figure 29 Mean (+/-SD) of Change from Baseline in Pyruvate Kinase Deficiency Impact Assessment (PKDIA) Score Over Time - NTD Subjects Full Analysis Set



Baseline is defined as the last complete assessment (with no missing item in response) before randomization for subjects randomized and not dosed or before start of study treatment for subjects randomized and dosed.

Subjects with Pyruvate Kinase Deficiency Who are Regularly Transfused

Transfusion Reduction Response

Table 63 Summary of RBC Units Transfused Standardised to 24 Weeks in Subjects with Pyruvate Kinase Deficiency Who Are Regularly Transfused (Full Analysis Set)

	Study 007/Study 011 Cohort 3 N=27
Historical RBC units transfused	
n	27
Mean (SD)	7.68 (3.981)
Median (Q1, Q3)	6.92 (5.08, 9.69)
Min, max	2.8, 20.3
RBC units transfused during Fixed-Dose Period	
n	26
Mean (SD)	5.51 (5.895)
Median (Q1, Q3)	4.17 (0.00, 7.81)
Min, max	0.0, 24.0
Reduction from historical RBC units transfused	
n	26
Mean (SD)	2.02 (3.427)
Median (Q1, Q3)	1.03 (-0.28, 4.62)
Min, max	-3.7, 10.1
Percent reduction from historical RBC units transfused	
n	26
Mean (SD)	35.29 (47.306)
Median (Q1, Q3)	20.83 (-4.00, 100.00)
Min, max	-46.8, 100.0
Percent reduction from historical RBC units category, n (%)	
<0	7 (25.9)
≥0 to <20%	6 (22.2)
≥20% to <33%	3 (11.1)
≥33% to <50%	2 (7.4)
≥50%	8 (29.6)
Not evaluable	1 (3.7)
Transfusion reduction responders, n (%)	9 (33.3)
95% CI	(16.5, 54.0)

Source: Table 18.2.2-2.1 (Integrated Summary of Efficacy).

Subjects Who Became Transfusion-Free

All 6 (22.2%) subjects who were transfusion-free in Study 007 remained transfusion-free in Study 011. One additional subject who was a transfusion reduction responder but was not transfusion-free in Study 007 was transfusion-free during Study 011.

Updated data at the DCO of Sept 2021: All data for subjects who were regularly transfused, received mitapivat for at least 12 weeks in the Fixed-Dose Period of Study AG348-C-007, and achieved TRR and transfusion-free response in study AG348-C-007 or study AG348-C-011 are included in the analysis below. With the updated cutoff date of 12 September 2021, 9 of the 10 transfusion reduction responders had ongoing transfusion reduction response up to 30.7 months with continued mitapivat treatment. In addition, 7 of the 8 transfusion-free responders maintained their transfusion-free response up to 31.8 months with continued mitapivat treatment.

Table 64 Summary of Duration of Transfusion Reduction Response (Transfusion Reduction Responders in the Full Analysis Set, Regularly Transfused)

	Total N=10
Duration of transfusion reduction response (months)	
n	10
Mean (SD)	21.1 (9.08)
Median (Q1, Q3)	24.0 (17.9, 28.0)
Min, Max	5.9, 30.7+

Source: AG348-C-007 MAA Day 80 Table 90-7.1 (Agios internal use only). Data cutoff date: 12SEP2021.
Abbreviations: RBC = red blood cell, SD = standard deviation.
The denominator used to calculate percentages is N, the number of transfusion reduction responders in the full analysis set.
Transfusion reduction response is defined as ≥33% reduction in the total number of RBC units transfused (transfusion burden) after the start of the Fixed Dose Period in Study 007 standardized to 24 weeks compared with the historical transfusion burden standardized to 24 weeks.
+ denotes ongoing transfusion reduction response.

Program Name: Z:\ag348\maa\Day 80 Response\final\programs\tables\t-summ-trr.sas Run Date: 22NOV2021 (13:31)

Table 65 Summary of Duration of Transfusion-free Response (Transfusion-free Responders in the Full Analysis Set, Regularly Transfused)

	Total N=8
Duration of transfusion-free response (months)	
n	8
Mean (SD)	24.7 (7.71)
Median (Q1, Q3)	27.0 (22.0, 30.0)
Min, Max	8.2, 31.8+

Source: AG348-C-007 MAA Day 80 Table 90-7.2 (Agios internal use only). Data cutoff date: 12SEP2021.
Abbreviations: RBC = red blood cell, SD = standard deviation.
The denominator used to calculate percentages is N, the number of transfusion-free responders in the full analysis set.
Transfusion-free responders are subjects who did not receive RBC transfusions for a period of ≥24 weeks any time between the start of the fixed dose period in Study 007 through the end of the on-treatment period.
+ denotes ongoing transfusion-free response.

Achievement of Normal Haemoglobin Concentrations

Table 66 Summary of Subjects with Pyruvate Kinase Deficiency Who Are Regularly Transfused Who Achieved Normal Haemoglobin Concentration (Full Analysis Set)

	Study 007/Study 011 Cohort 3 N=27
Subjects who achieved normal Hb concentrations, n (%)	6 (22.2)
95% CI	(8.6, 42.3)

Source: Table 18.2.2-4.1 (Integrated Summary of Efficacy).
Abbreviation: Hb = hemoglobin.
Notes: Subjects who achieved Hb levels in the normal range at least once during the Fixed-Dose Period of Study 007 and Study 011, 8 weeks or more after a transfusion.
CI is based on Clopper-Pearson method.

Iron Markers (data cutoff date of 12 September 2021 for Study AG348-C-011))

Table 82-7.2: Summary of Liver Iron Concentration by Magnetic Resonance Imaging at Baseline and Change from Baseline (Transfusion-free Responders and Transfusion Reduction Responders in the Full Analysis Set)

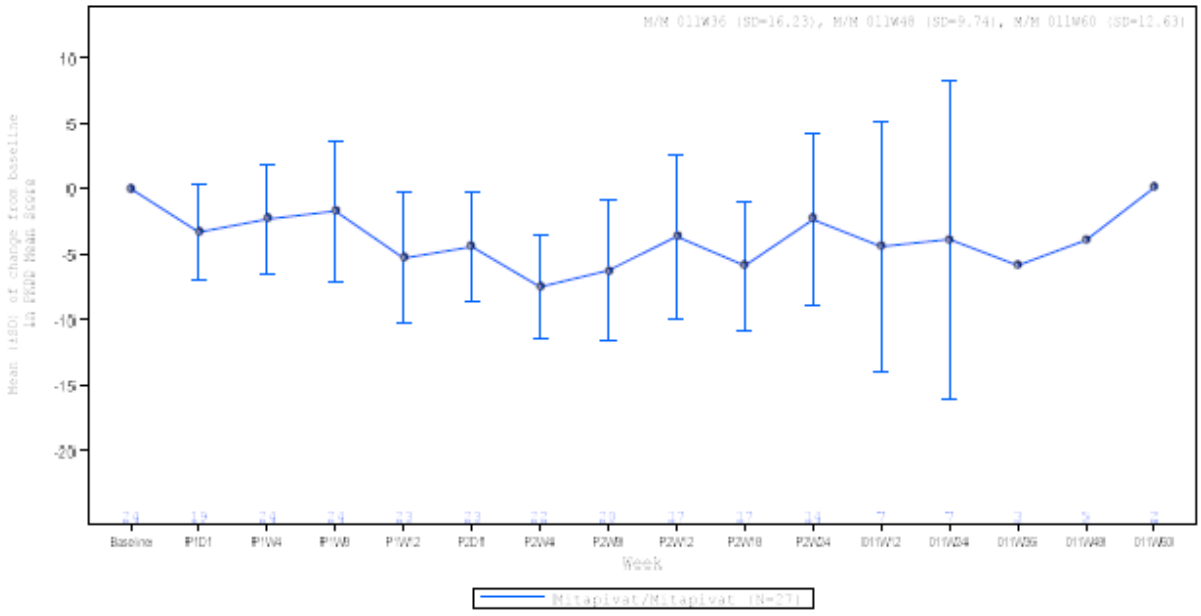
Visit	Transfusion-free Responders N=6	Transfusion Reduction Responders N=10
Baseline		
n	6	10
Mean (SD)	6.2 (5.87)	6.1 (5.05)
Median (Q1, Q3)	3.8 (2.8, 7.8)	3.8 (2.8, 7.8)
Min, Max	1, 17	1, 17
AG348-C-007 Part 2 Day 1		
Change from baseline		
n	5	8
Mean (SD)	-0.2 (1.89)	0.6 (2.98)
Median (Q1, Q3)	-0.2 (-0.3, 0.8)	0.1 (-1.0, 1.4)
Min, Max	-3, 2	-3, 7
AG348-C-007 Part 2 Week 24		
Change from baseline		
n	6	10
Mean (SD)	0.6 (1.22)	1.1 (3.05)
Median (Q1, Q3)	0.4 (-0.3, 0.8)	-0.1 (-0.4, 0.8)
Min, Max	0, 3	-1, 9
AG348-C-011 Week 24		
Change from baseline		
n	2	5
Mean (SD)	1.4 (0.14)	1.0 (0.89)
Median (Q1, Q3)	1.4 (1.3, 1.5)	1.3 (1.2, 1.5)
Min, Max	1, 2	-1, 2

Visit	Transfusion-free Responders N=6	Transfusion Reduction Responders N=10
AG348-C-011 Week 48		
Change from baseline		
n	4	7
Mean (SD)	-4.0 (5.56)	-1.5 (5.54)
Median (Q1, Q3)	-2.0 (-7.3, -0.7)	-1.5 (-2.4, 1.1)
Min, Max	-12, 0	-12, 6
AG348-C-011 Week 72		
Change from baseline		
n	3	6
Mean (SD)	-4.3 (7.35)	-1.6 (5.87)
Median (Q1, Q3)	-1.7 (-12.6, 1.4)	-0.9 (-1.7, 1.4)
Min, Max	-13, 1	-13, 5
AG348-C-011 Week 96		
Change from baseline		
n	1	4
Mean (SD)	-4.4 (NE)	3.4 (13.02)
Median (Q1, Q3)	-4.4 (-4.4, -4.4)	-2.5 (-4.0, 10.7)
Min, Max	-4, -4	-4, 23

Source: AG348-C-007 MAA Day 80 Table 82-7.2 (Agios internal use only). Data cutoff date: 12SEP2021.
Abbreviations : RBC = red blood cell, SD = standard deviation.
n is the number of subjects in the full analysis set within each response category with baseline and at least 1 postbaseline assessment at the visit.
Baseline is defined as the last assessment before start of study treatment.
Transfusion reduction responders: subjects who had ≥33% reduction in the number of RBC units transfused during the fixed-dose period standardized to 24 weeks compared with the historical number of RBC units transfused standardized to 24 weeks.
Transfusion-free responders: subjects who were transfusion-free in the fixed-dose period.

Pyruvate Kinase Deficiency Diary

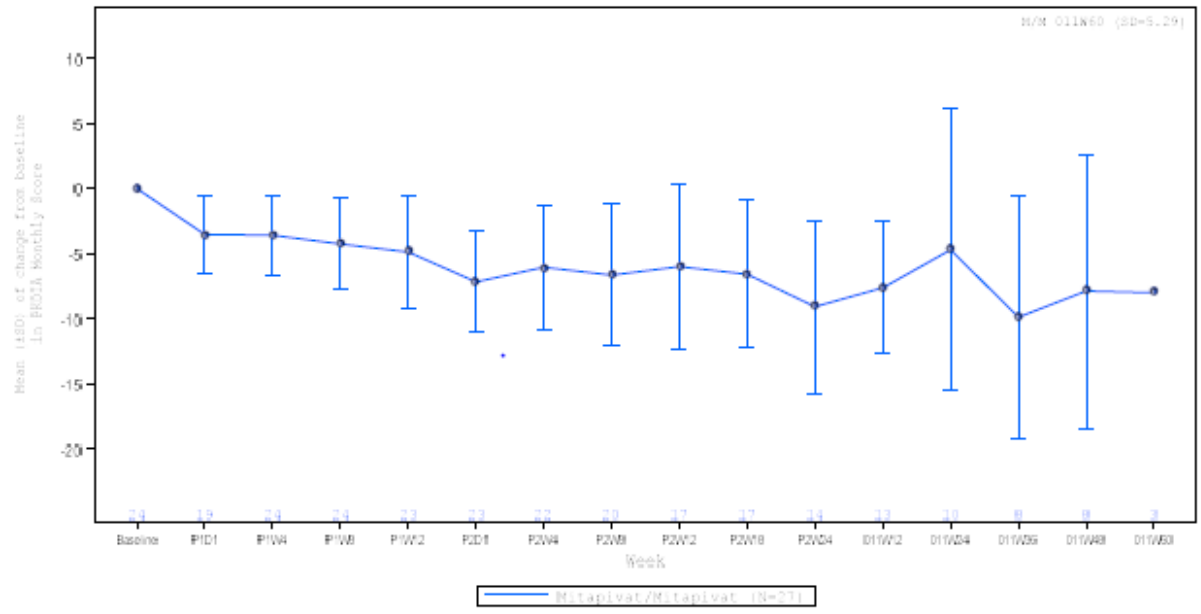
Figure 30 Mean (+/-SD) of Change from Baseline in Pyruvate Kinase Deficiency Diary (PKDD) Score Over Time - RT Subjects Full Analysis Set



aseline is defined as the last measurement before start of study treatment.
1Dx and P1Wx represent AG348-C-007 Part 1 study visits. P2Dx and P2Wx represent AG348-C-007 Part 2 study visits. 011Wx represents AG348-C-011 tudy visits.

Pyruvate Kinase Deficiency Impact Assessment

Figure 31 Mean (+/-SD) of Change from Baseline in Pyruvate Kinase Deficiency Impact Assessment (PKDIA) Score Over Time - RT Subjects Full Analysis Set



aseline is defined as the last complete assessment (with no missing item in response) before start of study treatment.
1Dx and P1Wx represent AG348-C-007 Part 1 study visits. P2Dx and P2Wx represent AG348-C-007 Part 2 study visits. 011Wx represents AG348-C-011 tudy visits.

Haemolysis Markers

For transfusion-free responders, there was a consistent and sustained improvement in haemolysis markers (indirect bilirubin, Hp, and LDH).

For non–transfusion-free responders, there were fluctuations in the levels of markers of haemolysis, but interpretation of these markers is limited by concomitant transfusions.

Reticulocyte Percentage

For transfusion-free responders, there was a consistent and sustained improvement in reticulocyte percentage; for non–transfusion-free responders, there were fluctuations in reticulocyte percentage.

2.5.5.1. Supportive studies

Study AG348-C-011 [Study 011]

This study is an ongoing multicentre, open-label extension study to evaluate the long-term safety, and efficacy of treatment with mitapivat in subjects who were previously enrolled in study 006 or Study 007.

Subjects were assigned to 1 of the following 3 cohorts:

• Cohort 1: Subjects who received placebo in Study 006

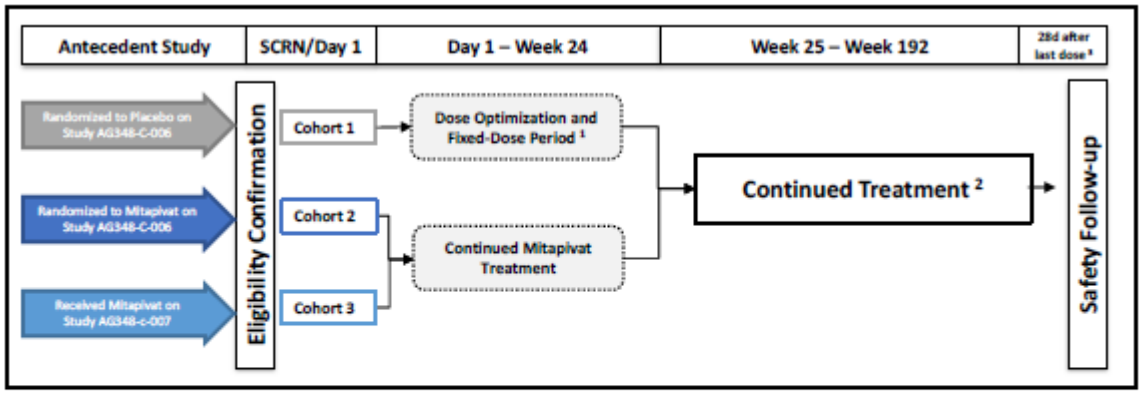
• Cohort 2: Subjects who received mitapivat in Study 006

• Cohort 3: Subjects who received mitapivat in Study 007

For Cohorts 2 and 3 only, subjects were required to have demonstrated clinical benefit from mitapivat in the antecedent study, in the opinion of the Investigator. Subjects are scheduled to receive mitapivat

treatment for up to a maximum of 192 weeks until study withdrawal criteria are met or the study is closed. For subjects in Cohort 1, individual dose titration was incorporated to allow each subject to escalate to a dose of mitapivat that maximised the subject's increase in Hb concentration (assessed every 4 weeks) while maintaining an acceptable safety profile.

Figure 32 Overview of Design for Study 011



Abbreviations: d = days; SCRN = Screening.
[1] Subjects in Cohort 1 initiated treatment with mitapivat in this extension study. Therefore, these subjects participated in a 12-week Dose Optimization Period followed by a 12-week Fixed-Dose Period during the first 24 weeks of this extension study.
[2] Dosing between Week 24 and Week 25 was continuous.
[3] All subjects who permanently discontinued mitapivat at any time were to attend a Safety Follow-up Visit 28 days (±4 days) after the last dose of mitapivat.

First subject enrolled: 21 March 2019

Data cutoff date: 12 November 2020

The primary objective of the study was to evaluate the long-term safety and tolerability of mitapivat.

The secondary endpoints of the study were as follows:

Cohort 1 Only: Subjects who received placebo in Study 006

• Proportion of subjects achieving an Hb response, defined as a ≥15 g/L (≥1.5 g/dL; 0.93 mmol/L) increase in Hb concentration from baseline that is sustained at 2 or more scheduled assessments at Weeks 16, 20, and 24.

• Average change from baseline in Hb concentration at Weeks 16, 20, and 24

All Cohorts

• Change from baseline in Hb concentration

• Change from baseline in markers of haemolysis: bilirubin, lactate dehydrogenase (LDH), and haptoglobin concentrations

• Change from baseline in markers of erythropoietic activity: reticulocyte percentages

• Change from baseline in the number of transfusion events, in the number of RBC units transfused

• Change from baseline in HRQOL PRO scores: Pyruvate Kinase Deficiency Diary (PKDD) and Pyruvate Kinase Deficiency Impact Assessment (PKDIA)

The Full Analysis Set (FAS) included all subjects who received at least 1 dose of study treatment. The Efficacy Analysis Set (EAS) is a subset of the FAS and included all subjects in Cohort 1 who received the first dose of mitapivat more than 24 weeks (≥169 days) before the data cutoff date.

As of the data cutoff date of 12 November 2020, 88 subjects were treated in the study, with 36 in Cohort 1, 35 in Cohort 2, and 17 in Cohort 3. Of the treated subjects in Cohort 1, 17 were included in the EAS.

Disposition of Subjects

A total of 10 (11.4%) subjects discontinued study treatment and 8 (9.1%) subjects discontinued the study prematurely. The primary reasons for discontinuation were lack of efficacy and withdrawal by subject.

Table 67 Summary of Subject Disposition (Full Analysis Set)

	Cohort 1 N=36 n (%)	Cohort 2 N=35 n (%)	Cohort 3 N=17 n (%)	Total N=88 n (%)
Disposition: End of treatment				
Discontinued	5 (13.9)	2 (5.7)	3 (17.6)	10 (11.4)
Reason for discontinuation				
Adverse event	0	1 (2.9)	0	1 (1.1)
Withdrawal by subject	3 (8.3)	0	1 (5.9)	4 (4.5)
Lack of efficacy	2 (5.6)	0	1 (5.9)	3 (3.4)
Other	0	1 (2.9)	1 (5.9)	2 (2.3)
Completed	0	0	0	0
Ongoing	31 (86.1)	33 (94.3)	14 (82.4)	78 (88.6)
Disposition: End of study				
Discontinued	4 (11.1)	1 (2.9)	3 (17.6)	8 (9.1)
Reason for discontinuation				
Withdrawal by subject	2 (5.6)	1 (2.9)	2 (11.8)	5 (5.7)
Lack of efficacy	2 (5.6)	0	1 (5.9)	3 (3.4)
Completed	0	0	0	0
Ongoing	32 (88.9)	34 (97.1)	14 (82.4)	80 (90.9)

Source: Table 14.1-1.2.1.
Note: The denominator used to calculate percentages is N, the number of subjects in the full analysis setwithin each treatment group.

