CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

216196Orig1s000

INTEGRATED REVIEW

Integrated Review

Table 1. Administrative Application Information

Category	Application Information
Application type	NDA
Application number(s)	216196
Priority or standard	Priority
Submit date(s)	6/17/2021
Received date(s)	6/17/2021
PDUFA goal date	2/17/2022
Division/office	Division of Nonmalignant Hematology (DNH)
Review completion date	2/16/2022
Established/proper name	Mitapivat
(Proposed) proprietary name	Pyrukynd
Pharmacologic class[1]	Pyruvate Kinase Activator
Code name	AG-348
Applicant	Agios Pharmaceuticals, Inc.
Dosage form(s)/formulation(s)	Oral Tablet
Dosing regimen	Starting dose of 5 mg per oral twice daily; titrate through sequential doses to 20 mg and to maximum of 50 mg twice daily
Applicant proposed indication(s)/ population(s)	Treatment of adult patients with pyruvate kinase deficiency
Proposed SNOMED indication	124331002 | Deficiency of pyruvate kinase (disorder)
Regulatory action	Approval
Approved dosage (if applicable)	Starting dose of 5 mg taken orally twice daily. To gradually increase hemoglobin, titrate Pyrukynd from 5 mg twice daily to 20 mg twice daily, and then to the maximum recommended dose of 50 mg twice daily, with these dose increases occurring every 4 weeks.
Approved indication(s)/ population(s) (if applicable)	Treatment of hemolytic anemia in adults with pyruvate kinase (PK) deficiency
Approved SNOMED term for indication (if applicable)	124331002 | Deficiency of pyruvate kinase (disorder)

[1] Mitapivat is a first-in-class new molecular entity that is an activator of pyruvate kinase. Therefore, there is no existing established pharmacological class (EPC) for mitapivat. The general EPC term “pyruvate kinase activator” is consistent with the in vitro and in vivo pharmacology of mitapivat and takes into consideration the nonspecific nature of pyruvate kinase activation by mitapivat.

Table of Contents

Table of Tables

Table of Figures

Glossary

2,3-DPG	2,3-bisphosphoglyceric acid
ADAM	advance dissolution, absorption, and metabolism
ADME	absorption, distribution, metabolism, excretion
ADP	adenosine diphosphate
AE	adverse event
ALT	alanine aminotransferase
ANCOVA	analysis of covariance
AST	aspartate aminotransferase
ATP	adenosine triphosphate
AUC	area under the concentration-time curve
BCRP	breast cancer resistance protein
BID	twice daily
BR_{max}	maximum percentage increase from baseline response value
BR_{min}	maximum percentage decrease from baseline response value
BSEP	bile salt export pump
CFR	Code of Federal Regulations
CI	confidence interval
CL	clearance
CL/F	apparent clearance
C_{max}	maximum plasma concentration
CMC	chemistry, manufacturing, and controls
CNS	central nervous system
CSR	critical supersaturation ratio
CV	coefficient of variation
CYP	cytochrome p450
DCOA	Division of Clinical Outcome Assessment
DDI	drug-drug interaction
DXA	dual-energy x-ray absorptiometry
EC50	half maximal effective concentration
ECG	electrocardiogram
eGFR	estimated glomerular filtration rate
EPC	established pharmacologic class
E-R	exposure-response
FAS	full analysis set
FDA	Food and Drug Administration
FMQ	Food and Drug Administration Medical Dictionary for Regulatory Activities query
GDT	gradual dose taper
GI	gastrointestinal
HCT	hematocrit
HD	high dose
HI	hepatic impairment
HLM	human liver microsomes
IC_{50}	half maximal inhibitory concentration

ICH	International Conference on Harmonisation
IND	investigational new drug
IVIVE	in vitro-in vivo extrapolation
LDH	lactate dehydrogenase
MAR	missing at random
MATE	multidrug and toxin extrusion
MedDRA	Medical Dictionary for Regulatory Activities
MI	multiple imputation
MMRM	mixed-effect model repeat measurement
MRHD	maximum recommended human dose
mRNA	messenger ribonucleic acid
NDA	new drug application
NOAEL	no observed adverse effect level
NTI	narrow therapeutic index
OAT	organic anion transporter
OATP	organic anion transporting polypeptide
OCT	organic cation transporter
OSI	Office of Scientific Investigations
PBPK	physiologically based pharmacokinetic
PD	pharmacodynamics
P-gp	p-glycoprotein
PK	pyruvate kinase
PKDD	Pyruvate Kinase Deficiency Diary
PKDIA	Pyruvate Kinase Deficiency Impact Assessment
PKL	L-type pyruvate kinase
PKLR	pyruvate kinase liver and red blood cell
PKM2	M2-type pyruvate kinase
PKPD	pharmacokinetics/pharmacodynamics
PK-R	red cell isoform of pyruvate kinase
PMR	postmarketing requirement
PP	per protocol
PRO	patient-reported outcome
PSA	prostate specific antigen
QD	once daily
QOD	every other day
QTcF	QT interval-Fridericia's method
RBC	red blood cell
RI	renal impairment
SAE	serious adverse event
SAP	statistical analysis plan
SD	standard deviation
SF1	solubility factor 1
SOC	system organ class
TEAE	treatment-emergent adverse event
T_{max}	time to maximum concentration
TRR	transfusion reduction response

TT	transfusion trigger
UGT	uridine 5'-diphospho-glucuronosyltransferase
ULN	upper limit of normal
U.S.	United States
UV	ultraviolet
V	volume of distribution
WT	wild-type

I. Executive Summary

1. Summary of Regulatory Action

Agios Pharmaceuticals, Incorporated (Applicant) submitted this new drug application (NDA) for mitapivat (tradename Pyrukynd), seeking approval for the treatment of adult patients with pyruvate kinase (PK) deficiency (PKD). The NDA was reviewed by a multidisciplinary review team and each discipline and the signatory recommends approval. Mitapivat is the first product to be approved for the treatment of PKD. Mitapivat will be approved for the treatment of hemolytic anemia in adults with PKD.

Substantial evidence of effectiveness for mitapivat in adults with PKD was established using data from two adequate and well-controlled trials. In Study AG-348-C-006 (Study 006) evaluating patients with PKD who were symptomatic but not receiving regular red blood cell transfusions, mitapivat demonstrated a substantial improvement in hemoglobin (Hb) response of at least 1.5 g/dL that was statistically significant compared to placebo. Almost all responders in the mitapivat arm had a ≥2 g/dL increase in Hb and six (38%) responders had a >4 g/dL increase in Hb. Most patients experienced Hb decrease in the placebo arm. Study 006 also met all of the prespecified hierarchical secondary endpoints, which included changes from baseline in the hemolysis parameters of lactate dehydrogenase, indirect bilirubin, haptoglobin, and reticulocytes. A meaningful clinical benefit was confirmed by demonstration of improvement in frequent and common signs and symptoms of PKD: fatigue, shortness of breath, and jaundice in a patient-reported outcome in the mitapivat arm compared with the placebo arm. In Study AG-348-C-007 (Study 007) in patients with PKD who are regularly receiving blood transfusions, demonstration of clinical benefit was achieved by a clinically significant reduction in transfusion burden, with 22% of patients becoming transfusion independent over the 24-week treatment period.