Analysis of Efficacy

Haemoglobin Response in Cohort 1

Table 68 Summary of Haemoglobin Response (Efficacy Analysis Set)

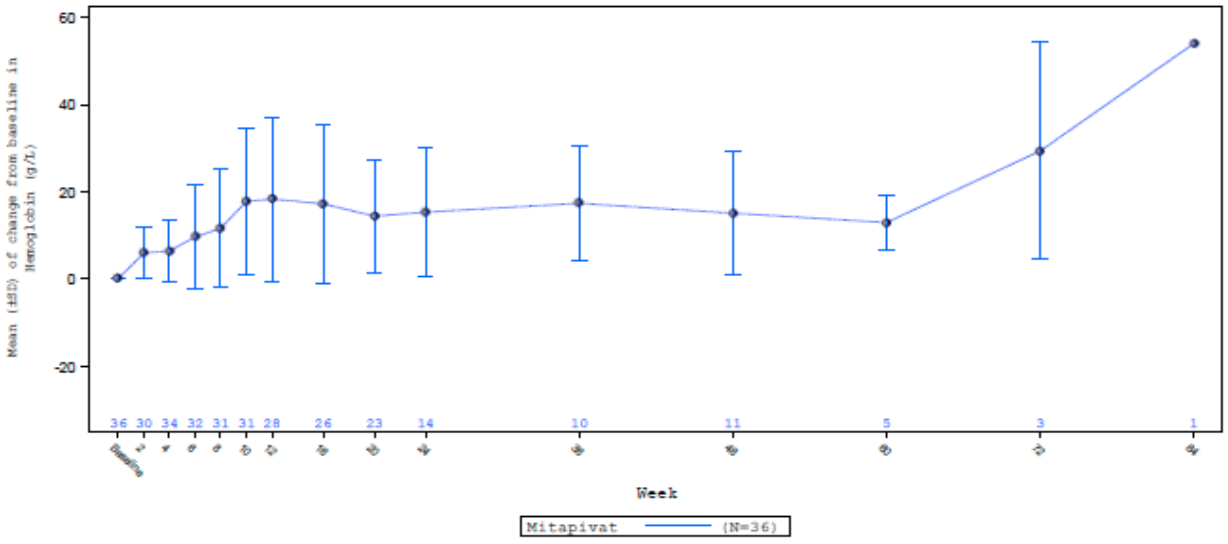
	Cohort 1 N=17
Hb Responders, n (%)	6 (35.3)
Average of weeks 16, 20 and 24 change from baseline	
n	17
Mean (SD)	14.51 (14.878)
Median (Q1, Q3)	10.00 (4.33, 21.00)
Min, Max	-7.0, 43.7

Source: Table 14.2-2.2.
Abbreviations: Hb = hemoglobin; max = maximum; min = minimum; Q1 = quartile 1; Q3 = quartile 3.
Notes: Hb responders: subjects who had a ≥15 g/L (1.5 g/dL) increase in Hb concentration from baseline that is sustained at 2 or more scheduled assessments at Weeks 16, 20, and 24 during the Fixed Dose Period, excluding those within 2 months (61 days) of transfusion.
The baseline value for Cohort 1 subjects is the average of all available measurements from the central laboratory within 45 (42+3) days before start of study treatment in Study AG348-C-011, excluding values within 61 days after a transfusion, or the baseline value from Study AG348-C-006 if no assessment is available.

Average Change from Baseline in Haemoglobin Concentration in Cohort 1

In the EAS of Cohort 1, the mean of the average change from baseline in Hb concentration across Weeks 16, 20, and 24 was 14.51 g/L.

Figure 33 Mean (±SD) of Haemoglobin over Time in Cohort 1 (Full Analysis Set)



Source: Figure 14.2-1.3.
Notes: The baseline value for Cohort 1 subjects is the average of all available measurements from the central laboratory within 45 (42+3) days before start of study treatment in Study AG348-C-011, excluding values within 61 days after a transfusion, or the baseline value from Study AG348-C-006 if no assessment was available.

In Cohort 1, mean Hb at baseline was 84.68 g/L, and the mean change from baseline was 15.29 g/L at Week 24.

Continued dosing with mitapivat (Cohort 2) resulted in sustained increases in Hb concentration compared with baseline; with a mean change from baseline of 16.14 g/L at Week 24.

In Cohort 3, 6 of 17 subjects with more than 12 weeks of mitapivat treatment in Study 011 who were transfusion-free achieved or maintained normal Hb as follows:

• One subject had at least 60 weeks of mitapivat treatment and reached normal Hb concentration once.

• Three subjects had at least 48 weeks of mitapivat treatment in study 011, with 1 subject reaching normal Hb concentrations once and 1 subject maintaining Hb at normal concentrations.

• Two subjects had at least 12 weeks of mitapivat treatment in Study 011, with 1 subject reaching normal Hb concentration once.

Study AG348-C-003

Refer to dose-response section for more details on methodology of the study.

Supportive and long-term efficacy data on the use of mitapivat for the treatment of patients with PK deficiency are provided in the Phase 2, open-label, 2-arm, multicentre, randomised, dose-ranging Study 003. All subjects were assigned mitapivat in this study, randomised 1:1 to a starting dose of 300 mg BID or 50 mg BID. The data cutoff date for the efficacy results is 28 August 2020.

As of the data cutoff date, enrollment has been completed; 52 subjects were randomised in the study, with 25 subjects randomised to the 300 mg arm and 27 subjects randomised to the 50 mg arm.

Efficacy Results

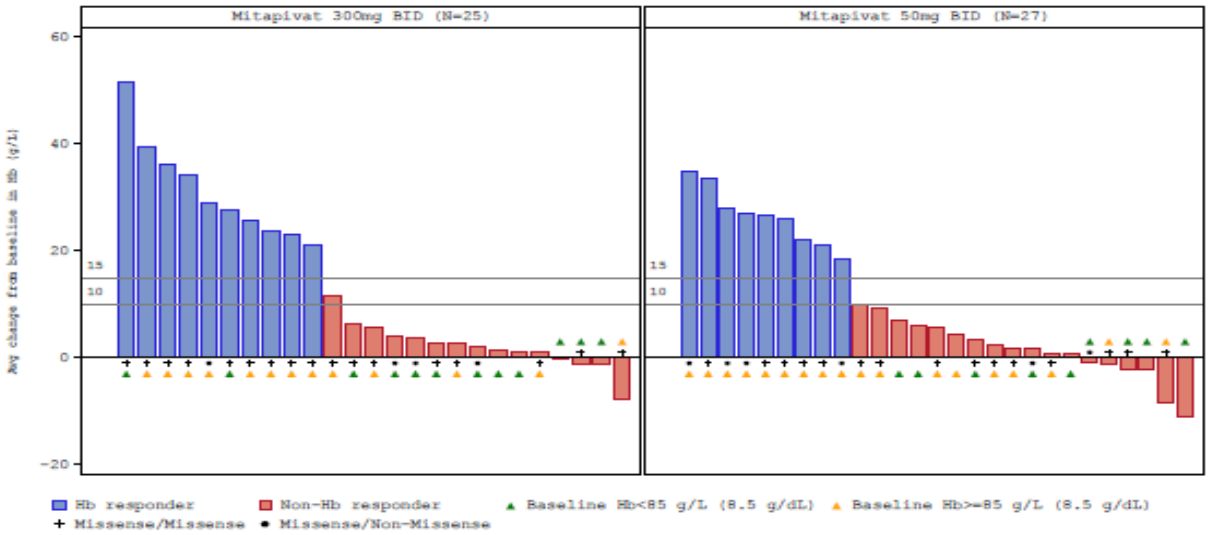
Haemoglobin

Table 9: Summary of Hemoglobin Response, Core Period (Full Analysis Set)

	Mitapivat 300 mg BID[1] N=25	Mitapivat 50 mg BID[1] N=27	Total N=52
Subjects with hemoglobin response, n (%)	10 (40.0)	9 (33.3)	19 (36.5)
95% CI[2]	(21.1, 61.3)	(16.5, 54.0)	(23.6, 51.0)

Source: Table 14.2-1.2 (data cutoff date: 28 August 2020).
Note: Hemoglobin response is defined as change from baseline in hemoglobin of ≥15 g/L (1.5 g/dL) at >50% of assessments in the Core Period, excluding those within 61 days after a transfusion.
1. Data are presented by treatment arm. Most subjects underwent dose modifications throughout the study, resulting in a broad range of doses administered throughout the study and received individually (as discussed in Section 10.1).
2. Based on Clopper-Pearson method.

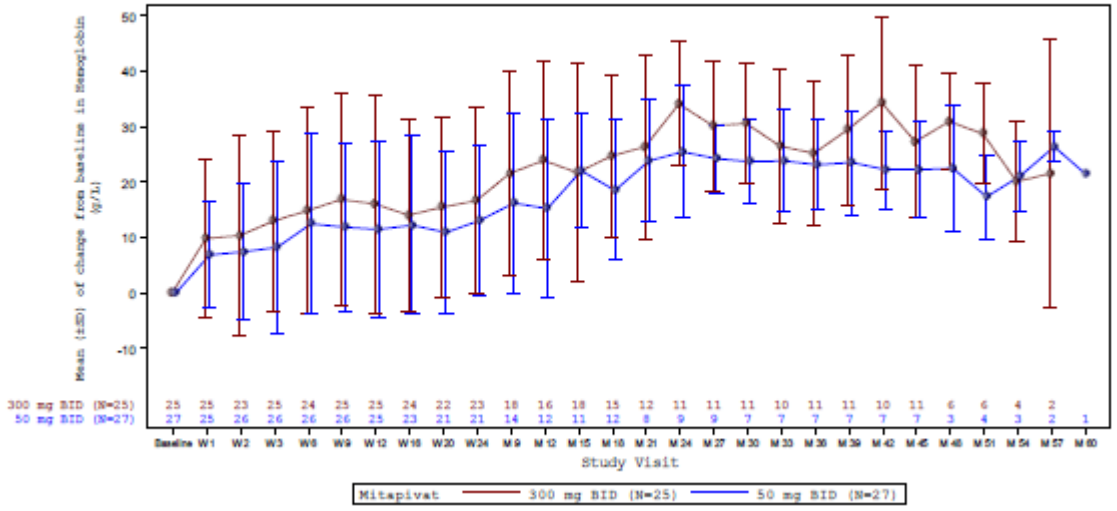
Figure 2: Waterfall Plot of Average Change From Baseline in Hemoglobin in Core Period (Full Analysis Set)



Source: Figure 14.2-5 (data cutoff date: 28 August 2020).
Abbreviations: Avg = average; BID = twice daily; Hb = hemoglobin.
Note: Baseline is defined as the average of all screening assessments within 45 (42+3) days before randomization for subjects randomized and not dosed or before start of study treatment for subjects randomized and dosed. Assessments collected within 61 days after a transfusion are excluded from the baseline derivation.

Mean Hb was low at baseline (89.5 g/L). Mitapivat treatment led to early and sustained increases in Hb concentrations throughout the core Period and in the extension Period.

Figure 3: Change From Baseline in Hemoglobin (Full Analysis Set)



All subjects who achieved Hb response in the Core Period and continued into the Extension Period maintained Hb levels that were increased from baseline in the Extension Period.

Haemoglobin response by mutation stratum group and PKLR mutation category is summarised in table below.

Table 69 Summary of Haemoglobin Response by Mutation Group – Core Period (Full Analysis Set)

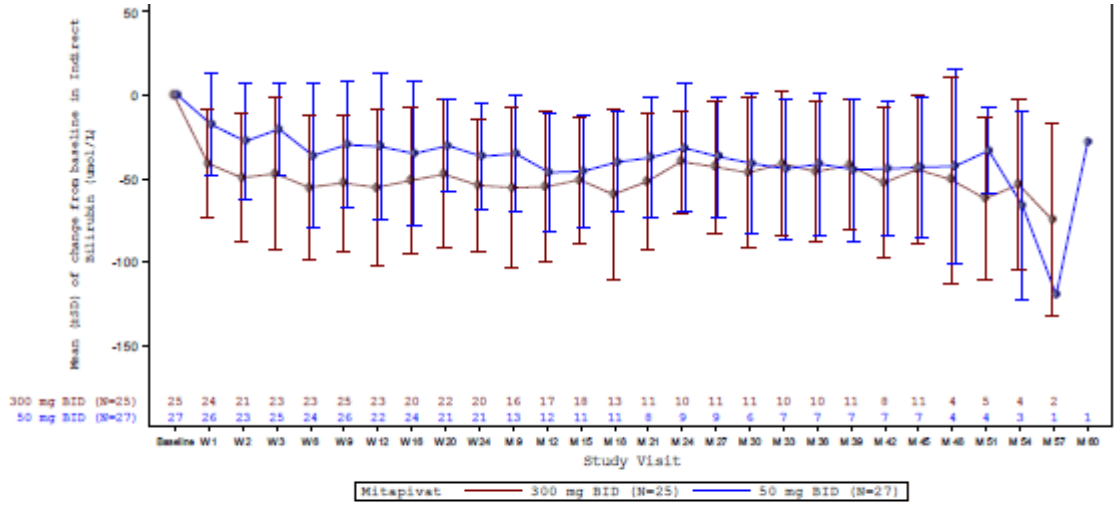
Table 14.2-1.3
Summary of Hemoglobin Response by Mutation Group - Core Period
Full Analysis Set

	Mitapivat 300 mg BID N=25		Mitapivat 50 mg BID N=27		Total N=52	
	Response Rate % (n/N1)	95% CI	Response Rate % (n/N1)	95% CI	Response Rate % (n/N1)	95% CI
Mutation stratum group						
R486W	100 (2/2)	(15.8, 100]	100 (3/3)	(29.2, 100]	100 (5/5)	(47.8, 100]
R510Q	28.6 (2/7)	(3.7, 71.0)	0 (0/7)	[0, 41.0)	14.3 (2/14)	(1.8, 42.8)
R479H	50.0 (2/4)	(6.8, 93.2)	0 (0/4)	[0, 60.2)	25.0 (2/8)	(3.2, 65.1)
OTHER	33.3 (4/12)	(9.9, 65.1)	46.2 (6/13)	(19.2, 74.9)	40.0 (10/25)	(21.1, 61.3)
PKLR mutation category						
Missense/Missense	52.9 (9/17)	(27.8, 77.0)	33.3 (5/15)	(11.8, 61.6)	43.8 (14/32)	(26.4, 62.3)
Missense/Non-Missense	25.0 (1/4)	(0.6, 80.6)	66.7 (4/6)	(22.3, 95.7)	50.0 (5/10)	(18.7, 81.3)
Non-Missense/Non-Missense	0 (0/4)	[0, 60.2)	0 (0/6)	[0, 45.9)	0 (0/10)	[0, 30.8)

Haemolysis

Mean indirect bilirubin was high at baseline (86.2 µmol/L).

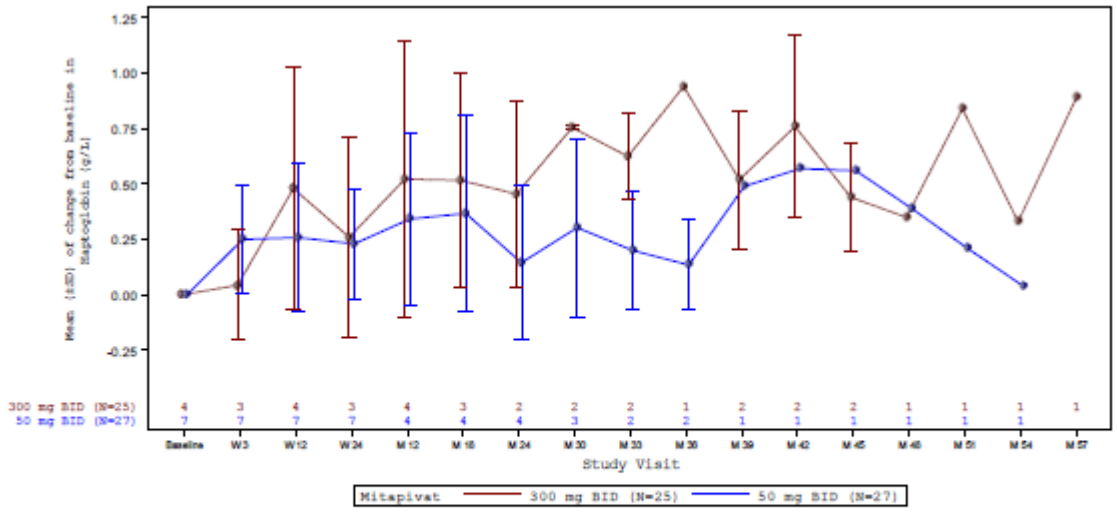
Figure 34: Change From Baseline in Indirect Bilirubin (Full Analysis Set)



Source: Figure 14.2-4 (data cutoff date: 28 August 2020).
Abbreviations: BID = twice daily; M = month; SD = standard deviation; W = week.
Note: Baseline is defined as the average of all screening assessments within 45 (42+3) days before randomization for subjects randomized and not dosed or before start of study treatment for subjects randomized and dosed. Assessments collected within 61 days after a transfusion are excluded from the baseline derivation.

Mean Hp remained normal (reference range [RR]: 0.3-2.0 g/L) throughout the study (Figure below).

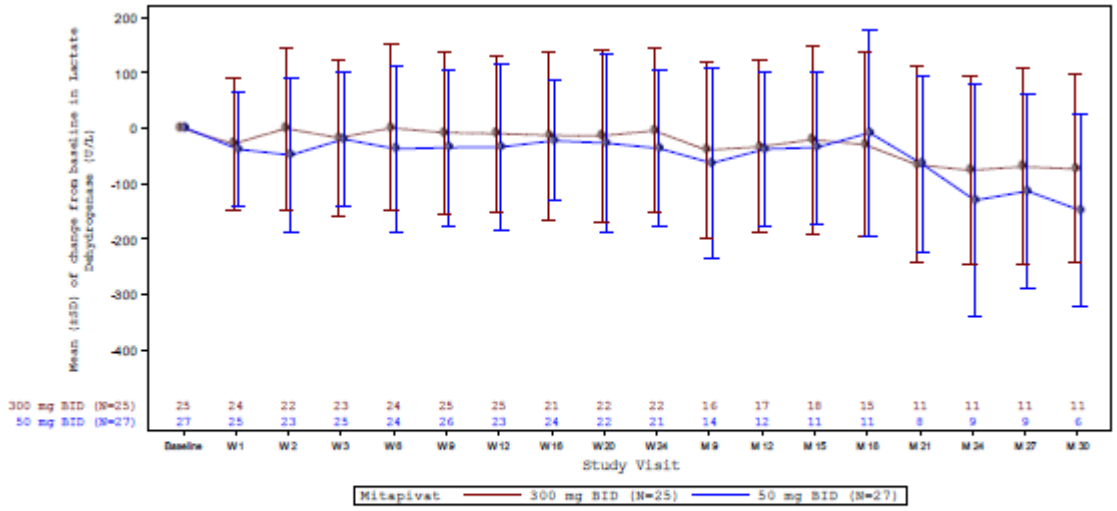
Figure 35: Change From Baseline in Haptoglobin (Full Analysis Set)



Source: Figure 14.2-4 (data cutoff date: 28 August 2020).
Abbreviations: BID = twice daily; M = month; SD = standard deviation; W = week.
Note: Baseline is defined as the average of all screening assessments within 45 (42+3) days before randomization for subjects randomized and not dosed or before start of study treatment for subjects randomized and dosed. Assessments collected within 61 days after a transfusion are excluded from the baseline derivation.

Mean LDH was high at baseline (272.9 U/L).