The available safety data show that mitapivat is safe for its intended use. The safety database is sufficient for evaluation of risk in the context of the rarity of the disease. Due to the risk of acute hemolysis with abrupt interruption or discontinuation of mitapivat, acute hemolysis will be included in the warnings and precautions section of the label and a gradual dose taper will be recommended. Common adverse reactions include reductions in estrone and estradiol levels in men (this could not be adequately assessed in women due to the variations in these hormones during the menstrual cycle and use of hormonal contraception), increased urate, back pain, and arthralgia. All the identified safety risks for mitapivat can be adequately mitigated through labeling and further evaluated during routine pharmacovigilance. Because mitapivat is a weak aromatase inhibitor and mitapivat will likely be given life-long to patients with this disease, the long-term safety of aromatase inhibition will be assessed in two clinical postmarketing requirements assessing reproductive hormones, decreased bone density and bone fractures, changes in blood lipid levels, and adverse events with long-term aromatase inhibition.

The NDA includes appropriate preapproval nonclinical and clinical pharmacology studies. One clinical pharmacology study will be conducted as a postmarketing requirement to assess

mitapivat pharmacokinetic exposures in patients with moderate hepatic function. In the meantime, labeling will state to avoid use in patients with moderate or severe hepatic function.

Based on review of all available efficacy and safety data, the benefits for mitapivat in terms of increasing hemoglobin, improving how a patient functions and feels with the disease, and decreasing the transfusion burden were clearly demonstrated and the risks appear manageable when mitapivat is used as recommended in the approved labeling. The availability of mitapivat will provide the first effective treatment option for adults with PKD.

2. Benefit-Risk Assessment

2.1. Benefit-Risk Framework

Table 2. Benefit-Risk Framework

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition	• Pyruvate kinase (PK) deficiency (PKD) is an autosomal recessive disorder caused by variants in the pyruvate kinase liver and red blood cell (PKLR) gene, resulting in deficiency of PK, which catalyzes the conversion of phosphoenolpyruvate and ADP to pyruvate and ATP in glycolysis. • The estimated prevalence of PKD in the Western (white European) population is 51 cases per million. • The glycolytic enzymopathy results in nonspherocytic hemolytic anemia with variable clinical presentations, ranging from mild to life-threatening and debilitating comorbidities. Over 80% of patients require transfusions either chronically or intermitentally due to anemia. • Signs and symptoms of PKD include fatigue, shortness of breath, bone pain, splenomegaly, and jaundice; its complications include iron overload, gallstones, infections, hemolytic crisis, bone fractures, extramedullary hematopoiesis, thrombosis, pulmonary hypertension, perinatal complications, endocrine disorders, and liver cirrhosis.	• PKD is a very rare, serious life-long disease with significant morbidity that can be life-threatening.
Current Treatment Options	• Treatment is mainly supportive and includes blood transfusions, folic acid, splenectomy, and iron chelation therapy for iron overload. Hematopoietic allogeneic stem cell transplantation can be curative; however, it is not widely used due to a significant risk of mortality and morbidity.	• There are no FDA-approved therapeutic agents for PKD; there is an unmet medical need.
Benefit	• The efficacy of mitapivat for the treatment of adults with PKD was established in two adequate and well-controlled trials: Study AG-348-C-006 (Study 006), a randomized, double-blind, placebo-controlled clinical study; and Study AG-348-C-007 (Study 007); a single-arm study with a historical transfusion rate. • Study 006 evaluated the efficacy of 24 weeks of treatment with mitapivat (n=40) versus placebo (n=40) in patients who were not regularly receiving blood transfusions (defined as four or fewer transfusion episodes in the prior 12 months and no transfusions in the prior 3 months before trial entry). Study 007 evaluated the efficacy of 40 weeks of treatment with	• Hemoglobin response of >1.5 g/dL and improvement in hemolysis markers (LDH, haptoglobin, reticulocyte, indirect bilirubin) in Study 006 demonstrate a clear treatment effect of mitapivat over placebo. • Mean treatment effects on the PKDD patient-reported outcome in patients who are not regularly