Figure 36: Change From Baseline in Lactase Dehydrogenase (Full Analysis Set)

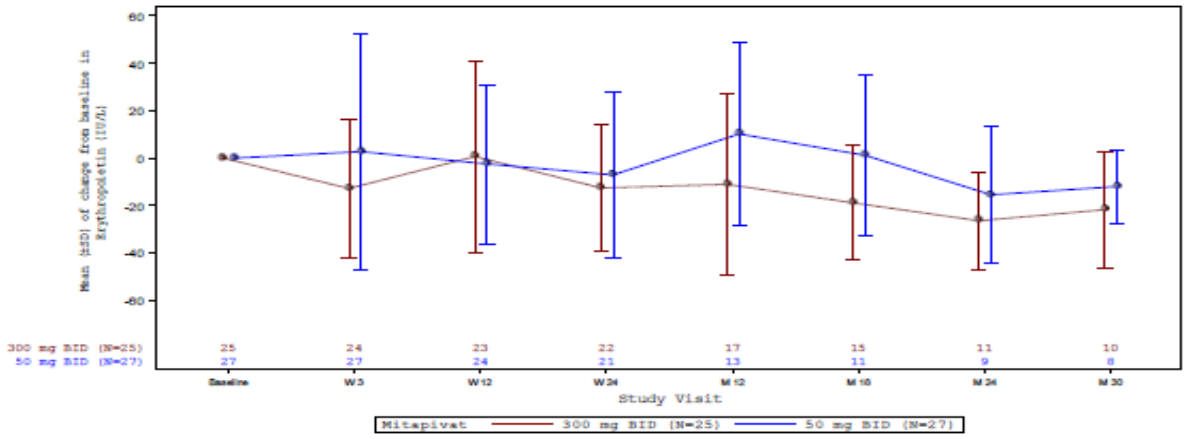


Source: Figure 14.2-4 (data cutoff date: 28 August 2020).
Abbreviations: BID = twice daily; M = month; SD = standard deviation; W = week.
Note: Baseline is defined as the average of all screening assessments within 45 (42+3) days before randomization for subjects randomized and not dosed or before start of study treatment for subjects randomized and dosed. Assessments collected within 61 days after a transfusion are excluded from the baseline derivation.

Change From Baseline in Markers of Erythropoietic Activity

Mean EPO was high (RR: 4.3-29.0 IU/L) at baseline (73.7 IU/L).

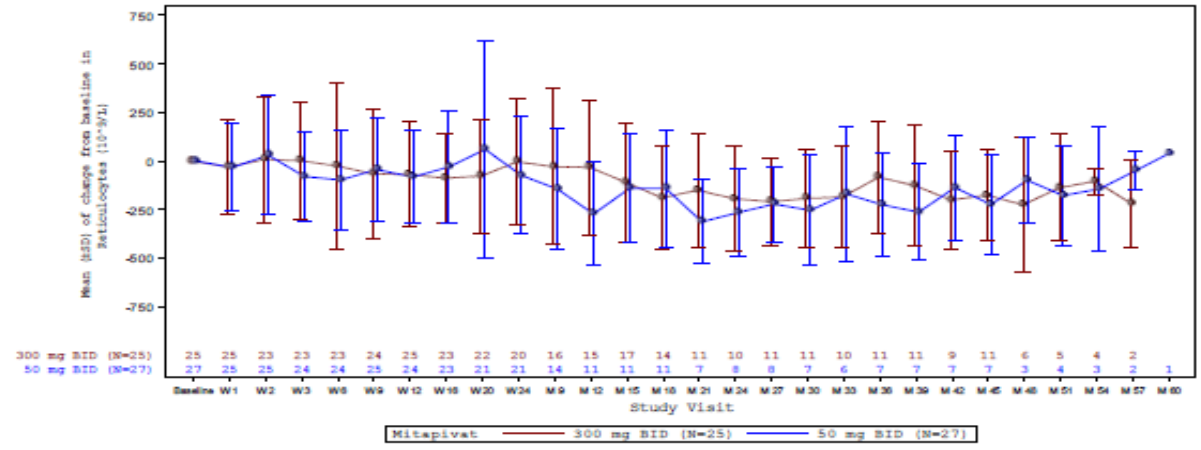
Figure 37: Change From Baseline in Erythropoietin (Full Analysis Set)



Source: Figure 14.2-4 (data cutoff date: 28 August 2020).
Abbreviations: BID = twice daily; M = month; SD = standard deviation; W = week.
Note: Baseline is defined as the average of all screening assessments within 45 (42+3) days before randomization for subjects randomized and not dosed or before start of study treatment for subjects randomized and dosed.
Assessments collected within 61 days after a transfusion are excluded from the baseline derivation.

Mean reticulocyte count was high at baseline (466.5 × 109/L).

Figure 38: Change From Baseline in Reticulocyte Count (Full Analysis Set)

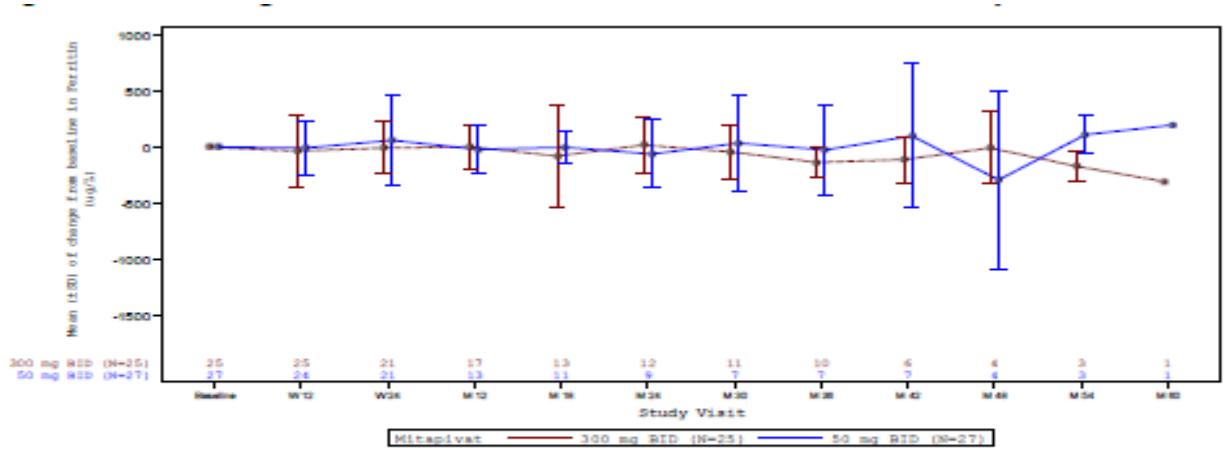


Source: Figure 14.2-4 (data cutoff date: 28 August 2020).
Abbreviations: BID = twice daily; M = month; SD = standard deviation; W = week.
Note: Baseline is defined as the average of all screening assessments within 45 (42+3) days before randomization for subjects randomized and not dosed or before start of study treatment for subjects randomized and dosed.
Assessments collected within 61 days after a transfusion are excluded from the baseline derivation

Markers of Iron Metabolism and Indicators of Iron Overload

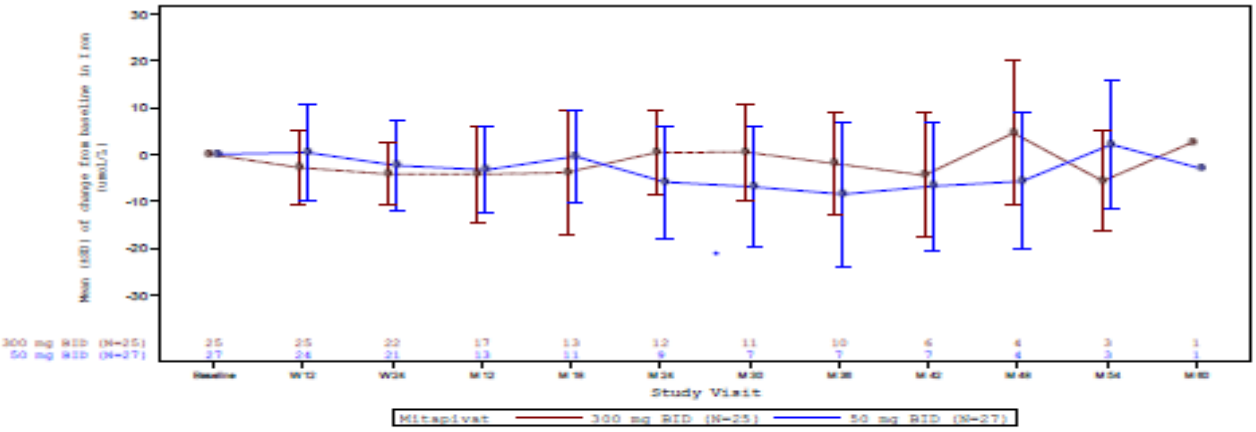
Levels of markers of iron metabolism and indicators of iron overload (ie, ferritin, transferrin saturation, iron, total iron-binding capacity [TIBC], hepcidin) either remained stable or improved during both the core Period and the extension Period.

Figure 39 Change from Baseline in Ferritin (AG348-C-003, Full Analysis Set)



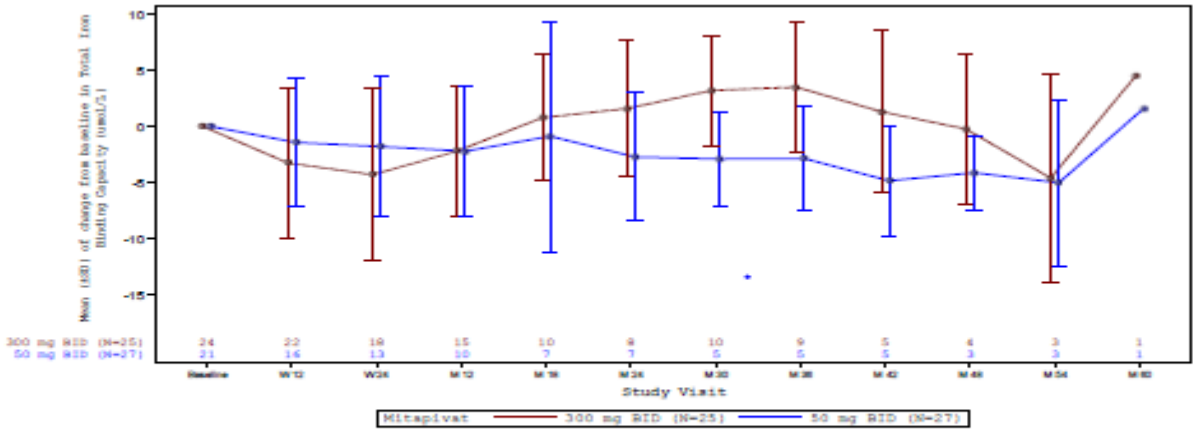
Source: CSR AG348-C-003, Figure 14.2-4 (data cutoff date: 28 August 2020).
Abbreviations: BID = twice daily; M = month; W = week.
Notes: Baseline is defined as the average of all screening assessments within 45 (42+3) days before randomization for subjects randomized and not dosed or before start of study treatment for subjects randomized and dosed. Assessments collected within 61 days after a transfusion are excluded from the baseline derivation.

Figure 40 Change from Baseline in Iron (AG348-C-003, Full Analysis Set)



Source: CSR AG348-C-003, Figure 14.2-4 (data cutoff date: 28 August 2020).
Abbreviations: BID = twice daily; M = month; W = week.
Notes: Baseline is defined as the average of all screening assessments within 45 (42+3) days before randomization for subjects randomized and not dosed or before start of study treatment for subjects randomized and dosed. Assessments collected within

Total iron-binding capacity levels remained stable throughout the Core Period and in the Extension Period. Change from baseline in hepcidin levels is provided in the figure below.



Source: AG348-C-003, Figure 14.2-4 (data cutoff date: 28 August 2020).
Abbreviations: BID = twice daily; M = month; W = week.
Notes: Baseline is defined as the average of all screening assessments within 45 (42+3) days before randomization for subjects randomized and not dosed or before start of study treatment for subjects randomized and dosed. Assessments collected within 61 days after a transfusion are excluded from the baseline derivation.

2.5.6. Discussion on clinical efficacy

A MAA for mitapivat has been submitted in the proposed indication "treatment of pyruvate kinase deficiency (PK deficiency) in adult patients".

The primary support for the proposed indication is based on efficacy and safety results from the pivotal studies AG348-C-006 and AG348-C-007, in subjects with PK deficiency who are not regularly transfused and who are regularly transfused, respectively. Supportive data are provided from the ongoing long-term study AG348-C-011 of subjects previously treated on studies 006 and 007 and the extension period of the phase 2 study AG348-C-003.

In Study 003, a relationship between genotype and haemoglobin response was observed: patients with at least 1 missense mutation had a higher likelihood of a haemoglobin response. None of the patients with 2 drastic mutations nonmissense/nonmissense (NM/NM) had a haemoglobin response. In addition, a relationship between red cell PK protein level and haemoglobin response was observed, likely indicating that a minimum amount of full-length PK protein is required for activation by mitapivat.

Design and conduct of clinical study AG348-C-006

Study AG348-C-006 is a phase 3, multicentre, randomised, double-blind, placebo-controlled efficacy and safety study of mitapivat compared with placebo in subjects with PK deficiency who were not regularly receiving blood transfusions. The study consisted of a dose optimisation period (Part 1 of 12 weeks) where patients received Mitapivat through sequential doses of 5 mg BID, 20 mg BID, and 50 mg BID with dose increases to the next dose level every 4 weeks, followed by a fixed dose period (Part 2 of 12 weeks). Subjects were randomised 1:1 to receive either mitapivat or matching placebo. The randomisation was stratified by average of screening Hb concentrations (Hb <85 g/L vs Hb ≥85 g/L) and the PKLR gene mutation category (missense/missense vs missense/nonmissense).

The study 006 enrolled adult subjects with clinical laboratory confirmed PK deficiency and harbouring at least one missense mutation. Patients must be not regularly transfused (no more than 4 transfusion episodes in the 12-month period up to the first day of study treatment and no transfusions in the 3 months before the first day of treatment). Patients must have an average screening Hb concentration of ≤100g/L (10 g/dl; 6.21 mmol/L). The applicant clarified that 'symptomatic anaemia' patients with different clinical severity were included in the trial.

Patients homozygous for the R479H mutation or with 2 non-missense mutations [NM/NM]), without the presence of another missense mutation, in PKLR gene were excluded from phase 3 trials given lack/absence of efficacy in the phase 2 study. This was clearly reflected in the product information. There are some limitations to this categorisation, according to the type of mutations (missense/missense, missense/non-missense, non-missense/non-missense). For example, some missense mutations may result in marked protein instability or functional inactivity, or, although not directly involved in splicing consensus sequences, may result in altered splicing. On the other hand, some non-missense variants may not have a significant impact on the protein structure (Bianchi et al. Am J Hematol. 2020). Due to the intrinsic difficulties of examining the relationship between genotype-phenotype in PK deficiency, future studies should consider studying the relationship between residual PK protein level and clinical phenotype. The primary endpoint was the number of Hb responders defined as a ≥15 g/L (1.5 g/dL; 0.93 mmol/L) increase in Hb concentration from baseline that is sustained at 2 or more scheduled assessments at Weeks 16, 20, and 24 during the fixed dose period. The measure of Hb was performed centrally. In case of unavailability, results from a local laboratory may be used. The key secondary endpoint was the average change from baseline in Hb concentration at weeks 16, 20, and 24. Other secondary endpoints include haemolysis and haematopoietic activity biomarkers (indirect bilirubin, reticulocyte percentage, lactate dehydrogenase and haptoglobin) and PRO score: Pyruvate Kinase Deficiency Diary (PKDD) and Pyruvate Kinase Deficiency Impact Assessment (PKDIA) that assessed and captured changes in symptom burden and impact of HRQOL in subjects with PK deficiency treated with mitapivat, respectively.

This is a double-blind study. However, the subject's treatment assignment was unblinded to the subject, investigator and site personnel once a subject entered the extension study, and after the completion of the week 24 assessment. The SAP was finalised after most subjects had already entered the extension study, i.e., after most of them were unblinded. In this context, the difference in the multiplicity adjustment procedures between the protocol and the SAP could be of concern, with six secondary endpoints (indirect bilirubin, reticulocyte percentage, LDH, haptoglobin, PKDD and PKDIA) added to the fixed sequence testing procedure in the SAP compared to the protocol. Nevertheless, the applicant further described the blinding requirements until after week 24 assessment, the data access restrictions to the secondary laboratory variables, and provided the blinding maintenance and unblinding plan (missing from the initial submitted documentation), which clarified the study team blinding up to the study database lock. Based on these considerations, it is agreed that the type I error is unlikely to be inflated.

The lack of specification and validation of the PRO endpoints prior to the confirmatory phase 3 trial is a limitation to the results interpretation.

The SAP introduced the use of local laboratory assessments in the absence of central laboratory assessments for efficacy analyses. As this is in contradiction with the protocol wording, supplementary analyses of all efficacy data based on central laboratory assessments were requested from the applicant. The results were consistent with the study report.

The MMRM analyses for key secondary and secondary endpoints assume that data are missing at random (MAR). However, some data are probably missing not at random (MNAR), therefore the applicant was requested to perform additional analyses based on methods that do not assume MAR (pattern-mixture model analysis with a control-based imputation). These sensitivity analyses showed similar results as the study secondary analysis results.

Several laboratory efficacy parameters (indirect bilirubin, reticulocyte percentage, LDH, haptoglobin) appear to be positively skewed based on the descriptive statistics. The applicant was requested to investigate whether log-transformation could improve the normality of the distribution, and if so, perform supplementary analyses based on log-transformed data. Log-transformation generally improved the normality of the laboratory parameter distributions; it is acknowledged that the improvement was less clear for indirect bilirubin. More importantly, the supplementary analyses did not impact the study conclusions, with similar significance levels achieved for all parameters in comparison with the study report.

A total of 80 subjects were randomised and 79 (98.8%) subjects completed the study. The first subject was enrolled on 01-Oct-2018 and the last subject completed on 09-Oct-2020.

Patients included are overall representative of patients with PK deficiency not regularly transfused. Of the 80 subjects enrolled, most subjects were white (75%) and 10% were Asian. There were a higher proportion of women than man (60.0% vs 40%). The median age was 33 years with 42 subjects (52.5%) that were <35 years of age; 4 subjects were older than 65 years. 55 subjects (68.8%) had a missense/missense PKLR gene mutation and 25 subjects (31.3%) had missense/non missense PKLR gene mutation.

Baseline Hb was similar in the mitapivat arm and the placebo arm. Most subjects (72.5%) had prior splenectomy and cholecystectomy. Most subjects had elevated ferritin levels at baseline. 74% of subjects (59 subjects) had no prior transfusion history, 19% of subjects (15 subjects) had 1 prior transfusion episode, and 5% of subjects (4 subjects) had 3 prior transfusion episodes in the 12-month period up to the first day of study treatment. Although subjects with Hb >100 g/L were excluded from the pivotal study 006, an Hb response was observed in 5 subjects among the 8 subjects with baseline Hb >100g/L enrolled in the phase 2 study 003 (Hb response rate of 62.5%, 95%CI: (24.5, 91.5)).

More subjects reported not receiving prior chelation therapy in the 12 months before the first day of study treatment (on both arms) compared with those who did receive it, with a lower percentage of subjects reporting prior chelation therapy in the mitapivat arm (12.5%) compared with the placebo arm (25.0%).

At baseline, more than 50% of subjects had decreased bone mineral density (T-score >-2.5 to <-1.0 or ≤-2.5) indicating osteopenia and osteoporosis. However, only 20 (25%) subjects had a medical history of osteopenia, and 11 (13.8%) subjects had a known medial history of osteoporosis.

The efficacy analysis was by intention to treat (N=80 subjects, with 40 subjects in each arm). 77.5% of the subjects randomised (n=62 subjects) were included in the PPS (82.5% in the mitapivat arm and 72.5% in the placebo arm) for sensitivity analysis.

Primary endpoint: For subjects with PK deficiency, the adjusted difference in Hb response rate was 39.3%, 95%CI (24.1, 54.6), 2-sided p-value<0.0001. 16 subjects (40%) in mitapivat arm were Hb responders. There were no Hb responders in the placebo arm.

Of the 16 subjects with Hb response, 13 subjects received a fixed dose of 50 mg BID, 2 subjects received 20 mg BID and 1 subject received 5 mg BID during the Fixed Dose Period. Resuts of sensitivity analyses of Hb response rate and change from baseline in Hb concentrations adjusting for concomitant transfusions received and excluding patients who received transfusions were consistent with the primary analysis.

Key secondary endpoint: The difference between both study arms in LS mean average change from baseline in Hb concentrations across Weeks 16, 20, and 24 was 18.21 g/L, 95% CI (12.41, 24.01), (2-sided p-value <0.0001).

Most subjects in the placebo arm experienced a decrease in Hb. Among subjects dosed with mitapivat, 16 subjects were responders and experienced increase in Hb more than 15 g/L, 19 subjects were non-responders but experienced some level of increase in Hb (<15 g/l) and 5 subjects experienced a decrease in Hb. Of 16 Hb responders in the mitapivat arm, the mean of average change from baseline in Hb concentration was 35.07 g/L. Of the 24 non-responders, the mean change was 4.45 g/L.

Among the 16 responders with PK deficiency, the median time to first ≥15 g/L increase in Hb concentration from baseline was 6.57 weeks (95% CI: 6.14, 8.14).