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	mitapivat (n=27) in adults with PKD who were regularly receiving blood transfusions (defined as at least six transfusion episodes in the 52-week period before trial entry). • The trials initiated mitapivat at 5 mg twice daily, which could be titrated up to a maximum dose of 50 mg twice daily based on hemoglobin response. • In Study 006, the primary endpoint was hemoglobin response, defined as a ≥1.5 g/dL increase from baseline. A total of 16 patients (40%) in the mitapivat arm and no patient in the placebo arm achieved this endpoint. The adjusted treatment difference was 39.3% (95% CI: 24.1, 54.6), p<0.0001, favoring mitapivat. Almost all mitapivat responders had a ≥2 g/dL increase in Hb, six of whom (38% of the responders) had a >4 g/dL increase in Hb. In contrast, most patients in the placebo arm experienced a decrease in Hb. • Study 006 also met all of the prespecified hierarchical secondary endpoints, which included improvements from baseline in hemolysis parameters: lactate dehydrogenase (LDH), indirect bilirubin, haptoglobin, and reticulocytes. • On the Pyruvate Kinase Deficiency Diary (PKDD), a patient-reported outcome that assessed some of the signs and symptoms of PK deficiency, the items with the greatest improvement with mitapivat compared to placebo were tired at its worst during the day, tiredness after finishing daily activities, jaundice and shortness of breath. However, the mean treatment differences in these scores were small and of unclear clinical meaningfulness (a difference of about 1 point or less between treatment groups, on a scale of 0-10 for tiredness and shortness of breath and a difference of 0.3 points on a scale of 0-4 for jaundice). Responder analyses showed that some mitapivat-treated patients can have more sizeable improvements on these signs and symptoms compared to the placebo-treated patients. • In Study 007, the primary endpoint was transfusion reduction burden, defined as ≥33% reduction in the number of red blood cell (RBC) units transfused compared with each patient's historical transfusion burden. A total of 9 patients (33.0% [95% CI: 16.5, 54.0]) achieved this endpoint and 6 (22.2% [95% CI: 8.6, 42.3]) became transfusion free. All patients who were transfusion free remained transfusion free in a long-term extension study (median duration of response 17 months). This reduction in transfusion is clinically meaningful, reducing exposure to iron overload and other transfusion-related risks.	transfused was small and of unclear clinical benefit. However, some mitapivat-treated patients can have more sizeable improvements on these signs and symptoms. • Reduction in transfusion burden and complete freedom from transfusion seen in some mitapivat-treated patients who had previously required regular red blood cell transfusions is clinically meaningful, reducing the need for this procedure and reducing exposure to iron overload and other transfusion complications. • The chronic hemolysis of PKD does not spontaneously improve because there is an underlying genetic defect. Therefore, a decrease in need for transfusions would not occur spontaneously. • There remains uncertainty whether rare subsets of patients (e.g., p.R479H/p.R479H genotype, nonmissense homozygosity) will respond to mitapivat. The limited information on these subgroups will be included in labeling so prescribers can decide whether to consider trying mitapivat in these patients and discontinuing treatment if the response is not adequate.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	• Both studies enrolled patients with at least two variant alleles in the PKLR gene, of which at least one was a missense variant; patients who were homozygous for the c.1436G > A (p.R479H) variant or had two nonmissense variants (without the presence of another missense variant) in the PKLR gene were excluded. The exclusion of the p.R479H/p.R479H genotype was because five patients with this genotype had a very low PK protein level at baseline and minimal to no change in Hb in another study. This response is expected when there is no or very low PK protein because PK protein is necessary for mitapivat's mehanism of action. • Having two nonmissense variants (without the presence of another missense variant) can encompass a wide variety of different types of variants (coding, promoter, intronic variants; deletions, insertions, nonsense, etc.) with a wide range of effects on the PK protein. Hb changes with mitapivat was not predictable in these patients in another study.	• Availability of mitapivat will provide a new and effective first-in-class treatment option for patients with PKD.