Subgroup analysis: Hb response rates and the average mean change from baseline in Hb at Weeks 16, 20, and 24 were higher in the mitapivat arm than the placebo arm across all pre-specified subgroups. However, subjects who did not have a prior splenectomy appear to draw more benefit in term of increase in Hb concentration than subjects who were splenectomised (difference of Hb response rate was 21.4% (95% CI: -4.2, 45.9) vs 83.3% (95% CI: 47.9, 97.9), difference in mean Hb average change from baseline was 11.63 g/l (95% CI: 6.22, 17.04) vs 34.26 g/l (95% CI: 24.62, 43.90)). The observed

difference in effect of mitapivat on Hb changes and transfusion burden in subjects with PK deficiency with and without splenectomy may be due to incompletely compensated haemolysis in patients with splenectomy.

Secondary endpoints: Consistent with the mechanism of action of mitapivat, it is noticed a moderate improvement in PD biomarkers of haemolysis (indirect bilirubin, LDH, and haptoglobin) and biomarkers of haematopoietic activity (reticulocyte percentage), in subjects treated with mitapivat compared with placebo during the fixed dose period. However, some missing assessments at several time are noticed.

Difference in LS mean average change from baseline in indirect bilirubin, LDH and haptoglobin across Weeks 16, 20, and 24 was -26.26 µmol/L (95% CI (-37.82, -14.70); 2-sided p-value <0.0001)), -70.81 U/L (95% CI (-115.88, -25.74), 2-sided p-value <0.0027) and 0.158 g/L (95% CI (0.043, 0.273), 2-sided p-value <0.0079), respectively.

Transfusions were indicated for clinically significant or poorly tolerated anaemia in 2 (5.0%) subjects in the mitapivat arm (2 units in 1 subject and 4 units in the other subject) and 5 (12.8%) subjects in the placebo arm (2 units per subject in 4 subjects and 1 unit in 1 subject).

No improvement is noticed in iron biomarkers with study 006. However, data interpretation of iron markers is limited due to short-term duration. The controlled part is limited to 24 weeks.

The difference in LS mean (SE) on the PKDD weekly mean score between the 2 arms at Week 24 was -3.11 (1.352), (p value=0.0247) not reaching the range for meaningful change. The difference in LS mean (SE) on the PKDIA score between the 2 arms at Week 24 was -3.25 (1.574) (p value =0.0421) not reaching the range of meaningful change.

PROs analyses are considered exploratory. The lack of specification and validation of the PRO endpoints (PKDD and PKDIA) prior to the confirmatory phase 3 trial is a limitation to the interpretation of results. New PRO instruments should be characterised and fully specified before the finalisation of the pivotal phase 3 protocol, especially if they were intended to be part of the confirmatory endpoints. The improvement observed in PKDD and PKDIA scores with mitapivat are not clinically significant. Results on PROs are thus not included in the SmPC.

Design and conduct of clinical study AG348-C-007

Study AG348-C-007 is a phase 3, multicentre, single-arm, open-label efficacy and safety study of mitapivat in subjects with PK deficiency who were regularly receiving blood transfusions. The study consisted of a dose optimisation period (Part 1 of 16 weeks) followed by a fixed-dose period (Part 2 of 24 weeks).

In study AG348-C-007, subjects who meet any of the following criteria during screening were not enrolled: be homozygous for the R479H mutation or have 2 non-missense mutations, without the presence of another missense mutation, in the PKLR gene or have a history of transfusions occurring on average more frequently than once every 3 weeks during the 52 weeks prior to signing the informed consent. However, there was no exclusion criterion limiting the number of RBC units transfused. Taking into consideration the MoA of mitapivat and the pathophysiology of the disease, it seems plausible that the treatment effect could be translated to these rare cases of patients who required transfusions more frequently than once every 3 weeks.

In PKD, there is currently no strategy of transfusing to keep the Hb above a set nadir with a goal of avoiding complications. Rather, transfusions are individualised, based on the patient's symptoms, level of activity, and assessment of the impact of the anaemia on their quality of life. In study 007, an Individual Transfusion Trigger (ITT) was calculated and the subject was to be transfused when they reached their ITT.

ITT is the mean (±0.5 g/dL or ±0.31 mmol/L) of subject's collected historical pre-transfusion Hb concentrations. The applicant confirmed that all subjects had complete records of transfusion history,

including transfusion dates, number of blood units transfused, and haemoglobin concentrations within 1 week before transfusion for at least 80% of transfusions.

The primary objective of the study was to evaluate the efficacy of treatment with mitapivat, as assessed by the reduction in transfusion burden which is considered a meaningful clinical endpoint in regularly transfused patients. Indeed, a substantial reduction of transfusion burden should decrease transfusional iron overload, prevention of iron accumulation and subsequent organ damage and, thus, the iron-chelation therapy requirements.

The proposed pivotal trial incorporates a variety of secondary endpoints that are relevant measures of improvement in TD patients with PK deficiency, including transfusion episode burden, proportion of subjects who became transfusion-free. PROs, markers of haemolysis, markers of iron metabolism and indicators of iron overload were exploratory endpoints.

The sample size was based on feasibility and left flexible with between 20 and 40 subjects to be enrolled. No criteria for stopping or continuing enrolment were pre-specified.

The null hypothesis, added with protocol amendment 1, uses a reference TRR rate of 10% for which no justification could be found. No further clarification was provided by the applicant on the quantification of the specific 10% value of the selected threshold. As a consequence, the corresponding hypothesis test (against the 10% TRR rate) remains unjustified, and the associated p-value was removed from the SmPC.

The definition of the primary endpoint was updated with the first protocol amendment and the sample size was increased with the second amendment. Based on these updates and the flexibility in the total number of subjects, the study results should be considered exploratory.

Due to the change in primary analysis pre-specification, the applicant was requested to repeat the primary endpoint analysis as per the original protocol analysis description, i.e., including the entire study Part 2 period regardless of treatment discontinuation / dose taper (not only the fixed dose period), and considering subjects who discontinued the study before the end of Part 2 as non-responders. The requested analysis has been performed, with results identical to the primary analysis results. It can be concluded that the change in analysis period definition had no impact on the study results.

Efficacy data and additional analyses

A total of 27 subjects received at least 1 dose of mitapivat, 21 (77.8%) subjects completed study treatment and 20 (74.1%) subjects completed the study.

A total of 20 study sites participated in this study. The first subject was enrolled on 26 June 2018 and the last subject completed on 12 November 2020.

Primary endpoint: Among the 27 subjects who received treatment, 10 patients (37% (95% CI: 19.4%, 57.6%)) were responders and had ≥ 33% reduction in total number of RBC units transfused during the fixed-dose period compared with the historical number of RBC units transfused standardised to 24 weeks.

The mean percent reduction from historical RBC units transfused was 37.1%. One responder achieved reduction between 33% and 50%, while the remaining 9 responders achieved a ≥50% reduction. 6 subjects did not experience any reduction from historical RBC units and 8 subjects experienced a slight (<20%) reduction.

As requested, the applicant provided an analysis of time to transfusion requirement during the study compared with the historical transfusion burden. The results showed that the time between transfusion requirements becomes longer, by approximately 1 month on average, with continued mitapivat treatment compared to that in the 52 weeks before informed consent.

As requested, a discussion of the cases in which a subject on study AG348-C-007 missed at least 1 transfusion after reaching their individual TT (mean individual pretransfusion haemoglobin [Hb] during the 52-week historical period, ±0.5 g/dL) has been presented by the applicant. A sensitivity analysis

(which classified as non-responder subjects who did not complete 12 weeks of treatment and subjects who missed a transfusion after reaching their TT for reasons other than "clinically asymptomatic") was performed. Two subjects were considered non-responders for purposes of this sensitivity analysis. The analysis showed that 8 subjects out of 27 were responders (29.6%; 95% CI: 13.8, 50.2, p= 0.0039).

Secondary endpoints: There were 6 transfusion-free responders, during fixed-dose period.

The percent mean reduction of transfusion episodes during the fixed-dose period compared with the historical RBC transfusion episodes standardised to 24 weeks was 39.57%, which was consistent with the mean percent reduction in the number of RBC units.

Three (11.1%) subjects achieved Hb concentrations in the normal range at least once, 8 weeks or more after last receiving a transfusion in the fixed-dose period. All 3 of these subjects were transfusion-free responders.

Subgroup analysis: Results of subgroup analysis of transfusion reduction responders (TRR) were homogenous across different subgroups based on sex, race, and baseline Hb level. Although, in the context of a single arm study with a small number of patients and some unbalanced subgroups it is difficult to draw any conclusion. However, a difference in the magnitude of TRR rate is observed in subgroups based on prior splenectomy status.

Exploratory endpoints: The mean (SD) reduction in PKDD score from baseline to dose optimisation week 12 was -5.3 (11.63) and was -2.4 (11.30) at fixed-dose period week 24 and did not reach the range of meaningful change. The mean (SD) reduction in PKDIA score from baseline to dose optimisation Period Week 12 was -4.9 (9.97) and was -9.1 (11.50) at fixed-dose period Week 24, reaching the range of meaningful change. These results are overall sustained for most time points during the extension study 011. However, a high proportion of missing data was noticed (N=14 at week 24 vs 24 patients at baseline). Overall results on improvement of signs and symptoms and disease impact as measured by PKDD and PKDI are very difficult to interpret in the context of single arm study and these results remains completely exploratory.

For transfusion-free responders, there was an improvement in haemolysis markers (indirect bilirubin, haptoglobin, and LDH) during both the Dose Optimisation Period and the Fixed-Dose Period (with most subjects reaching values within the normal range for these markers). However, due to the limited number of subjects, no conclusions can be drawn.

For non–transfusion-free responders, there were fluctuations in the levels of markers of haemolysis, but interpretation of these markers is limited by concomitant transfusions.

Study 011 is an ongoing multicentre, open-label extension study to evaluate the long-term safety, and efficacy of treatment in subjects who were previously enrolled in study 006 or Study 007. Subjects who received placebo in study 006 were assigned in cohort 1. Subjects who received mitapivat in study 006 were assigned in cohort 2. Subjects who received mitapivat in Study 007 were assigned in cohort 3.

The pooled analysis of cohort 1 and 2 at the DCO date of 12 November 2020 showed that after 24 weeks, the mean change from baseline was 15.98 g/L in Hb, 0.188 g/L in Hp, -22.43 µmol/L in indirect bilirubin, and -88.05 U/L in LDH for subjects treated with mitapivat. Slight to moderate improvement in markers of haemolysis was observed. However, it is difficult to contextualise the effect observed to clinical benefit particularly at long-term on the complications of the disease.

Updated long-term efficacy data for study 011 as of the cut-off date of 12 September 2021 were provided. Among the 31 NTD Hb responders, Hb response was ongoing for 26 Hb responders up to 29.1 months with continued mitapivat treatment. The median duration of Hb response was 15.1 months 95%CI (10.84, 19.58). Out of the 5 Hb responders who experienced loss of Hb response, 3 subjects continued to have Hb concentration increased by ≥15 g/L with ongoing treatment with mitapivat after the initial loss of Hb response (iei.e., loss of Hb response was due to transient dip in Hb).

In cohort 3 of the study 011, nine out of the 10 transfusion reduction responders had ongoing transfusion reduction response up to 30.7 months with continued mitapivat treatment. Among the 10 TD responders, the median duration of TRR was 24.0 months 95%CI (17.9, 28.0). In addition, 7 out of the 8 transfusion-

free responders maintained their transfusion-free response up to 31.8 months with continued mitapivat treatment. The median duration of transfusion-free response was 27.0 months.

Available long-term data suggest a slight non-significant improvement over time in some iron markers, as mean iron levels, hepcidin levels and transferrin saturation with mitapivat treatment but these does not show a change in ferritin levels.

At cut-off date of 12 September 2021 for study AG348-C-011, ferritin remained largely stable among NTD subjects treated with mitapivat. However, slight improvements in LIC by MRI were seen with continued mitapivat treatment in both Hb responders and Hb non-responders of the study 006. These improvements were unexpectedly more pronounced in Hb non-responders patients; mean change from baseline in LIC at week 48 was -0.39 (2.468) among Hb responders and -3.66 (13.511) among non-responder patients. The available data on use of chelators are not informative.

At cut-off date of 12 September 2021 for study AG348-C-011, updated data on iron burden (ferritin and LIC) were provided in transfusion free responders and transfusion reduction responders. Of the 2 transfusion-free responders with baseline LIC high (≥5 mg Fe/g dry weight), both subjects showed decreased levels of LIC to <5 mg F3/g dry weight after treatment with mitapivat. Out of 4 transfusion reduction responders with high baseline LIC levels, 3 subjects decreased LIC to <5 mg Fe/g dry weight after treatment with mitapivat. Although a longer follow-up is needed to highlight a significant impact on iron burden in transfused patients, the data provided support the conclusion that long-term treatment with mitapivat could have a beneficial impact on iron overload in patients with pyruvate kinase deficiency. In any case, a longer follow-up is needed to confirm these data.

None of the 6 transfusion-free responders increased their dose of iron chelation therapy; 1 decreased their dose of chelation therapy over the course of the study, and 2 discontinued chelation therapy completely. In addition, the responder who became transfusion-free in Study 011 also had a decrease in dose of chelation therapy during the study.

2.5.7. Conclusions on the clinical efficacy

In NTD patients, the adjusted difference in Hb response rate (mitapivat vs placebo) was 39.3%, (95% CI (24.1, 54.6), 2-sided p-value<0.0001). These results are overall sustained with the long treatment regimen. The reported results on HRQoL and improvement of symptoms of anaemia are not clinically meaningful and are considered exploratory given the lack of specification and validation of the PRO endpoints (PKDD and PKDIA) prior to the confirmatory study 006.

Among the 27 TD subjects who received treatment in study 007, 10 patients (37%) had ≥ 33% reduction in total number of RBC units transfused and there were 7 (26%) transfusion free responders. These results are overall sustained with long-term therapy.

The potential benefit of this therapy on iron overload was not sufficiently demonstrated. Long-term outcomes (i.e., gallstones, endocrine dysfunctions, leg ulcers and osteopenia, extramedullary haematopoiesis and pulmonary hypertension) are still unknown. The choice of the trial population, with the exclusion of patients based on genotype; those who had 2 non missense mutations without the presence of another missense mutation or were homozygous for the R479H mutation in PKLR gene, significantly limit the representativeness of PK deficient patients into the trial.

2.5.8. Clinical safety

Safety data for oral mitapivat (AG-348) in adult subjects with pyruvate kinase (PK) deficiency are mainly taken from 4 studies in adults:

- Study AG348-C-006 (Study 006 - ACTIVATE): Phase 3, multicenter, randomized, double-blind, placebo-controlled study evaluating the efficacy, safety, pharmacokinetics, and impacts on

HRQOL of mitapivat in adults with PK deficiency not regularly receiving transfusions (26 weeks - 80 subjects, completed);

- Study AG348-C-007 (Study 007 – ACTIVATE-T): Phase 3, multicenter, open-label study evaluating the efficacy and safety of mitapivat in adults with PK deficiency regularly receiving transfusions (42 weeks - 27 subjects, completed);
- Study AG348-C-003 (Study 003): Phase 2, multicenter, randomized, open-label, dose ranging study evaluating the safety and tolerability, pharmacokinetics, pharmacodynamics, and clinical activity of mitapivat in adults with PK deficiency not regularly receiving transfusions (52 subjects, ongoing);
- Study AG348-C-011 (Study 011): Phase 3, multicenter, open-label, long-term extension study evaluating the safety and efficacy of mitapivat in adults with PK deficiency previously enrolled in Study AG348 C-006 or AG348-C-007 (88 subjects, ongoing)
    - Cohort 1 : non regularly transfused subjects from the placebo arm of Study 006
    - Cohort 2 : non regularly transfused subjects from the experimental arm of Study 006
    - Cohort 3 : regularly transfused subjects from Study 007

Additional supportive safety data come from 7 completed phase 1 studies in healthy volunteers (n=249) and an ongoing phase 2 study (Study 010) in adult subjects (n=20) with non-transfusion-dependent thalassemia.

Only subjects from the pivotal studies 006 and 007 received mitapivat at the regimen considered for MA: a dose titration from 5 to 50 mg BID. Subjects from ongoing supportive studies 003 and 010 received fixed doses ranged from 50 mg BID to 300 mg BID.

- ***Patient exposure***

Overall, 400 adult subjects have received at least one dose of mitapivat:

- 225 healthy subjects with single doses ranged from 5 to 2.500 mg and multiple doses of 15 to 700 mg BID for 14 days
- 20 subjects with non-transfusion-dependant thalassemia (NTDT) with doses ranged from 50 to 100 mg BID for up to 29 weeks (~7.2 months)
- 155 subjects with PK deficiency, of whom 27 were regularly transfused and 128 were not. These subjects received doses ranged from 5 mg once daily (QD) to 300 mg twice a day (BID) for up to 58.7 months (~ 4.9 years)

Of the 155 subjects with PK deficiency, 103 received the MA treatment schedule (studies 006, 007 and 011), the subjects from Study 003 received either 50 mg BID (n=18) or 300 mg BID (n=34).

The median duration of exposure was comparable between regularly transfused (Study 007) and not regularly transfused (Study 006) subjects: 12.42 months (3.7, 28.6) and 11.55 months (6.0, 25.4), respectively. Exposure duration was longer in supportive Study 003.

In subjects treated at the MA regimen, most of dose reductions were due to planned discontinuation following withdrawal of consent (to be further discussed below). A sixth of dose reductions (5.8% of subjects) occurred following an adverse event.

The use of concomitant medications was comparable within the safety analysis set and in line with standard supporting treatment for PK deficiency (i.e. folic acid, vitamin B12, iron chelation therapy and antipyretics/analgesics).

Overall, demographics and baseline disease characteristics were homogeneous between groups and studies, confirming that the safety analysis set is a representative sample of the target population.

- ***Adverse events***

Safety data are presented over two analysis periods:

- the 24-week period, which corresponds to the on-treatment period
- the cumulative period, which is the on-treatment period along with 28 days after the end of study treatment.

As subjects who initially received placebo in Study 006 were eligible for mitapivat treatment in extension Study 011, no meaningful comparisons could be made between the mitapivat and the placebo arms of Study 006 for the cumulative period. Therefore, safety assessment will mostly be based on the summaries of data provided for the 24-week period.

Overview of TEAEs

Table 70 Overall Summary of Treatment-Emergent Adverse Events – 24-Week Period (Safety Analysis Set)

	Mitapivat							Placebo
	Study 003		Study 007	Study 011 Cohort 1	Study 006	Total		Study 006
Number (%) Subjects With	≤50 mg BID N=20 n (%)	>50 mg BID N=32 n (%)	≤50 mg BID N=27 n (%)	≤50 mg BID N=36 n (%)	≤50 mg BID N=40 n (%)	≤50 mg BID N=123 n (%)	All Doses N=155 n (%)	N=39 n (%)
Any TEAEs	19 (95.0)	32 (100)	24 (88.9)	35 (97.2)	35 (87.5)	113 (91.9)	145 (93.5)	35 (89.7)
Grade ≥3 TEAEs	2 (10.0)	8 (25.0)	4 (14.8)	3 (8.3)	10 (25.0)	19 (15.4)	27 (17.4)	5 (12.8)
Treatment-related TEAEs	10 (50.0)	29 (90.6)	17 (63.0)	17 (47.2)	23 (57.5)	67 (54.5)	96 (61.9)	14 (35.9)
Grade ≥3 treatment-related TEAEs	0	4 (12.5)	1 (3.7)	0	3 (7.5)	4 (3.3)	8 (5.2)	0
Serious TEAEs	2 (10.0)	4 (12.5)	2 (7.4)	2 (5.6)	4 (10.0)	10 (8.1)	14 (9.0)	2 (5.1)
Serious treatment-related TEAEs	0	2 (6.3)	0	0	1 (2.5)	1 (0.8)	3 (1.9)	0
TEAEs leading to discontinuation of study drug	0	3 (9.4)	0	0	0	0	3 (1.9)	0
TEAEs leading to dose reduction of study drug	2 (10.0)	7 (21.9)	1 (3.7)	0	0	3 (2.4)	10 (6.5)	0
TEAEs leading to interruption of study drug	2 (10.0)	5 (15.6)	0	1 (2.8)	0	3 (2.4)	8 (5.2)	2 (5.1)
TEAEs leading to death	0	0	0	0	0	0	0	0
Treatment-related TEAEs leading to death	0	0	0	0	0	0	0	0

Abbreviations: BID = twice daily; TEAE = treatment-emergent adverse event.

Note: The denominator used to calculate percentages is N, the number of subjects in the Safety Analysis Set within each treatment group.

Common adverse events

Table 71 Summary of Most Common (Any Grade in ⩾10% Subjects Any Treatment Group) Treatment-Emergent Adverse Events by MedDRA Preferred Term and Worst CTCAE Grade – 24-Week Period (Safety Analysis Set)

	Mitapivat							Placebo
	Study 003		Study 007	Study 011 Cohort 1	Study 006	Total		Study 006
Preferred Term (PT)	≤50 mg BID N=20 n (%)	>50 mg BID N=32 n (%)	≤50 mg BID N=27 n (%)	≤50 mg BID N=36 n (%)	≤50 mg BID N=40 n (%)	≤50 mg BID N=123 n (%)	All Doses N=155 n (%)	N=39 n (%)
Subjects with events	19 (95.0)	32 (100)	24 (88.9)	35 (97.2)	35 (87.5)	113 (91.9)	145 (93.5)	35 (89.7)
Headache	6 (30.0)	17 (53.1)	10 (37.0)	17 (47.2)	6 (15.0)	39 (31.7)	56 (36.1)	13 (33.3)
Nausea	8 (40.0)	11 (34.4)	5 (18.5)	3 (8.3)	6 (15.0)	22 (17.9)	33 (21.3)	8 (20.5)
Nasopharyngitis	5 (25.0)	5 (15.6)	3 (11.1)	4 (11.1)	5 (12.5)	17 (13.8)	22 (14.2)	6 (15.4)
Back pain	4 (20.0)	3 (9.4)	4 (14.8)	1 (2.8)	5 (12.5)	14 (11.4)	17 (11.0)	3 (7.7)
Fatigue	3 (15.0)	6 (18.8)	5 (18.5)	1 (2.8)	4 (10.0)	13 (10.6)	19 (12.3)	4 (10.3)
Arthralgia	3 (15.0)	5 (15.6)	0	4 (11.1)	4 (10.0)	11 (8.9)	16 (10.3)	2 (5.1)
Diarrhoea	3 (15.0)	3 (9.4)	2 (7.4)	2 (5.6)	4 (10.0)	11 (8.9)	14 (9.0)	7 (17.9)
Dizziness	5 (25.0)	2 (6.3)	1 (3.7)	1 (2.8)	4 (10.0)	11 (8.9)	13 (8.4)	3 (7.7)
Alanine aminotransferase increased	1 (5.0)	1 (3.1)	8 (29.6)	1 (2.8)	1 (2.5)	11 (8.9)	12 (7.7)	6 (15.4)
Middle insomnia	0	0	2 (7.4)	5 (13.9)	3 (7.5)	10 (8.1)	10 (6.5)	3 (7.7)
Initial insomnia	1 (5.0)	0	2 (7.4)	7 (19.4)	0	10 (8.1)	10 (6.5)	4 (10.3)
Oropharyngeal pain	3 (15.0)	4 (12.5)	2 (7.4)	0	3 (7.5)	8 (6.5)	12 (7.7)	2 (5.1)
Insomnia	4 (20.0)	17 (53.1)	1 (3.7)	0	2 (5.0)	7 (5.7)	24 (15.5)	0
Vomiting	2 (10.0)	5 (15.6)	4 (14.8)	0	1 (2.5)	7 (5.7)	12 (7.7)	1 (2.6)
Abdominal pain	0	2 (6.3)	2 (7.4)	1 (2.8)	4 (10.0)	7 (5.7)	9 (5.8)	2 (5.1)
Hot flush	2 (10.0)	7 (21.9)	1 (3.7)	0	3 (7.5)	6 (4.9)	13 (8.4)	0
Cough	3 (15.0)	5 (15.6)	0	0	3 (7.5)	6 (4.9)	11 (7.1)	2 (5.1)
Influenza	4 (20.0)	3 (9.4)	1 (3.7)	0	1 (2.5)	6 (4.9)	9 (5.8)	0
Pain in extremity	2 (10.0)	2 (6.3)	0	2 (5.6)	2 (5.0)	6 (4.9)	8 (5.2)	3 (7.7)

	Mitapivat							Placebo
	Study 003		Study 007	Study 011 Cohort 1	Study 006	Total		Study 006
Preferred Term (PT)	≤50 mg BID N=20 n (%)	>50 mg BID N=32 n (%)	≤50 mg BID N=27 n (%)	≤50 mg BID N=36 n (%)	≤50 mg BID N=40 n (%)	≤50 mg BID N=123 n (%)	All Doses N=155 n (%)	N=39 n (%)
Nasal congestion	3 (15.0)	2 (6.3)	0	1 (2.8)	2 (5.0)	6 (4.9)	8 (5.2)	3 (7.7)
Hypertriglyceridaemia	1 (5.0)	4 (12.5)	1 (3.7)	0	3 (7.5)	5 (4.1)	9 (5.8)	0
Dry mouth	2 (10.0)	0	1 (3.7)	1 (2.8)	1 (2.5)	5 (4.1)	5 (3.2)	0
Viral upper respiratory tract infection	0	0	4 (14.8)	0	1 (2.5)	5 (4.1)	5 (3.2)	1 (2.6)
Dyspepsia	1 (5.0)	4 (12.5)	2 (7.4)	0	1 (2.5)	4 (3.3)	8 (5.2)	2 (5.1)
Asthenia	2 (10.0)	2 (6.3)	0	1 (2.8)	1 (2.5)	4 (3.3)	6 (3.9)	1 (2.6)
Aspartate aminotransferase increased	0	1 (3.1)	3 (11.1)	0	1 (2.5)	4 (3.3)	5 (3.2)	3 (7.7)
Upper respiratory tract infection	2 (10.0)	1 (3.1)	1 (3.7)	1 (2.8)	0	4 (3.3)	5 (3.2)	4 (10.3)
Rash	0	4 (12.5)	2 (7.4)	1 (2.8)	0	3 (2.4)	7 (4.5)	3 (7.7)
Dysmenorrhoea	1 (5.0)	6 (18.8)	0	0	1 (2.5)	2 (1.6)	8 (5.2)	3 (7.7)
Pyrexia	0	6 (18.8)	0	2 (5.6)	0	2 (1.6)	8 (5.2)	2 (5.1)
Chest discomfort	1 (5.0)	4 (12.5)	0	0	1 (2.5)	2 (1.6)	6 (3.9)	0
Sneezing	2 (10.0)	0	0	0	0	2 (1.6)	2 (1.3)	0
Night sweats	0	4 (12.5)	0	0	0	0	4 (2.6)	1 (2.6)

Abbreviations: BID = twice daily; CTCAE = Common Terminology Criteria for Adverse Events; MedDRA = Medical Dictionary for Regulatory Activities.
Notes: Summarised in order of decreasing frequency of subjects with events based on the frequencies observed for total subjects in mitapivat ≤50 mg BID group.
The denominator used to calculate percentages is N, the number of subjects in the Safety Analysis Set within each treatment group.
Subjects with multiple adverse events within a PT are counted only once in that PT.
For subjects with multiple occurrences of an adverse event, the adverse event with the maximum CTCAE grade is included in the summary.
MedDRA Version 23.1 and CTCAE Version 4.03 are used.

Grade ≥3 adverse events

During the 24-week period, 10 subjects (25.0%) who received mitapivat in Study 006 had a Grade ≥3 TEAE. Hypertriglyceridaemia and Hypertension each occurred in 2 subjects (5.0% each). Events of Syncope, Musculoskeletal pain, Rib fracture occurred once each in the same treatment group (2.5% each).

In regularly transfused subjects (Study 007), there were 4 subjects (14.8%) with Grade ≥3 TEAEs. Each event occurred once: Jugular vein occlusion, Back Pain, Joint swelling and Ovarian cyst (3.1% each).

During the 24-week period, more Grade ≥3 TEAEs have been reported in not regularly transfused subjects (Study 006) compared to regularly transfused ones (Study 007). This difference is smoothed over the cumulative period.

Treatment-related TEAEs

Table 72 Summary of Treatment-Emergent Adverse Events Related to Study Treatment, by System Organ Class and Preferred Term –24-Week Period (Safety Analysis Set)

Table 18.3.1-2.5a
Summary of Treatment-Emergent Adverse Events Related to Study Treatment, by System Organ Class and Preferred Term - 24-Week Period
Safety Analysis Set

	Mitapivat							Placebo
	Study 003		Study 007	Study 011 Cohort 1	Study 006	Total		Study 006
System Organ Class (SOC) Preferred Term (PT)	≤50 mg BID N=20 n (%)	>50 mg BID N=32 n (%)	≤50 mg BID N=27 n (%)	≤50 mg BID N=36 n (%)	≤50 mg BID N=40 n (%)	≤50 mg BID N=123 n (%)	All Doses N=155 n (%)	N=39 n (%)
Subjects with events	10 (50.0)	29 (90.6)	17 (63.0)	17 (47.2)	23 (57.5)	67 (54.5)	96 (61.9)	14 (35.9)
Gastrointestinal disorders	7 (35.0)	14 (43.8)	6 (22.2)	4 (11.1)	9 (22.5)	26 (21.1)	40 (25.8)	6 (15.4)
Nausea	7 (35.0)	9 (28.1)	4 (14.8)	2 (5.6)	6 (15.0)	19 (15.4)	28 (18.1)	4 (10.3)
Dry mouth	2 (10.0)	0	1 (3.7)	1 (2.8)	1 (2.5)	5 (4.1)	5 (3.2)	0
Diarrhoea	1 (5.0)	2 (6.3)	0	1 (2.8)	1 (2.5)	3 (2.4)	5 (3.2)	1 (2.6)
Dyspepsia	1 (5.0)	2 (6.3)	1 (3.7)	0	1 (2.5)	3 (2.4)	5 (3.2)	1 (2.6)
Abdominal distension	1 (5.0)	1 (3.1)	0	0	1 (2.5)	2 (1.6)	3 (1.9)	0
Constipation	1 (5.0)	1 (3.1)	0	0	1 (2.5)	2 (1.6)	3 (1.9)	0
Vomiting	1 (5.0)	1 (3.1)	1 (3.7)	0	0	2 (1.6)	3 (1.9)	0
Abdominal pain	0	1 (3.1)	0	0	1 (2.5)	1 (0.8)	2 (1.3)	0
Gastrooesophageal reflux disease	0	0	0	0	1 (2.5)	1 (0.8)	1 (0.6)	0
Gastric ulcer	0	0	1 (3.7)	0	0	1 (0.8)	1 (0.6)	0
Impaired gastric emptying	0	0	1 (3.7)	0	0	1 (0.8)	1 (0.6)	0
Lip ulceration	0	0	1 (3.7)	0	0	1 (0.8)	1 (0.6)	0
Abdominal pain upper	0	2 (6.3)	0	0	0	0	2 (1.3)	0
Gastrointestinal mucosal disorder	0	0	0	0	0	0	0	1 (2.6)
Nervous system disorders	4 (20.0)	10 (31.3)	8 (29.6)	7 (19.4)	6 (15.0)	25 (20.3)	35 (22.6)	6 (15.4)
Headache	3 (15.0)	9 (28.1)	5 (18.5)	7 (19.4)	5 (12.5)	20 (16.3)	29 (18.7)	6 (15.4)
Dizziness	2 (10.0)	1 (3.1)	0	0	0	2 (1.6)	3 (1.9)	2 (5.1)
Paraesthesia	0	1 (3.1)	0	0	1 (2.5)	1 (0.8)	2 (1.3)	0
Psychomotor hyperactivity	0	0	1 (3.7)	0	0	1 (0.8)	1 (0.6)	0
Restless legs syndrome	0	0	1 (3.7)	0	0	1 (0.8)	1 (0.6)	0
Somnolence	0	0	1 (3.7)	0	0	1 (0.8)	1 (0.6)	0
Dysgeusia	0	1 (3.1)	0	0	0	0	1 (0.6)	0
Taste disorder	0	1 (3.1)	0	0	0	0	1 (0.6)	0
Psychiatric disorders	3 (15.0)	17 (53.1)	7 (25.9)	9 (25.0)	5 (12.5)	24 (19.5)	41 (26.5)	2 (5.1)
Middle insomnia	0	0	2 (7.4)	4 (11.1)	3 (7.5)	9 (7.3)	9 (5.8)	2 (5.1)
Initial insomnia	1 (5.0)	0	2 (7.4)	4 (11.1)	0	7 (5.7)	7 (4.5)	0
Insomnia	2 (10.0)	15 (46.9)	1 (3.7)	0	1 (2.5)	4 (3.3)	19 (12.3)	0
Abnormal dreams	1 (5.0)	1 (3.1)	1 (3.7)	1 (2.8)	0	3 (2.4)	4 (2.6)	0
Terminal insomnia	0	0	1 (3.7)	1 (2.8)	0	2 (1.6)	2 (1.3)	0
Anxiety	1 (5.0)	1 (3.1)	0	0	0	1 (0.8)	2 (1.3)	0
Adjustment disorder	0	0	0	0	1 (2.5)	1 (0.8)	1 (0.6)	0
Nightmare	1 (5.0)	0	0	0	0	1 (0.8)	1 (0.6)	0
Bradyphrenia	0	1 (3.1)	0	0	0	0	1 (0.6)	0
Sleep terror	0	1 (3.1)	0	0	0	0	1 (0.6)	0
Musculoskeletal and connective tissue disorders	3 (15.0)	6 (18.8)	2 (7.4)	0	6 (15.0)	11 (8.9)	17 (11.0)	1 (2.6)
Arthralgia	2 (10.0)	0	0	0	2 (5.0)	4 (3.3)	4 (2.6)	1 (2.6)
Back pain	2 (10.0)	0	0	0	1 (2.5)	3 (2.4)	3 (1.9)	1 (2.6)
Pain in extremity	1 (5.0)	0	0	0	1 (2.5)	2 (1.6)	2 (1.3)	0
Muscular weakness	0	2 (6.3)	1 (3.7)	0	0	1 (0.8)	3 (1.9)	0

Muscle spasms	0	1 (3.1)	0	0	1 (2.5)	1 (0.8)	2 (1.3)	0
Musculoskeletal pain	0	0	0	0	1 (2.5)	1 (0.8)	1 (0.6)	0
Osteopenia	0	0	0	0	1 (2.5)	1 (0.8)	1 (0.6)	0
Joint swelling	0	0	1 (3.7)	0	0	1 (0.8)	1 (0.6)	0
Musculoskeletal chest pain	0	2 (6.3)	0	0	0	0	2 (1.3)	0
Joint stiffness	0	1 (3.1)	0	0	0	0	1 (0.6)	0
Limb discomfort	0	1 (3.1)	0	0	0	0	1 (0.6)	0
Myalgia	0	1 (3.1)	0	0	0	0	1 (0.6)	0
Skin and subcutaneous tissue disorders	2 (10.0)	10 (31.3)	2 (7.4)	0	4 (10.0)	8 (6.5)	18 (11.6)	2 (5.1)
Dry skin	1 (5.0)	0	1 (3.7)	0	1 (2.5)	3 (2.4)	3 (1.9)	1 (2.6)
Pruritus	1 (5.0)	3 (9.4)	0	0	1 (2.5)	2 (1.6)	5 (3.2)	0
Hyperhidrosis	0	1 (3.1)	0	0	1 (2.5)	1 (0.8)	2 (1.3)	0
Photosensitivity reaction	0	1 (3.1)	1 (3.7)	0	0	1 (0.8)	2 (1.3)	0
Onychoclasis	0	0	0	0	1 (2.5)	1 (0.8)	1 (0.6)	0
Night sweats	0	4 (12.5)	0	0	0	0	4 (2.6)	0
Rash	0	1 (3.1)	0	0	0	0	1 (0.6)	1 (2.6)
Acne	0	1 (3.1)	0	0	0	0	1 (0.6)	0
Rash erythematous	0	1 (3.1)	0	0	0	0	1 (0.6)	0
Rash maculo-papular	0	1 (3.1)	0	0	0	0	1 (0.6)	0
Skin ulcer	0	1 (3.1)	0	0	0	0	1 (0.6)	0
Nail disorder	0	0	0	0	0	0	0	1 (2.6)
Investigations	1 (5.0)	3 (9.4)	3 (11.1)	1 (2.8)	1 (2.5)	6 (4.9)	9 (5.8)	3 (7.7)
Alanine aminotransferase increased	1 (5.0)	1 (3.1)	1 (3.7)	0	1 (2.5)	3 (2.4)	4 (2.6)	2 (5.1)
Aspartate aminotransferase increased	0	1 (3.1)	1 (3.7)	0	1 (2.5)	2 (1.6)	3 (1.9)	0
Blood glucose increased	0	0	0	1 (2.8)	0	1 (0.8)	1 (0.6)	1 (2.6)
Blood triglycerides increased	0	0	1 (3.7)	0	0	1 (0.8)	1 (0.6)	0
Heart rate increased	0	0	1 (3.7)	0	0	1 (0.8)	1 (0.6)	0
Blood cholesterol increased	0	1 (3.1)	0	0	0	0	1 (0.6)	0
Electrocardiogram T wave inversion	0	1 (3.1)	0	0	0	0	1 (0.6)	0
Vascular disorders	3 (15.0)	9 (28.1)	1 (3.7)	0	1 (2.5)	5 (4.1)	14 (9.0)	0
Hot flush	2 (10.0)	6 (18.8)	1 (3.7)	0	1 (2.5)	4 (3.3)	10 (6.5)	0
Flushing	1 (5.0)	3 (9.4)	0	0	0	1 (0.8)	4 (2.6)	0
General disorders and administration site conditions	2 (10.0)	9 (28.1)	1 (3.7)	0	1 (2.5)	4 (3.3)	13 (8.4)	1 (2.6)
Fatigue	1 (5.0)	4 (12.5)	1 (3.7)	0	0	2 (1.6)	6 (3.9)	1 (2.6)
Asthenia	1 (5.0)	1 (3.1)	0	0	1 (2.5)	2 (1.6)	3 (1.9)	0
Thirst	1 (5.0)	0	1 (3.7)	0	0	2 (1.6)	2 (1.3)	0
Chest discomfort	1 (5.0)	4 (12.5)	0	0	0	1 (0.8)	5 (3.2)	0
Feeling abnormal	0	0	0	0	1 (2.5)	1 (0.8)	1 (0.6)	0
Chills	0	2 (6.3)	0	0	0	0	2 (1.3)	0
Non-cardiac chest pain	0	2 (6.3)	0	0	0	0	2 (1.3)	0
Malaise	0	1 (3.1)	0	0	0	0	1 (0.6)	0
Pain	0	1 (3.1)	0	0	0	0	1 (0.6)	0
Peripheral swelling	0	1 (3.1)	0	0	0	0	1 (0.6)	0
Pyrexia	0	1 (3.1)	0	0	0	0	1 (0.6)	0
Cardiac disorders	1 (5.0)	3 (9.4)	2 (7.4)	0	1 (2.5)	4 (3.3)	7 (4.5)	1 (2.6)
Palpitations	0	2 (6.3)	1 (3.7)	0	0	1 (0.8)	3 (1.9)	1 (2.6)
Tachycardia	0	1 (3.1)	1 (3.7)	0	0	1 (0.8)	2 (1.3)	0
Arrhythmia	0	0	0	0	1 (2.5)	1 (0.8)	1 (0.6)	0
Ventricular extrasystoles	1 (5.0)	0	0	0	0	1 (0.8)	1 (0.6)	0
Reproductive system and breast disorders	0	5 (15.6)	1 (3.7)	0	2 (5.0)	3 (2.4)	8 (5.2)	0
Breast discomfort	0	0	0	0	2 (5.0)	2 (1.6)	2 (1.3)	0
Premenstrual pain	0	0	0	0	1 (2.5)	1 (0.8)	1 (0.6)	0
Pelvic pain	0	0	1 (3.7)	0	0	1 (0.8)	1 (0.6)	0
Dysmenorrhoea	0	2 (6.3)	0	0	0	0	2 (1.3)	0
Breast enlargement	0	1 (3.1)	0	0	0	0	1 (0.6)	0
Breast swelling	0	1 (3.1)	0	0	0	0	1 (0.6)	0
Menorrhagia	0	1 (3.1)	0	0	0	0	1 (0.6)	0