Risk and Risk Management	• The safety database of mitapivat for treatment of PKD is small but acceptable in the context of a rare disease with 40 patients treated with mitapivat in the placebo-controlled study; 27 patients in the single-arm study; 52 patients in a dose-ranging study; and 36 patients with longer-term treatment in the extension study. Thirty-two patients received mitapivat for more than a year. • Acute hemolysis with subsequent anemia following abrupt interruption or discontinuation of mitapivat occurred in two patients receiving six times the maximum recommended mitapivat dose. No such cases occurred with the use of a taper in Study 006 and 007. It is unclear whether the taper or the reduced mitapivat dose or a combination of both led to no such cases. A taper will be recommended in labeling. • Mitapivat is a weak aromatase inhibitor in vitro. Patients treated with mitapivat experienced adverse reactions that could be consistent with those observed with aromatase inhibitors; the most common adverse reactions (including laboratory abnormalities [≥10%]) were estrone and estradiol decreased (in men; the effect of mitapivat on these hormones could not be reliably assessed in women due to the physiologic variability in the menstrual cycle and use of hormonal contraception), increased urate, back pain, and arthralgia. Aromatase inhibition could also potentially cause decreases in bone mineral density. The trials were too short to assess this risk. • Serious adverse reactions observed in the clinical studies were single cases of atrial fibrillation, gastroenteritis, rib fracture, and musculoskeletal pain in Study 006; and single cases of ovarian cyst, renal colic, and blood triglycerides increased in Study 007. • There were no deaths reported in the safety database. • Several drug interactions have the potential to affect mitapivat exposures, including drugs that are inhibitors or inducers of CYP3A, UGT1A1, and P-gp substrates. These drugs could increase mitapivat exposures (increasing risks), or in the case of inducers, reduce mitapivat exposures (reducing efficacy). Labeling will recommend to avoid use of mitapivat with these drugs. • Mitapivat undergoes extensive hepatic metabolism, and moderate and severe hepatic impairment is expected to increase the systemic exposure of mitapivat. The pharmacokinetics of mitapivat in patients with hepatic impairment has not been studied.	• The safey database is adequate for mitapivat for the proposed indication, doage regimen and duration. • Adverse reactions observed in the clinical trials were generally manageable with monitoring, dose adjustment and appropriate clinical management. • The potential risk of acute hemolysis with abrupt interruption or discontinuation will be included in Warnings and Precautions in labeling, and a gradual dose taper of mitapivat will be recommended when discontinuing treatment. • Labeling will include medications to avoid due to drug-drug interactions.. • The safety data do not indicate need for a risk evaluation and mitigation strategy (REMS). Risks are adequately mitagated when mitapivat is used according to labeling. • Long-term safety to include reproductive hormone, decreased bone density and bone fractures, changes in blood lipid levels, and adverse events with long-term aromatase inhibition will be assessed in two postmarketing requirements (PMRs). • An additional PMR will evaluate moderate hepatic impairment on mitpativat pharmacokinetics. In the meantime labeling will state to avoid use in patients with moderate or severe hepatic impairment.

'Abbreviations: ADP, adenosine diphosphate; ATP, adenosine triphosphate; CYP3A, cytochrome 450 family 3 subfamily A; LDH, lactate dehydrogenase; MMRM, mixed model for repeated measures; P-gp, P-glycoprotein 1; PK, pyruvate kinase; PKDD, pyruvate kinase deficiency diary; PKDIA, pyruvate deficiency impact assessment; PKLR, pyruvate kinase liver and red blood cell gene; PMR, post marketing requirement; PRO, patient-reported outcome; RBC, red blood cell; UGT1A1, UDP glucuronosyltransferase family 1 member A1 gene

2.2. Conclusions Regarding Benefit-Risk

PK deficiency is a rare and serious autosomal recessive disorder affecting the function of the pyruvate kinase (PK) enzyme. The abnormal red blood cell (RBC) PK enzyme isoform leads to insufficient generation of adenosine triphosphate (ATP) in RBCs, shortened RBC lifespan, and chronic hemolysis. PKD is a lifelong condition with variable clinical presentations ranging from mild to life-threatening. Signs and symptoms include fatigue, shortness of breath, bone pain, splenomegaly, and jaundice; the complications include iron overload from repeated blood transfusions, gallstones, infections, hemolytic crisis, bone fractures, extramedullary hematopoiesis, thrombosis, pulmonary hypertension, perinatal complications, endocrine disorders, and liver cirrhosis. Treatment is supportive and includes blood transfusions, folic acid supplementation, splenectomy, and iron chelation therapy for iron overload. There are no FDA-approved therapies for PK deficiency.

Mitapivat is a PK activator that allosterically binds to the PK tetramer. The clinical data submitted clearly demonstrate that mitapivat has a large treatment effect of increasing hemoglobin and improving the hemolysis parameters lactate dehydrogenase, haptoglobin, indirect bilirubin, and reticulocytes compared to placebo in adults with PK deficiency who are not regularly transfused. For example, 40% of these mitapivat-treated patients had at least a 1.5 g/dL increase in hemoglobin (a measure of red blood cell mass) compared to none of the placebo-treated patients. More than one-third of the mitapivat responders had a >4 g/dL increase in hemoglobin. For context, a 1 unit red blood cell transfusion typically raises the hemoglobin by about 1 g/dL. On the Pyruvate Kinase Deficiency Diary (PKDD), a patient-reported outcome that assessed some of the signs and symptoms of PK deficiency in this study, the items with the greatest improvement with mitapivat compared to placebo were tired at its worst during the day, tiredness after finishing daily activities, jaundice and shortness of breath. However, the mean treatment differences in these scores were small and of unclear clinical meaningfulness (a difference of about 1 point or less between treatment groups, on a scale of 0-10 for tiredness and shortness of breath and a difference of 0.3 points on a scale of 0-4 for jaundice). Responder analyses showed that some mitapivat-treated patients can have more sizeable improvements on these signs and symptoms than placebo-treated patients.

In patients who required regular transfusions, clinical benefit was demonstrated by a decrease in transfusion burden. In this trial, the baseline number of transfusion units was 7 (range 3-20) standardized to the 24-weeks prior to study enrollment. Thirty-three percent of the patients in this trial achieved at least a one-third reduction in their number of transfusions during treatment with mitapivat compared to their historical transfusion burden and 22% of the patients in this trial remained transfusion-free over the 24-week assessment period. A decrease in transfusion burden or transfusion independence would not be expected to occur spontaneously. The clinical benefit of reduced transfusions includes less need for this procedure and less exposure to its risks including iron overload.

The safety database for mitapivat was adequate for the proposed dosing regimen and intended patient population. Overall, mitapivat has a favorable safety profile, and the safety findings can be adequately mitigated with labeling and by routine pharmacovigilance. The drug was well-tolerated, and there were no deaths in the clinical program. The most frequent adverse reactions were reductions in estrone and estradiol in men (the effect of mitapivat on these hormones in women could not be adequately assessed due to physiologic variations during the menstrual cycle and use of hormonal contraception), increased urate, back pain, and arthralgia. There is a risk of acute hemolysis with abrupt cessation or discontinuation of the drug, which will be included as a Warning and Precaution in labeling, with a recommendation to gradually taper the dose if stopping the medication. Mitapivat weakly inhibits aromatase, which could potentially further decrease bone mineral density in a population that is already at risk for osteoporosis. The trials were too short to adequately assess this risk. Long-term safety data will be collected in two postmarketing requirements that evaluate reproductive hormones, changes in blood lipid levels, bone fractures and other adverse events caused by long-term aromatase inhibition.

Drugs that are inhibitors of cytochrome P450 3A have the potential to increase mitapivat exposure (increasing mitapivat's risks). Inducers of cytochrome P450 3A have the potential to decrease mitapivat exposure (decreasing mitapivat's efficacy). Labeling will recommend avoiding concomitant administration of these drugs with mitapivat.