Metabolism and nutrition disorders	1 (5.0)	4 (12.5)	0	0	2 (5.0)	3 (2.4)	7 (4.5)	0
Hypertriglyceridaemia	0	2 (6.3)	0	0	2 (5.0)	2 (1.6)	4 (2.6)	0
Decreased appetite	1 (5.0)	2 (6.3)	0	0	0	1 (0.8)	3 (1.9)	0
Eye disorders	2 (10.0)	2 (6.3)	0	0	1 (2.5)	3 (2.4)	5 (3.2)	0
Eye pruritus	1 (5.0)	0	0	0	1 (2.5)	2 (1.6)	2 (1.3)	0
Periorbital oedema	0	0	0	0	1 (2.5)	1 (0.8)	1 (0.6)	0
Dry eye	1 (5.0)	0	0	0	0	1 (0.8)	1 (0.6)	0
Eye irritation	1 (5.0)	0	0	0	0	1 (0.8)	1 (0.6)	0
Photophobia	1 (5.0)	0	0	0	0	1 (0.8)	1 (0.6)	0
Mydriasis	0	1 (3.1)	0	0	0	0	1 (0.6)	0
Vision blurred	0	1 (3.1)	0	0	0	0	1 (0.6)	0
Infections and infestations	1 (5.0)	1 (3.1)	2 (7.4)	0	0	3 (2.4)	4 (2.6)	0
Nasopharyngitis	1 (5.0)	1 (3.1)	0	0	0	1 (0.8)	2 (1.3)	0
Pharyngitis	0	0	1 (3.7)	0	0	1 (0.8)	1 (0.6)	0
Sialoadenitis	0	0	1 (3.7)	0	0	1 (0.8)	1 (0.6)	0
Influenza	0	1 (3.1)	0	0	0	0	1 (0.6)	0
Respiratory, thoracic and mediastinal disorders	1 (5.0)	2 (6.3)	0	0	1 (2.5)	2 (1.6)	4 (2.6)	0
Dyspnoea	1 (5.0)	1 (3.1)	0	0	0	1 (0.8)	2 (1.3)	0
Oropharyngeal pain	0	0	0	0	1 (2.5)	1 (0.8)	1 (0.6)	0
Productive cough	0	0	0	0	1 (2.5)	1 (0.8)	1 (0.6)	0
Cough	0	1 (3.1)	0	0	0	0	1 (0.6)	0
Dyspnoea exertional	0	1 (3.1)	0	0	0	0	1 (0.6)	0
Orthopnoea	0	1 (3.1)	0	0	0	0	1 (0.6)	0
Pleural effusion	0	1 (3.1)	0	0	0	0	1 (0.6)	0
Ear and labyrinth disorders	1 (5.0)	3 (9.4)	0	0	0	1 (0.8)	4 (2.6)	1 (2.6)
Tinnitus	1 (5.0)	1 (3.1)	0	0	0	1 (0.8)	2 (1.3)	0
Vertigo	0	1 (3.1)	0	0	0	0	1 (0.6)	1 (2.6)
Ear congestion	0	1 (3.1)	0	0	0	0	1 (0.6)	0
Ear pain	0	1 (3.1)	0	0	0	0	1 (0.6)	0
Blood and lymphatic system disorders	0	3 (9.4)	0	0	0	0	3 (1.9)	0
Haemolysis	0	2 (6.3)	0	0	0	0	2 (1.3)	0
Haemolytic anaemia	0	2 (6.3)	0	0	0	0	2 (1.3)	0
Renal and urinary disorders	0	3 (9.4)	0	0	0	0	3 (1.9)	0
Chromaturia	0	2 (6.3)	0	0	0	0	2 (1.3)	0
Bilirubinuria	0	1 (3.1)	0	0	0	0	1 (0.6)	0
Hepatobiliary disorders	0	1 (3.1)	0	0	0	0	1 (0.6)	0
Ocular icterus	0	1 (3.1)	0	0	0	0	1 (0.6)	0
Immune system disorders	0	1 (3.1)	0	0	0	0	1 (0.6)	0
Drug hypersensitivity	0	1 (3.1)	0	0	0	0	1 (0.6)	0
Injury, poisoning and procedural complications	0	1 (3.1)	0	0	0	0	1 (0.6)	0
Scratch	0	1 (3.1)	0	0	0	0	1 (0.6)	0

Treatment-related Grade ≥3 TEAEs

More than half of headaches and nauseas reported in the regularly transfused subjects (pivotal Study 007) were considered related to study treatment (18.5% and 14.8%, respectively). Two cases of Rash were also considered related to treatment, as well as cases of vomiting (3.7%), ASAT and ALAT increase (7.4% each). The severity of these events was mild (Grade 1 or 2). The only reported Grade ≥3 TEAE in this study was a Grade 3 Joint swelling. Grade ≥3 Jugular vein occlusion, Ovarian Cyst and Back pain were not considered to be related to treatment.

In subjects who received mitapivat during the core period of Study 006, the following AEs were considered related mitapivat: nauseas and transaminases increases (15.0% and 5% respectively), as well as most headaches (12.5%), an half of arthralgia (5.0%), a third of hypertriglyceridaemia (2 subjects, 5.0%) and a quarter of abdominal pain (2.5%). All these cases were mild in severity (Grade 1 or 2). In addition, one Grade 3 Musculoskeletal pain (2.5%) and the two Grade ≥3 Hypertriglyceridaemia (5.0%) were considered related to study treatment.

- ***Serious adverse events, deaths, and other significant events***

Deaths

No deaths have been reported in any study with mitapivat.

Serious adverse events

Table 73 Summary of Serious Treatment-Emergent Adverse Events by System Organ Class and Preferred Term –24-Week Period (Safety Analysis Set)

Table 18.3.1-3.1a
Summary of Serious Treatment-Emergent Adverse Events by System Organ Class and Preferred Term - 24-Week Period
Safety Analysis Set

	Mitapivat							Placebo
	Study 003		Study 007	Study 011 Cohort 1	Study 006	Total		Study 006
System Organ Class (SOC) Preferred Term (PT)	≤50 mg BID N=20 n (%)	>50 mg BID N=32 n (%)	≤50 mg BID N=27 n (%)	≤50 mg BID N=36 n (%)	≤50 mg BID N=40 n (%)	≤50 mg BID N=123 n (%)	All Doses N=155 n (%)	N=39 n (%)
Subjects with events	2 (10.0)	4 (12.5)	2 (7.4)	2 (5.6)	4 (10.0)	10 (8.1)	14 (9.0)	2 (5.1)
Infections and infestations	1 (5.0)	2 (6.3)	0	1 (2.8)	1 (2.5)	3 (2.4)	5 (3.2)	1 (2.6)
Gastroenteritis	0	0	0	0	1 (2.5)	1 (0.8)	1 (0.6)	0
Cytomegalovirus infection	0	0	0	1 (2.8)	0	1 (0.8)	1 (0.6)	0
Influenza	1 (5.0)	0	0	0	0	1 (0.8)	1 (0.6)	0
Pharyngitis	0	2 (6.3)	0	0	0	0	2 (1.3)	0
Metapneumovirus infection	0	0	0	0	0	0	0	1 (2.6)
Renal and urinary disorders	0	0	1 (3.7)	1 (2.8)	0	2 (1.6)	2 (1.3)	0
Nephrolithiasis	0	0	0	1 (2.8)	0	1 (0.8)	1 (0.6)	0
Renal colic	0	0	1 (3.7)	0	0	1 (0.8)	1 (0.6)	0
Cardiac disorders	0	0	0	0	1 (2.5)	1 (0.8)	1 (0.6)	0
Atrial fibrillation	0	0	0	0	1 (2.5)	1 (0.8)	1 (0.6)	0
Injury, poisoning and procedural complications	0	0	0	0	1 (2.5)	1 (0.8)	1 (0.6)	0
Rib fracture	0	0	0	0	1 (2.5)	1 (0.8)	1 (0.6)	0
Musculoskeletal and connective tissue disorders	0	0	0	0	1 (2.5)	1 (0.8)	1 (0.6)	0
Musculoskeletal pain	0	0	0	0	1 (2.5)	1 (0.8)	1 (0.6)	0
Reproductive system and breast disorders	0	0	1 (3.7)	0	0	1 (0.8)	1 (0.6)	0
Ovarian cyst	0	0	1 (3.7)	0	0	1 (0.8)	1 (0.6)	0
Hepatobiliary disorders	1 (5.0)	0	0	0	0	1 (0.8)	1 (0.6)	0
Cholelithiasis	1 (5.0)	0	0	0	0	1 (0.8)	1 (0.6)	0
Blood and lymphatic system disorders	0	2 (6.3)	0	0	0	0	2 (1.3)	0
Haemolytic anaemia	0	2 (6.3)	0	0	0	0	2 (1.3)	0
Haemolysis	0	1 (3.1)	0	0	0	0	1 (0.6)	0
Gastrointestinal disorders	0	0	0	0	0	0	0	1 (2.6)
Obstructive pancreatitis	0	0	0	0	0	0	0	1 (2.6)

Treatment-related SAEs

Treatment-related SAEs were reported in 1.9% of all subjects who received mitapivat during the 24-week period. The incidence of treatment-related SAEs was highest (6.3%) in subjects in Study 003 who received mitapivat >50 mg BID. There were no treatment-related SAEs in subjects in Study 007 or subjects who received placebo in Study 006.

One (0.6%) subject in Study 003 who received mitapivat >50 mg BID experienced treatment-related SAEs of Haemolytic anaemia and Haemolysis due to abrupt withdrawal of mitapivat. One (0.6%) subject in Study 003 who received mitapivat >50 mg BID experienced a treatment-related SAE of Haemolytic anaemia; this subject did not respond to treatment with mitapivat and required blood transfusion. One (0.6%) subject in Study 006 who received mitapivat ≤50 mg BID had a treatment-related SAE of Musculoskeletal pain.

Adverse events of special interest (AESIs)

Transaminase increase

The AESI transaminase increase is defined as an increase of more than 2.5 × the baseline value for subject or an increase to Grade 2. Transaminase increase was reported as AESI only in the Extension Period of Study 003, which does not allow relevant comparison to Studies 006 and 007. In these two studies, identification of transaminase increase as AESI is based on retrospective review of liver test monitoring performed during the core study.

Eleven subjects who received mitapivat (7.0%) presented elevated transaminases. They all had baseline increased bilirubin, which during treatment decreased or remained at baseline values in 9 of them.

During the 24-week period, a lower incidence of transaminase increase was observed in subjects who received mitapivat (1 subject, 2.5%) compared to subjects in the placebo arm (6 subjects, 15.4% including one subject with Grade 3 aspartate aminotransferase [AST] increase) and regularly transfused subjects (Study 007, 7 subjects [25.9%]).

These elevations generally resolved without the need for dose modification or discontinuation of treatment.

Similar results were observed during the cumulative period. No serious TEAEs related to transaminases increase have been reported. None of the events of transaminase increase led to a dose reduction nor discontinuation of study treatment.

Adverse events

Acute haemolysis

As Hb would exceed the upper limit normal (ULN) in two subjects who received 300 mg BID of mitapivat (Study 003), sudden withdrawal of mitapivat was performed and caused acute haemolysis; Grade 3 SAE Haemolysis rapidly followed by Grade 3 Haemolytic Anaemia in the first subject and a Grade 2 nonserious TEAE Haemolysis in the second one. Another sudden withdrawal of treatment has been performed in a female subject who became pregnant (≥50 mg BID).

During the Extension Period of Study 003, 14 subjects of the >50 mg arm underwent the newly implemented dose taper (starting at 300 mg BID and decreasing by 100 mg increments for 3 weeks) to minimise the haemolytic risk. Hb decreased was observed at 1.1 g/dL per week at most, with stabilisation at the new dose in eleven of them.

Based on these findings, an adapted dose taper was implemented in the pivotal studies (see table below). After Week 24, five subjects of Study 006 (3 of them were non-responders regarding increase in Hb or decrease of key markers of haemolysis) underwent this dose taper and no haemolytic episode was reported.

Table 74 Dose Taper Scheme

Dose at the Time of Taper	First Step × 7 Days	Second Step × 7 Days
5 mg BID	5 mg QD	NA
20 mg BID	20 mg QD	5 mg QD
50 mg BID	50 mg QD	20 mg QD
Abbreviations: BID = twice daily; NA = not applicable; QD = once daily.		

Aromatase inhibition

Aromatase activity is inhibited by mitapivat. As this enzyme is responsible for the transformation of androgens to estrogens, a decrease of estrogens and an increase in androgens in male subjects were expected in male subjects.

During the 24-week period, 17 male subjects who received mitapivat had lower limit normal (LLN) estrone levels on treatment and 26 subjects had high levels of free testosterone. Estradiol levels decreased but not below the LLN.

These trends were confirmed during the cumulative period. The changes were reversible upon treatment discontinuation.

In female subjects, no clear changes could have been identified due to confounding factors such as menstrual cycle variations, type of contraceptives (hormonal or non-hormonal), and the production of estrogens by ovaries in women of childbearing potential.

Also, according to T- and Z-scores; no meaningful changes in bone mineral density were observed, regardless of the gender.

Insomnia

Mitapivat is thought to have inhibitory activity on histamine H3 receptors without penetrating the blood brain barrier.

During the 24-week period, events of insomnia were widely reported in the target population (Study 006, mitapivat arm: 15.0% and placebo arm: 17.9% and Study 007: 22.0%), with a considerably higher incidence was observed in supportive Study 003 for doses greater than 50 mg (64.0%). The occurrence of these events increased during the cumulative period.

In the 4 main studies of the safety analysis set, Insomnia TEAEs were generally transient (resolution over 40 days) non serious and rarely considered related to study treatment. In supportive Study 010, an ongoing phase 2 study in subjects with non-transfusion dependent thalassemia (NTDT), the 11 reported events of Insomnia were considered to be related to the study drug.

Gastrointestinal disorders

During the 24-week period, GI events concerned at least 40% subjects in all treatment groups. The rate of these events were comparable between the placebo arm of Study 006 and Study 007 (48.7% and 48.1% respectively). A higher incidence was observed in Study 003, regardless of the dose. Nauseas were the most reported events (>15% in each treatment group), some of them considered drug-related in Study 003.

In healthy subjects, such events occurred in 14 subjects (6.2%) and the frequency of GI events is significant (24 subjects, 10.7%).

Triglycerides increase

Events of triglycerides increase were more frequent in subjects who received mitapivat, especially in Study 003 (≥50 mg, 27% during the cumulative period). Such events were mostly mild and transient. Also, no case of pancreatitis has been reported in the target population.

Hypersensitivity

Among the safety analysis set, some TEAEs of hypersensitivity have been reported, especially in Study 003 for doses greater than 50 mg. Some have been considered drug-related but were mild and transient.

Other significant events

Overdose

A case of overdose was reported in clinical studies: a subject originally enrolled in the mitapivat arm of Study 006 took 3 tablets at once (150 mg BID) instead of 50 mg BID during the long-term extension Study 011.

Withdrawal and rebound

Subjects who abruptly interrupt or discontinue mitapivat may be at risk of developing acute haemolysis; a gradual reduction in dosing (dose taper) is recommended. Events of acute haemolysis that were observed with mitapivat have been discussed above.

Drug abuse

As data cutoff, no case of drug abuse or drug dependence has been reported.

Effects on ability to drive or operate machinery or impairment of mental ability

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.

- ***Laboratory findings***

No other unexpected safety finding was raised from clinical chemistry, haematology and coagulation parameters. Transaminase increase, triglycerides and acute haemolysis have been discussed above as AESIs.

Also, no unexpected signal was raised from ECG abnormalities.

- ***In vitro biomarker test for patient selection for safety***

Not applicable.

- ***Safety in special populations***

Pregnant and breastfeeding women

Pregnant women were excluded from studies, however two pregnancies were reported from mitapivat clinical trial.

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.

Intrinsic factors

Age, sex, race

Table 75 Overall Summary of Adverse Events by Age for Subjects Who Received Mitapivat – Cumulative Period (Safety Analysis Set)

	Age Group				
	<65 yr N=150 n (%)	**65 to <75 yr N=4 n (%)**	**75 to <85 yr N=1 n (%)**	**≥85 yr N=0 n (%)**	**Overall N=155 n (%)**
Total AEs	147 (98.0)	4 (100.0)	1 (100.0)	0	152 (98.1)
Serious AEs - total	28 (18.7)	2 (50.0)	0	0	30 (19.4)
Fatal	0	0	0	0	0
Hospitalisation/prolong existing hospitalisation	19 (12.7)	1 (25.0)	0	0	20 (12.9)
Life-threatening	2 (1.3)	0	0	0	2 (1.3)
Disability/incapacity	2 (1.3)	1 (25.0)	0	0	3 (1.9)
Other (medically significant)	14 (9.3)	0	0	0	14 (9.0)
AE leading to drop-out	7 (4.7)	0	0	0	7 (4.5)

	Age Group				
	<65 yr N=150 n (%)	**65 to <75 yr N=4 n (%)**	**75 to <85 yr N=1 n (%)**	**≥85 yr N=0 n (%)**	**Overall N=155 n (%)**
Psychiatric disorders (SMQ)	1 (0.7)	0	0	0	1 (0.6)
Nervous system disorders (SOC)	76 (50.7)	2 (50.0)	0	0	78 (50.3)
Accidents and injuries (SMQ)	16 (10.7)	0	0	0	16 (10.3)
Cardiac disorders (SOC)	14 (9.3)	0	1 (100.0)	0	15 (9.7)
Vascular disorders (SOC)	24 (16.0)	0	0	0	24 (15.5)
Cerebrovascular disorders (HLGT)	1 (0.7)	0	0	0	1 (0.6)
Infections and infestations (SOC)	96 (64.0)	0	0	0	96 (61.9)
Anticholinergic syndrome (SMQ)	0	0	0	0	0
Quality of life decreased	0	0	0	0	0
Sum of postural hypotension, falls, black outs, syncope, dizziness, ataxia, fractures[1]	84 (56.0)	2 (50.0)	0	0	86 (55.5)

Abbreviations: AE = adverse event; HLGT = high-level group term; MedDRA = Medical Dictionary for Regulatory Activities; PT = Preferred Term; SMQ = Standardised MedDRA Query; SOC = System Organ Class.

Notes: The denominator used to calculate percentages is N, the number of subjects in the Safety Analysis Set within each subgroup.

Subjects with multiple AEs within a PT are counted only once in that PT.

Subjects with multiple AEs within an SOC are counted only once in that SOC.

MedDRA Version 23.1 is used.

[1] Includes all PT in Accidents and injuries (SMQ) narrow, Anticholinergic syndrome (SMQ) narrow, and Torsade de pointes/QT prolongation (SMQ) narrow and all PTs in Nervous system disorders (SOC).

The vast majority of subjects with PK deficiency is under 65 years of age (96.8%, median around 36 years of age). Due to restrictive inclusion criteria of the pivotal studies, limited data in subjects with renal or hepatic impairment have been provided.

Regarding gender, there was a slightly greater amount of female subjects but no clear trend favouring men or women has been observed when assessing safety data. Concerning ethnicity, most subjects were White (126 subjects, 77.4%) or Asian (16 subjects, 10.3%). The other reported races were represented in smaller proportions, which did not allow meaningful comparisons.

Splenectomy status

About 75% of the target population had prior splenectomy.

During the 24-week period, a slightly higher incidence of TEAEs in splenectomised subjects who received mitapivat was observed: 94.9% compared to 89.7% in non-splenectomised ones. Rates of TEAEs were comparable in both subject subgroups who received placebo. The occurrence of Grade ≥3 TEAEs was higher in non-splenectomised subjects who received mitapivat (27.0% and 14.4%, respectively) or placebo (17.2% or 0%).

During the cumulative period, the trends are reinforced. In the absence of splenectomy, more GI events were reported in the absence of splenectomy: Nausea (35.1% and 24.6%, respectively), Diarrhoea (21.6% and 10.2%, respectively), and Vomiting (18.9% and 8.5%, respectively) along with Bone pain (13.5% and 0%, respectively). Events of Grade ≥3 Insomnia were also more reported in non-splenectomised subjects (5.4% and 0%, respectively).

Baseline Hb

57.4% of subjects who received mitapivat and 42.6% of subjects who received placebo had baseline Hb levels ≥ 8.5 g/dL. During the 24-week period, the overall incidence of TEAEs was comparable in subjects who received mitapivat in Study 006, regardless of baseline Hb levels (<8.5 g/dL: 89.5%, ≥8.5 g/dL: 85.7%). Regularly transfused subjects from Study 007 were more prone to TEAEs when baseline Hb levels were greater than 8.5 g/dL (95% compared to 79.4% for Hb levels >8.5 g/dL).

Extrinsic factors

Adverse events by region

Clinical studies with mitapivat were conducted worldwide. Most subjects with PK deficiency came from Western Europe (56.8%), North America (36.1%) or Asia (4.51%). Considering the unbalanced distribution of subjects across regions, no meaningful comparisons could be made regarding the incidence of TEAEs.

- ***Immunological events***

Not applicable.

- ***Safety related to drug-drug interactions and other interactions***

No conclusion can be drawn on safety related to drug-drug interactions as the dedicated studies have not been performed.

- ***Discontinuation due to adverse events***

7 subjects who received mitapivat had a TEAE that led to discontinuation of study drug. Most of the subjects (6 of them) who discontinued study drug due to a TEAE were those who received mitapivat >50 mg BID in Study 003. Similar trends were observed concerning dose interruptions and dose reductions.

- ***Post marketing experience***

Not applicable.

2.5.9. Discussion on clinical safety

The overall safety experience for mitapivat is derived from a clinical development program consisting of 12 studies.

The primary safety data in adults with PK deficiency are provided by two pivotal studies:

- Study 006, a placebo-controlled Phase 3 study in non-transfusion-dependant (NTD) subjects
- Study 007, an open-label Phase 3 study in transfusion-dependant (TD) subjects.

Main supportive safety data come from Study 011, the long-term extension of pivotal studies and Study 003, an open-label phase 2 study in NTD subjects.

Additional supportive safety data from an ongoing phase 2 study in subjects with NTD thalassemia and seven studies in healthy volunteers have also been provided.

Out of the 155 subjects with PK deficiency included the safety analysis set, 106 were included in pivotal studies: 40 mitapivat and 39 placebo subjects of Study 006 and 27 subjects of Study 007 who received 5 to 50 mg BID of mitapivat.

This sample of subjects was considered overall representative of the target population in terms of demographics and baseline disease characteristics. However, the data in subjects over 65 years is very limited.

Patient exposure

The median duration of treatment was comparable between both studies 006 and 007: 12.42 months (3.7, 28.6) and 11.55 months (6.0, 25.4), respectively.

As of the 12 November 2020, due to the rarity of the disease, a limited number of subjects have been exposed to study treatment: only 45% and 51.9% of recruited participants in the pivotal studies 006 and 007, respectively, have completed at least 12 months of treatment at data cut-off. As mitapivat is intended to be used on a daily basis and for the long-term treatment of pyruvate kinase deficiency anaemia, longer-term safety data from the ongoing Study 011 were provided during the procedure in order better characterise mitapivat safety profile, based on the identification of potential chronic toxicity, rare and/or longer-term complications.

The updated safety data from Study 011 have been provided (new data cut-off: 12 September 2021). Study 011 is the Phase 3, multicentre, open-label, long-term extension study in adults with PK deficiency originally enrolled in Study 006 or 007 with 3 distinctive cohorts:

- Cohort 1: non transfusion-dependent (NTD) subjects from the placebo arm of Study 006
- Cohort 2: NTD subjects from the experimental arm of Study 006
- Cohort 3: transfusion-dependent (TD) subjects from Study 007

The updated median duration of exposure was 18.55 months (range 1.9 to 35.6 months) with comparable rates in TD and NTD transfused subjects (19.25 ± 11.084 months and 21.26 ± 8.720 months, respectively). 72% of subjects received mitapivat for more than 12 months and 29% of subjects received treatment for more than 24 months.

Treatment discontinuation now concerned a third of the target population (35 subjects, 33.3% versus 24.3% initially), still driven by TD subjects from the pivotal Study 007 (15 subjects [55.6%] versus 48.1 % initially).

Overall, these events were still driven by withdrawal of consent (19/35, 18.1% of subjects from pivotal studies), which raised the question of the long-term tolerability of the treatment.

Treatment discontinuation due to AE remained rare; an additional case was reported, bring up the total to two NTD subjects.

Adverse events

TEAEs were presented by individual study based on the highest dose taken (≤50 mg BID or >50 mg BID) over two analysis periods: the 24-week on-treatment period and the cumulative period, which includes an additional period of 28 days of post-treatment follow-up.

TEAEs were presented by individual study based on the highest dose taken (≤50 mg BID or >50 mg BID) over two analysis periods: the 24-week on-treatment period and the cumulative period, which includes an additional period of 28 days of post-treatment follow-up.

Initially, the overall incidence of TEAEs was similar between groups and studies: 89.7% in the placebo arm; 87.5% in the mitapivat arm of Study 006 and 88.9% in the open-label study 007.

Treatment-related TEAEs were more frequent in regularly transfused subjects (63.0% in Study 007 vs 57.5% in Study 006), which was expected due to repeated transfusions. Also, a low number of treatment-related Grade ≥ 3 TEAEs was observed in pivotal studies: 1 in regularly transfused subjects and 3 in occasionally transfused subjects, including 1 SAE.

Most common TEAEs, all doses included, were: Headache (36.1%), Nausea (21.3%), Insomnia (15.5%), Nasopharyngitis (14.2%), Fatigue (12.3%), Back pain (11.0%) and Arthralgia (10.3%).

As of the updated data cutoff (12 September 2021), the overall incidence of TEAEs increased but remained similar between groups and studies: 97.4% in Cohort 1; 95.0% Cohort 2 and 100.0% in Cohort 3.

As of the updated data cutoff, overall in the target population reported most common TEAEs were consistent with what has been previously observed.

Whereas treatment-related TEAEs were initially more frequent in TD, such events became comparable between the three cohorts at time of updated data cutoff, driven by a largest increase in Cohort 1 (7 additional subjects) followed by Cohort 2 (4 additional subjects) and then Cohort 1 (1 additional subject). This was not unexpected considering that subjects in Cohort 1 started mitapivat treatment in Study 011 (i.e. receiving placebo in the parent study).

Furthermore, the number of Grade ≥ 3 treatment-related TEAEs remained low (6 subjects, 5.7%).

Transfusion-dependant subjects (Cohort 3) remained more susceptible to infections and experienced more nausea (40.7%), vomiting and fatigue (25.9% each), probably due to recurrent and persistent transfusions. Aminotransferase levels were also higher in this subgroup (ALAT: 40.7%, ASAT: 22.2%), which was expected since transfusions cause iron overload which disrupts liver function. When assessed as related to treatment, these events were mild to moderate in severity.

The only reported treatment-related Grade ≥3 TEAE was a Grade 3 Joint swelling. At time of updated DLP, the additional case of treatment-related Grade 3 ASAT increased does not impact mitapivat safety profile.

Initially, in the pivotal Study 006, events of Arthralgia, Abdominal pain (10.0% each), Hot flush and Hypertriglyceridaemia (7.5% each) were more frequent compared with other treatment groups. The following AEs were considered as related to mitapivat: nauseas and transaminases increases (15.0% and 5% of subjects respectively), headaches (12.5% of subjects), an half of arthralgia (5.0% of subjects), hypertriglyceridaemia (2 subjects, 5.0% of subjects) and abdominal pain (2.5% of subjects). All these cases were mild in severity (Grade 1 or 2). In addition, one Grade 3 Musculoskeletal pain and both events of Grade ≥3 Hypertriglyceridaemia (5.0%) were considered related to study treatment.

The two treatment-related Grade 3 events of Hypertriglyceridaemia reported in Study 006 are no longer of concern since these events occurred in overweight subjects presenting elevated triglycerides at baseline, thus ruling out a potential causal relationship between severe triglycerides increase and mitapivat treatment.

All the treatment-related TEAEs under the SOC 'Musculoskeletal or connective tissue disorders' were assessed as not related to mitapivat treatment by the applicant despite assessed as related by the investigators; the applicant considered that these cases were confounded by past medical history and/or the underlying disease. This is acknowledged but causal relationship still cannot be ruled out. Further longer-term data will hopefully allow a better characterisation of BMD decrease potentially associated with mitapivat treatment, and close monitoring in PSURs should be ensured.

Also, as of 12 September 2021, Osteopenia became frequent within the safety analysis set: events were reported in both populations who received mitapivat in the parent studies (1 case [3.7%] in Cohort 3 and 6 cases [15.0%] in Cohort 2). These events cannot be disentangled from the mitapivat inhibitory effect on aromatase (please refer to the section dedicated to AESIs).

In NTD subjects who initially received placebo (Cohort 1), the frequency of headaches and initial insomnia was higher compared to the other groups, which is consistent with previous observations.

The additional treatment-related TEAEs occurred mainly in this cohort, led by sleep disturbance (Middle insomnia, Initial insomnia) and general condition disturbance (Headache, Restless leg syndrome, Muscle spasms and Gastroenteritis). They were generally mild in severity.

Five additional subjects with SAEs have been reported, mostly in the same cohort: Seizure, Varicella zoster virus (VZV) infection, Syncope, Haemolysis and Gastroenteritis (1 subject each, 2.6%), of whom the events of Seizure and Gastroenteritis (resolved with treatment so not of concern) were considered treatment-related.

Provided data suggest that events of Hot Flush occurred on treatment onset, regardless reported changes in sex hormones.

Also, a common cause underlying headache and hot flush could be either vascular or endocrine in nature, or both. Changes in sex hormones have been investigated given the known aromatase inhibitory activity of the drug. Quite peculiarly, the effect of mitapivat on vital signs, including blood pressure and heart rate, had not been formally investigated, even though cases of hypertension have been described (2.6% in the group of all doses included vs 0% in placebo) that were among the most common causes of Grade ≥3 events (1.7%).

During the procedure, information on the two (out of 40) patients in the mitapivat group who experienced hypertension as AE were provided, revealing comorbidities as confounding factors. However, a trend towards BP increase in this short-term course of treatment was observed. The small sample size might be insufficient to detect clinically relevant effects associated to chronic therapy and in particular groups of patients depending on age, sex and co-morbidities. Considering that aromatase inhibition with oestrogen suppression affects endothelial function and vascular tone, and the resulting level of vascular resistance, a potential effect of mitapivat on blood pressure cannot be ruled out. Therefore, cardiovascular parameters should be monitored to better define the effect of mitapivat in the treated population. Collection of BP data through the ongoing long-term extension PK deficiency studies and review of any cardiovascular events through routine pharmacovigilance can be considered sufficient to detect relevant signals.

Furthermore, special considerations merit the potential risk of carcinogenicity that has emerged from preclinical studies. In the intended population, iron overload due to the underlying disease represents and additional risk factor for tumorigenesis.

Although data accumulated so far indicate improvement in iron overload related clinical parameters, the risk factor persists despite treatment due to the underlying disease. Considering the predisposing conditions, the relevance of the preclinical findings is of difficult interpretation and a potential risk of tumorigenesis cannot be excluded, at this stage. Therefore, the proposal for monitoring carcinogenicity AEs as part of the planned Category 3 PASS, together with routine pharmacovigilance activity as contemplated in the RMP, is considered appropriate. A report specifically addressing the carcinogenicity AEs is expected in the future as part of the Category 3 PASS study report and as well from routine pharmacovigilance activity.

Adverse events of special interest

Transaminase increase

Eleven subjects who received mitapivat (7.0%) presented elevated transaminases. They all had baseline increased bilirubin which during treatment decreased or remained at baseline values in 9 of them.

A lower incidence of transaminase increase was observed in subjects who received mitapivat (1 subject, 2.5%) compared to subjects in the placebo arm (6 subjects, 15.4) and regularly transfused subjects (Study 007, 7 subjects [25.9%]).

Elevated liver enzymes were expected in placebo-treated subjects as hepatic activity is increased in subjects with PK deficiency. Indeed, the liver and spleen work together to destroy aging and defective RBCs, resulting in intra-tissue haemolysis. In addition, a higher incidence of transaminase increases was

also expected in regularly transfused subjects as repeated transfusions cause iron overload which affects liver function.

These elevations generally resolved without the need for dose modification or discontinuation of treatment. Also, no serious TEAEs related to transaminases increase have been reported. None of the events of transaminase increase led to a dose reduction nor discontinuation of study treatment.

Considering the timing of this AESI, its reversibility and the associated underlying haemolysis, it is considered that there is no causal relationship between mitapivat administration and transaminase increases in pivotal Study 006; a lower incidence of increased ALT/AST was observed in the mitapivat group, independent of responder status.

In the absence of a control arm in Study 007 with subjects undergoing transfusion, the effect of mitapivat on liver function could not have been fully disentangled from the underlying hepatic dysfunction and detrimental effect of blood transfusions. The two cases of treatment-related transaminase increases reported in Study 007 have been discussed. Mitapivat causality has been excluded for the first one due to confounding factors, however the second one remains of concern. Indeed, a clinically relevant transaminase increase was observed upon treatment discontinuation, suggesting a possibly related haemolysis following mitapivat dose reduction.

Acute haemolysis

Sudden withdrawal of mitapivat was performed in two subjects from Study 003 (>50 mg BID) when Hb levels would exceed the upper limit normal (ULN), leading to acute haemolysis. Implementation of a dose taper (starting at 300 mg BID and decreasing by 100 mg increments for 3 weeks) in 14 subjects from this arm induced a decrease in Hb levels, which stabilised in eleven of them and helped avoiding such situation.

A different dose taper (see SmPC) was implemented in pivotal studies and performed in 5 subjects from Study 006 and signs of mild haemolysis were reported in some Hb responders and non-responders. Also, two cases of haemolysis were reported in Study 007.

Clarifications on subjects who missed at least a dose of study treatment, underwent a dose taper and/or presented signs of haemolysis (even mild signs) were provided.

Data provided during the procedure showed that the reported Grade 2 or 3 cases of Haemolysis were not considered related to treatment.

Mild signs of haemolysis were reported either following increased Hb response or the final recommended dose taper in subjects not entering the extension. Generally, haemolytic markers returned at baseline without action be taken with mitapivat treatment.

The clinical and safety impact of missed doses was discussed. Most events occurred at doses >50 mg BID without any emerging safety signals. Therefore, no additional RMMs are deemed necessary.

Acute haemolysis is mentioned in SmPC, sections 4.4 and 4.8 and is listed as an important identified risk in the RMP. The recommended dose taper is present in section 4.2.

Aromatase inhibition

Aromatase activity is inhibited by mitapivat. As this enzyme is responsible for the transformation of androgens to estrogens, a decrease of estrogens and an increase in androgens in male subjects are expected in male subjects. Indeed, these events were observed: 17 male subjects (44.7%) who received mitapivat had lower limit normal (LLN) estrone levels on treatment and 26 male subjects (66.4%) had high levels of free testosterone. Estradiol levels decreased but not below the LLN.

In female subjects, no clear changes could have been identified due to confounding factors such as menstrual cycle variations, type of contraceptives, and production of estrogens by ovaries in women of childbearing potential.

In addition, as estrogens play a key role in bone growth and maturation, mitapivat-mediated aromatase inhibition could be associated with increased bone fragility, which is of concern and a thorough

investigation of the relationship between aromatase inhibition, menstrual disorders and bone density loss, along with discussion of corresponding TEAEs was performed during the procedure. The data as presented do not allow to determine the potential effects of the off-target aromatase inhibition on the safety profile of the male and female subjects who experienced changes in sex hormones. Difference in sample sizes in men and confounding factors in women did not allow to draw any meaningful conclusions. However 'Changes in sex hormones' should be closely monitored in upcoming PSURs, as part of PSUR safety concerns in order to better characterise this risk in real life settings.

Furthermore, updated data show that the subjects' T-scores mainly remained into the same baseline category during mitapivat treatment. However, it was not specified whether or not these scores decreased during treatment. If so, provided results would not be inconsistent with a possible worsening of pre-existing bone fragility in patients treated with mitapivat, especially in those who have been receiving treatment for the longest time. As this reinforces the concern about mitapivat long-term tolerability, longer-term data and close monitoring of BMD decrease are needed to rule out a potential causal relationship.

Insomnia

Mitapivat is thought to have inhibitory activity on histamine H3 receptors without penetrating the blood brain barrier, which is unlikely since these receptors, involved in wakefulness, are widely distributed in the striatum, nucleus accumbens and cerebral cortex.

Events of insomnia were frequently reported in the target population (Study 006, mitapivat arm: 15.0% and placebo arm: 17.9% and Study 007: 22.0%), with a considerably higher incidence observed in supportive Study 003 with doses greater than 50 mg (64.0%).

Insomnia TEAEs were generally transient (resolution over 40 days), non-serious and rarely considered related to study treatment. Interestingly, in the supportive Study 010, an ongoing phase 2 study in subjects with non-transfusion dependent thalassemia (NTDT), the 11 reported events of Insomnia were considered to be related to the study drug.

In view of the numerous cases of insomnia reported in clinical studies, the inclusion of this adverse reaction in the SmPC is considered adequate and the proposed wording is acceptable.

Gastrointestinal disorders

GI events concerned at least 40% subjects in all treatment groups. The rate of these events was comparable between the placebo arm of Study 006 and Study 007 (48.7% and 48.1% respectively). A higher incidence was observed in Study 003, regardless of the dose. Nausea were the most reported GI events (>15% in each treatment group). The possible causes for this significant occurrence of nausea in the treatment arms and in the placebo arm (especially with regard to its composition) along with listing of this event at the appropriate frequencies in the product information were discussed.

Considering the review of excipients provided by the applicant, the potential relationship between the placebo composition and the significant frequency of nausea in the placebo arm is not a concern.

This AE has been listed in the SmPC, as requested.

Triglycerides increase

Events of triglycerides increase were more frequent in subjects who received mitapivat, especially in Study 003 (≥50 mg, 27% during the cumulative period). Such events were mostly mild and transient. Also, no case of pancreatitis has been reported in the target population. Based on provided narratives of subjects with Grade 3 Hypertriglyceridemia that occurred in subjects with confounding factors, no update of the SmPC regarding this risk is finally deemed necessary.

Hypersensitivity

Mild and transient TEAEs of hypersensitivity have been reported, mostly for doses greater than 50 mg BID. The considered treatment schedule for MA, along with the mention in section 4.3 of the SmPC, are considered adequate to minimise the risk.

Other significant events

'Events of endocrinological interest' were initially not investigated. Such events apparently occurred in 16 subjects (15.2%), including 4 additional subjects as of the updated data cut-off. These subjects were all from Cohort 1 and experienced either Alopecia (no case in the original MAA) or Dysmenorrhea (2 subjects each, 5.3%).

In the absence of comparative data from the initial data cut-off, it was difficult to draw any meaningful conclusions regarding the evolution in the trend in these AEs based on these results.Longer-term data should be provided post-approval in order to allow for an evaluation of the impact of mitapivat treatment on 'events of endocrinological interest'.

It should be noted that no new unexpected safety finding was raised from ECG abnormalities and laboratory findings, i.e. clinical chemistry haematology and coagulation parameters.

Overdose

A case of overdose was reported in Study 011: a subject took 150 mg BID instead of 50 mg BID during 70 days.

This dose error was not associated with any major AEs considered related to mitapivat.

In order to minimise this risk identified during the clinical studies, the tablets are packaged in such a way as to avoid any confusion. Also, the wording proposed in section 4.9 of the SmPC is acceptable.

Effects on ability to drive or operate machinery or impairment of mental ability

The effect of mitapivat on the ability to drive vehicles and operate machinery has not been evaluated, this remains unknown. Considering listing of Insomnia as 'very common' in SmPC section 4.8, the following text has been included in SmPC section 4.7 as per QRD template: at least minor influence on the ability to drive and use machines should be considered.

Safety in special populations

There are no or very limited available data on mitapivat use in pregnant women to evaluate for drug-associated risks. Animal studies indicate harmful effects with respect to reproductive toxicity. In addition, clinical data on off-target aromatase inhibition are very limited and are considered an issue when mitapivat is used during pregnancy. Cases of pregnancy occurred during the clinical experimentation of mitapivat. No safety issues have been identified. However, pre-clinical data demonstrated embryo-foetal toxicity. Consequently and according to the GVP, the risk "embryo-fetal toxicity" has been classified as an Important Potential Risk in the RMP of mitapivat.

No unexpected safety finding was raised from sex, race, region and Hb levels subgroups. Concerns regarding subjects over 65 years of age have been already mentioned above: considering the limited available data, the safety profile of mitapivat in this population should be further discussed considering the questioned effect of the drug on the vascular system, bone density as related to hormonal changes, the potential carcinogenicity and drug interactions. A subgroup analysis for the 5 subjects above 65 years of age, with a comparison to younger patients was provided. However, the data were not conclusive due to difference in sample sizes. An appropriate wording was added in section 5.1 of the SmPC to clearly inform physicians on the limited data currently available in this subpopulation.

In addition, due to restrictive inclusion criteria of the pivotal studies, limited data in subjects with renal or hepatic impairment have been provided. As a study in subjects with hepatic impairment is currently ongoing, only the safety profile of mitapivat in subjects with mild to moderate renal impairment has been discussed.

As few subjects with impaired renal function were included in mitapivat studies in PK deficiency (i.e. 28 mitapivat and 4 placebo subjects versus 125 mitapivat and 35 placebo subjects with normal renal

function), no meaningful comparison can be made from the submitted subgroup analysis. Therefore, the wording proposed in section 4.2 to reflect this lack of data is acceptable.

Regarding splenectomy status, the overall incidence of TEAEs was slightly higher in splenectomised subjects: 94.9% vs 89.7% in non-splenectomised ones.

Since the spleen is deeply involved in the immune system, splenectomised subjects are by essence more susceptible to infections and therefore more fragile. However, these subjects presented less GI events and were less prone to insomnia compared with subjects without prior splenectomy.

Regarding GI events, the observed difference in the frequency of subjects with GI events observed in non-splenectomised subjects who received mitapivat appears to be eliminated in the placebo arm, thus suggesting a study bias due to inter-individual variability.

Moreover, a greater frequency of Insomnia in non-splenectomised subjects was observed in all treatment and placebo groups, as outlined by initial CHMP assessment (for ≤50 mg BID: 35.5% vs. 21.1% in splenectomised subjects). Such results are unlikely to be related to a study bias but may be linked to aromatase inhibition. Insomnia is already listed in the SmPC. The applicant discussed whether or not the effects of aromatase inhibition are greater in non-splenectomised subjects. However, due to difference in sample sizes, no conclusion could have been drawn from presented data.

Safety related to drug-drug interactions and others interactions

No conclusion can be drawn on safety related to drug-drug interactions as the dedicated studies have not been performed.

Dose modifications and discontinuations due to adverse events

Few discontinuations or dose modifications due to adverse events occurred under the MA regimen, this supporting the relevance of the dose titration used in pivotal studies.

2.5.10. Conclusions on the clinical safety

The safety profile of mitapivat is adequately characterised through both pivotal studies and the supportive extension study. The safety profile is manageable.

2.6. Risk Management Plan

2.6.1. Safety concerns

Table 76 Summary of safety concerns

Summary of Safety Concerns	
Important identified risks	Acute haemolysis
Important potential risks	Embryo-fetal toxicity
Missing information	Use in patients with hepatic impairment Long-term use

2.6.2. Pharmacovigilance plan

Table 77 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 authorisation or a marketing authorisation under exceptional circumstances				
None				
Category 3 - Required additional pharmacovigilance activities				
AG348-C-0HEP A Phase 1, Single-Dose, Open-label Study to Evaluate the Pharmacokinetics, Safety, and Tolerability of Mitapivat in Subjects With Moderate Hepatic Impairment or Normal Hepatic Function Planned	Evaluate the pharmacokinetics, safety, and tolerability of mitapivat in subjects with moderate hepatic impairment or normal hepatic function	Use in patients with hepatic impairment	Final study report	31 March 2024
AG348-C-011 A Phase 3, Multicentre, 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 haemolysis Long-term use	Final study report	30 November 2025

Table 77 Ongoing and Planned Additional Pharmacovigilance Activities

Title and Study Status	Summary of Objectives	Safety Concerns Addressed	Milestones	Due Dates
AG348-C-008 (Peak Registry) An ongoing Agios-sponsored, global, retrospective and prospective, longitudinal observational study of paediatric 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	Final study report for patients that received mitapivat	30 September 2028

2.6.3. Risk minimisation measures

Table 78 Summary Table of Pharmacovigilance Activities and Risk Minimisation Activities by Safety Concern

Safety Concern	Risk Minimisation Measures	Pharmacovigilance Activities
Acute haemolysis	Routine risk minimisation measures: • Acute haemolysis is listed as a special warnings and precautions for use in the Summary of Product Characteristics (SmPC) Section 4.4 • Acute haemolysis is described as a selected adverse reaction in the SmPC Section 4.8 • Acute haemolysis is listed as a warning and precaution in Package Leaflet (PL) Section 2 • Acute haemolysis, after abrupt interruption or discontinuation of Pyrukynd, is described in PL Section 4 • Warning and precaution that acute haemolysis with subsequent anaemia has been observed following abrupt interruption or discontinuation of Pyrukynd in SmPC Section 4.4 • Warning that to minimise the risk of acute haemolysis, avoid abrupt interruption or discontinuation of Pyrukynd in SmPC Sections 4.2 and 4.4	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.

	• Advice on the dose taper schedule to be followed when discontinuing Pyrukynd in SmPC Section 4.2 • Warning to monitor patients for signs of acute haemolysis with worsening of anaemia if discontinuing treatment in 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 PL 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 SmPC Section 6.5 and PL Section 6 Additional risk minimisation measures: • None	

Embryo-fetal toxicity	Routine risk minimisation 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 • 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 PL 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 PL Section 2 Additional risk minimisation measures: • None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: • Pregnancy-, lactation-, embryo-fetal toxicity-follow up form Additional pharmacovigilance activities: • None

Use in patients with hepatic impairment	Routine risk minimisation measures: • Information that the pharmacokinetics of mitapivat in patients with mild, moderate, or severe hepatic impairment have not been studied in SmPC Section 5.2 • Advice that Pyrukynd has not been studied in patients with hepatic impairment and no dose recommendations can be made in SmPC Sections 4.2 and 5.2 Additional risk minimisation measures: • None	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: • None Additional pharmacovigilance activities: • Hepatic impairment Study AG348-C-0HEP; final study report available: 31 March 2024
Long-term Use	Routine risk minimisation measures: • Information that the median duration of treatment with Pyrukynd was 24.1 weeks in AG348-C-006 and median duration of treatment in AG348-C-007 was 40.3 weeks in SmPC Section 5.1 • 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 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.

2.6.4. Conclusion

The CHMP considers that the risk management plan version 0.5 is acceptable.

2.7. Pharmacovigilance

2.7.1. Pharmacovigilance system

The CHMP considered that the pharmacovigilance system summary submitted by the applicant fulfils the requirements of Article 8(3) of Directive 2001/83/EC.

2.7.2. Periodic Safety Update Reports submission requirements

The requirements for submission of periodic safety update reports for this medicinal product are set out in the Annex II, Section C of the CHMP Opinion. The applicant did request alignment of the PSUR cycle with the international birth date (IBD). The IBD is 17.02.2022. The new EURD list entry will therefore use the IBD to determine the forthcoming Data Lock Points.

2.8. Product information

2.8.1. User consultation

The results of the user consultation with target patient groups on the package leaflet submitted by the applicant 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.*

2.8.2. 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

The claimed therapeutic indication is "the treatment of pyruvate kinase deficiency (PK deficiency) in adult patients". Pyruvate kinase deficiency (PKD) is a glycolytic defect causing congenital non-spherocytic haemolytic anaemia.

The aim of the treatment is to improve haemolytic anaemia associated with the disease.

3.1.1. Available therapies and unmet medical need

There are no standard guidelines for treating patients with PK deficiency. Current management remains supportive treating the symptoms of lifelong haemolytic anaemia and associated complications, with red cell transfusions, chelation and splenectomy. Decisions to transfuse and/ or splenectomise must be individualised. Haematopoietic stem cell transplant has been pursued in a small number of patients with mixed outcomes.

There is an unmet need for a safe and effective therapy that specifically targets the underlying pathophysiology of PK deficiency, and thereby improves chronic haemolytic anaemia, prevents or reduces the need for transfusions, reduces iron loading and decreases disease burden, improves quality of life (QOL), and has the potential to prevent long-term complications.

3.1.2. Main clinical studies

The clinical development consisted of two phase 3 pivotal studies in adult subjects with PK deficiency across a broad range of transfusion needs. Study AG348-C-006 was a randomised, double-blind, controlled, multicentre study in non-transfusion dependent adult subjects with PK deficiency, to evaluate the efficacy, and safety of mitapivat compared to placebo with a primary endpoint the rate of Hb response defined as ≥1.5 g/dl increase in Hb concentration (N=80). Study AG348-C-007 was a phase 3, single-arm, open-label study in transfusion-dependent subjects with a primary endpoint, transfusion reduction rate, defined as a ≥33% reduction in the number of RBC units transfused during standardised period.

3.2. Favourable effects

At the DCO date of the study 006, 40% (16 subjects) of subjects treated with mitapivat achieved an Hb response compared with 0% on placebo. The randomisation stratification adjusted difference in response rate was 39.3%, 95%CI (24.1, 54.6), 2-sided p-value <0.0001.

The difference between both study arms in LS mean average change from baseline in Hb concentrations across Weeks 16, 20, and 24, was 18.21 g/L, 95% CI (12.41, 24.01) (2-sided p-value <0.0001).

In regard to biomarkers of haemolysis, the difference in LS mean average change from baseline in indirect bilirubin, LDH, and haptoglobin across Weeks 16, 20, and 24 were -26.26 µmol/L (95% CI: -37.82, -14.70), -70.81 U/L, (95% CI:-115.88, -25.74) and 0.158 g/L, (95% CI: 0.043, 0.273), respectively. The difference in LS mean (SE) on the PKDD weekly mean score and PKDIA score between the 2 arms at Week 24 was -3.11 (1.352), and -3.25 (1.574).

At the DCO date of study 007, out of the 27 TD patients with PK deficiency, 10 patients were responders and had ≥ 33% reduction in RBC units transfused during the fixed-dose period standardised to 24 weeks compared with the historical number of RBC units (TRR rate: 37%, 95% CI: 19.4%, 57.6%). The mean percent reduction from historical RBC units transfused was 37.1%. Among the 10 responders, 9 subjects achieved a ≥50% reduction in RBC units transfused and 6 (22.2%) subjects were transfusion-free. The

percent mean reduction of transfusion episodes during the Fixed-Dose Period compared with the historical RBC transfusion episodes was 39.57%.

At the DCO date of study 011 (12 September 2021), among the 31 NTD Hb responders, Hb response was ongoing for 26 Hb responders up to 29.1 months with continued mitapivat treatment. The median duration of Hb response was 15.1 months 95%CI (10.84, 19.58). Out of the 5 Hb responders who experienced loss of Hb response, 3 subjects continued to have Hb concentration increased by ≥15 g/L with ongoing treatment with mitapivat after the initial loss of Hb response.

Nine out of the 10 transfusion reduction responders had ongoing transfusion reduction response up to 30.7 months with continued mitapivat treatment. Among the 10 TD responders, the median duration of TRR was 24.0 months 95%CI (17.9, 28.0). In addition, 7 out of the 8 transfusion-free responders maintained their transfusion-free response up to 31.8 months with continued mitapivat treatment.

3.3. Uncertainties and limitations about favourable effects

While the pivotal studies 006 and 007 enrolled subjects with different transfusion needs to allow measurement of the respective primary endpoints, there are serious concerns about the representativeness of the trial population given restrictions in selection of patients related to genotype in PKLR gene. Patients who had 2 non missense mutations without the presence of another missense mutation or were homozygous for the R479H mutation in PKLR gene were excluded from both trials. It is therefore uncertain if these patients may respond to treatment. The lack of specification and validation of the PRO endpoints (PKDD and PKDIA) prior to the confirmatory phase 3 study 006 is a limitation to the results interpretation. No clinically significant effect of mitapivat on symptoms and disease burden was achieved and results are only of exploratory nature given above raised methodological concerns.

The study 007 is a single-arm clinical trial aimed at investigating the efficacy of mitapivat in TD patients. There are obvious uncertainties related to the design of a single arm study due to the lack of comparator and potential bias related to selection and measurements.

Analysis of iron markers in pivotal studies are of exploratory nature. Controlled results on ferritin and LIC in NTD patients are limited to only 24 weeks and no improvement was observed. Based on the current long-term non-controlled data, ferritin remained largely stable. Slight improvements in LIC by MRI were seen with continued mitapivat treatment in some patients.

It is difficult to contextualise the effect observed on haemolysis/haematopoetic markers to clinical benefit particularly at long-term on the complications of the disease. The relation between the improvement of haemolytic anaemia and improvement of disease course or long-term outcomes is still not known.

3.4. Unfavourable effects

155 subjects with PK deficiency were included the safety analysis set, 108 from both pivotal studies. Overall, this sample of subjects was considered overall representative of the target population for the safety analysis, in terms of demographics and baseline disease characteristics.

TEAEs were presented by individual study based on the highest dose taken (≤50 mg BID or >50 mg BID) over two analysis periods: the 24-week on-treatment period and the cumulative period, which includes an additional period of 28 days of post-treatment follow-up.

As of the updated data cut-off (12 September 2021), the overall incidence of TEAEs increased but remained similar between groups and studies: 97.4% in Cohort 1; 95.0% Cohort 2 and 100.0% in Cohort 3 (versus 89.7%; 87.5% and 88.9%, respectively).

Overall, in the target population, reported most common TEAEs were consistent with what has been previously observed.

Also, Osteopenia became frequent within the safety analysis set: events were reported in both populations who received mitapivat in the parent studies (1 case [3.7%] in Cohort 3 and 6 cases [15.0%] in Cohort 2). These events cannot be disentangled from the mitapivat inhibitory effect on aromatase.

Whereas treatment-related TEAEs were initially more frequent in TD subjects (63.0% in Study 007 vs 57.5% in Study 006), due to repeated transfusions, such events became comparable between the three cohorts, driven by a largest increase in Cohort 1 (7 additional subjects) followed by Cohort 2 (4 additional subjects) and then Cohort 1 (1 additional subject).

Initially, a low number of treatment-related Grade ≥ 3 TEAEs was observed in pivotal studies: 1 TD subjects (Joint Swelling) and 3 in NDT subjects (Musculoskeletal pain, 2 cases of Hypertriglyceridaemia), including 1 SAE (Grade 3 Hypertriglyceridaemia). The number of such events remained low (6 subjects, 5.7%): two additional events were reported in Cohort 1 (Grade 3 Gastroenteritis and Grade 3 Seizure).

Among the five additional SAEs reported, only the two above mentioned SAEs were considered treatment-related.

Few discontinuations or dose modifications due to adverse events occurred under the MA regimen, this supporting the relevance of the dose titration used in pivotal studies.

Acute haemolysis was an identified risk associated with mitapivat. Sudden withdrawal of mitapivat was performed in two subjects from supportive Study 003 (>50 mg BID) when Hb levels would exceed the upper limit normal (ULN), leading to acute haemolysis. Implementation of a dose taper (starting at 300 mg BID and decreasing by 100 mg increments for 3 weeks) in 14 subjects from this arm induced a decrease in Hb levels, which stabilised in eleven of them.

A different dose taper was implemented in pivotal studies and successfully performed in 5 subjects (of which 3 Hb non-responders) from Study 006.

Moreover, mitapivat inhibits aromatase activity. Through the conversion of androgens to estrogens, this enzyme plays a key role in bone growth and maturation. Changes in sex hormones (increase of androgens, decrease of estrogens) have been observed in male subjects. These changes were reversible upon treatment discontinuation.

In addition, GI events initially concerned at least 40% subjects in all treatment groups. The rate of these events was comparable between the placebo arm of Study 006 and Study 007 (48.7% and 48.1% respectively). Nausea were the most reported GI events (>15% in each treatment group), which was confirmed by updated safety data.

3.5. Uncertainties and limitations about unfavourable effects

Considering the updated safety data, the chronic toxicity of treatment should be further investigated in appropriately designed post-marketing studies/registries.

Also, few subjects over 65 years of age (5 subjects, 4.6%) were included. An appropriate wording has been added in SmPC, section 5.1 to reflect that it was not possible to determine whether the safety profile of mitapivat in this sub-population was comparable to that of the under 65 years of age.

The potential for an effect on blood pressure of mitapivat has been recognised based on biological plausibility and considering the observed results. Cardiovascular parameters in patients will be monitored through Study 011 to better define the effect of mitapivat in the treated population.

Regarding acute haemolysis, the dose taper implemented in supportive Study 003 to reduce the risk of haemolysis seems effective. Also, the clinical impact of reported missed doses was remote, as depicted in the data provided by the applicant.

Regarding the AESI aromatase inhibition, in female subjects, the adverse events identified as possibly associated with changes in sex hormones were discussed. Due to confounding factors, it is not possible to determine whether or not the reported events are related to mitapivat.

In addition, although no meaningful changes in bone mineral density have been observed in both gender, impact of mitapivat treatment on these subjects with fragile bones still cannot be ruled out. Longer-term data will generate more information on this aspect post-approval.

Nauseas were the most reported GI event in subjects who received mitapivat, independently of the transfusion burden (25.9% in TD subjects versus 25.0% in NTD subjects). This AE has been listed in the SmPC.

Also, the following risks will be closely monitored and discussed in the PSURs: Changes in sex hormones and Bone mineral density decrease due to aromatase inhibition and associated AEs, as part of PSURs safety concerns.

3.6. Effects Table

Table 79 Effects Table for mitapivat in the study AG348-C-006 (data cut-off: 20 November 2020), study AG348-C-007 (data cut-off: 15 January 2021) and study AG348-C-011 (data cut-off: 12 September 2021)

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Favourable Effects						
HbR Hb response	⩾ 1.5 g/dL increase in Hb concentration from baseline	%	40	0		Study 006
			Adjusted difference in HbR: 39.3 95% CI (24.1, 54.6) p<0.0001			
Hb LS mean	Average change from baseline in Hb level at Weeks 16, 20, and 24	g/L	16.73	-1.48		
			Difference in LS mean: 18.21 95% CI (12.41, 24.01)			
TRR Transfusion reduction response	≥33% reduction in RBC units transfused	% (95% CI)	37 (19.4, 57.6)	NA	Single-arm non-controlled study, small number of patients (N=27).	Study 007
T0	transfusion-free responders	% (95% CI)	22.2 (8.6, 42.3)	NA		
Unfavourable Effects						
AEs	All grades included	Incidence in %	Cohort 1: 97.4% Cohort 2: 95.0% Cohort 3: 100.0%	NA		Studies 006, 007 & 011

Effect	Short Description	Unit	Treatment	Control	Uncertainties/ Strength of evidence	References
Aromatase inhibition	-	-	-	-	Uncertainties on the impact on menstrual cycle disorders and bone mineral density	Studies 006, 007 & 011
Nausea	Grade 1-2	Incidence in %	Cohort 2: 25.0% Cohort 3: 25.9%	-	The high rate in subjects who received mitapivat in parent studies is of concern	Studies 006, 007 & 011

3.7. Benefit-risk assessment and discussion

3.7.1. Importance of favourable and unfavourable effects

Sustained Hb response was observed with long-term treatment, with most subjects maintaining their response of increase in Hb (study 006) and transfusion reduction (study 007).

Nevertheless, the available data do not provide sufficient evidence of a clear benefit of mitapivat on the clinical manifestation of the disease and iron overload in patients who are not regularly transfused.

Although study AG348-C-007 had a single-arm design, patients with PK deficiency who are regularly transfused do not spontaneously become transfusion free or achieve normal Hb concentrations independent of transfusions. Being transfusion free for an extended period with sustained treatment could decrease "transfusional" iron overload and may thus mitigate the limitations of a single-arm study and uncertainties related to the estimation of the primary endpoint (TRR rate).

The absence of efficacy results in a subset of patients excluded from both clinical trials, based on mutations is a significant concern on study population representativeness. This is clearly reflected in the product information.

The safety profile identified from the safety dataset is manageable. Most of AEs were non serious, no fatal AE was reported. Few discontinuations or dose modifications due to adverse events occurred under the MA regimen, this supporting the relevance of the dose titration used in pivotal studies.

Acute haemolysis was an identified risk associated with mitapivat, mainly in case of sudden withdrawal of mitapivat. A dose taper was then successfully implemented. However, the safety impact of missed doses remains unknown.

Moreover, mitapivat inhibits aromatase activity, which plays a key role in bone growth and maturation, and unbalances in sex hormones. Although no meaningful changes in bone mineral density have been observed in both gender, impact of mitapivat treatment on these subjects with fragile bones still cannot be ruled out.

In addition, Nausea concerned at least 20% subjects from mitapivat groups of parent studies, which is still of concern.

3.7.2. Balance of benefits and risks

The clinical efficacy of mitapivat has been clearly shown in terms of Hb level, haemolysis parameters and transfusion need in TD and NTD patients (study 006 and study 007, respectively) even though the available data do not provide sufficient evidence of clear beneficial effect on symptoms and iron overload.

The absence of efficacy results in a subset of patients excluded from both clinical trials based on mutations is a significant concern in terms of study population representativeness. A warning and information related to genotype is added in the product information.

Long-term outcomes are still unknown, and the applicant has committed to provide final results of the long-term study AG348-C-011; an ongoing, global, retrospective and prospective, longitudinal observational study of paediatric and adult patients with pyruvate kinase deficiency (PK deficiency) as a post-approval measure via the relevant regulatory procedure.

3.8. Conclusions

The overall benefit/risk balance of Pyrukynd is positive, subject to the conditions stated in section 'Recommendations'.

4. Recommendations

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 is favourable in the following indication(s):

> Pyrukynd is indicated for the treatment of pyruvate kinase deficiency (PK deficiency) in adult patients.

The CHMP therefore recommends the granting of the marketing authorisation subject to the following conditions:

Conditions or restrictions regarding supply and use

Medicinal product subject to medical prescription.

Other 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.

The marketing authorisation holder shall submit the first periodic safety update report for this product within 6 months following authorisation.

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.

New Active Substance Status

Based on the CHMP review of the available data, the CHMP considers that mitapivat is to be qualified as a new active substance in itself as it is not a constituent of a medicinal product previously authorised within the European Union.