Mitapivat undergoes extensive hepatic metabolism, and moderate and severe hepatic impairment is expected to increase the systemic exposure of mitapivat. A postmarketing requirement will evaluate the impact of hepatic impairment on the pharmacokinetics of mitapivat in subjects with moderate hepatic impairment. In the meantime, labeling will state to avoid use of mitapivat in patients with moderate or severe hepatic impairment.

Based on review of all available efficacy and safety data, the benefits of mitapivat outweigh the risks for treatment of anemia in patients with PKD, when mitapivat is used according to labeling. The availability of mitapivat will provide the first pharmacologic treatment option for this patient population.

II. Interdisciplinary Assessment

3. Introduction

Agios Pharmaceuticals seeks approval of mitapivat for the treatment of adults with pyruvate kinase (PK) deficiency (PKD). PKD is a rare, inherited, autosomal recessive disorder causing life-long hemolytic anemia with variable clinical presentations, ranging from mild to life-threatening and debilitating. Signs and symptoms include fatigue, shortness of breath, bone pain, splenomegaly, and jaundice. Complications include splenectomy, iron overload from repeated transfusions, gallstones, infections, hemolytic crisis, osteopenia/osteoporosis/bone fracture, extramedullary hematopoiesis, thrombosis, pulmonary hypertension, and liver cirrhosis (Grace et al. 2019). Patients with PKD have significantly higher lifetime rates of osteoporosis, liver cirrhosis, and pulmonary hypertension compared to a matched population (Boscoe et al. 2021). Perinatal complications (need for preterm birth intrauterine transfusion, growth retardation, preterm labor) also occur. In Western populations, the prevalence of clinically diagnosed PKD is estimated to be between 3.2 and 8.5 cases per million; the prevalence of the disease including the undiagnosed could be as high as 51 cases per million (Bianchi and Fermo 2020).

Currently, there are no approved therapeutic agents for the treatment of patients with PKD; the treatment is mainly supportive and includes blood transfusions, folic acid, splenectomy, and chelation therapy for iron overload. Hematopoietic allogeneic stem cell transplantation (HSCT) can be curative; however, it is not widely used due to significant mortality and morbidity.

Clinical PKD is caused by compound heterozygous or homozygous variants in the pyruvate kinase liver and red blood cell (PKLR) gene, which lead to deficiency in PK. To date, over 300 variants (mostly missense) in the PKLR gene have been reported (Bianchi and Fermo 2020). PK catalyzes the conversion of phosphoenolpyruvate and adenosine diphosphate (ADP) to pyruvate and adenosine triphosphate (ATP) in glycolysis. Since mature red blood cells (RBCs) depend on the ATP generated by this process for maintaining cell integrity and function, insufficient energy production leads to reduced ATP levels, shortened RBC lifespan, and chronic hemolysis.

Mitapivat is a first-in-class PK activator of the red cell isoform of PK (PK-R). Mitapivat acts by binding to the PK-R tetramer and inducing the active R-state conformation of the PK-R tetramer, resulting in enhanced activity of the glycolytic pathway, improving adenosine triphosphate (ATP) levels and reducing 2,3-bisphosphoglyceric acid (2,3-DPG) levels. Mutations in PK cause deficiency in PK-R, preventing adequate red blood cell glycolysis, leading to buildup of the upstream glycolytic intermediate 2,3-DPG and deficiency in ATP.

Mitapivat is a new molecular entity that was granted orphan drug designation in March 2015 for the treatment of PKD. The drug product is presented as 5 mg, 20 mg, and 50 mg tablets. The proposed starting dose of mitapivat is 5 mg orally twice daily (with gradual titration based on hemoglobin levels through sequential doses increases) to 20 mg twice daily and to a maximum dose of 50 mg twice daily.

This submission was granted Fast-Track Designation for the treatment of patients with PKD and was reviewed under the priority review timeline.

3.1. Review Issue List

The review team identified key review issues relevant to the evaluation of benefit and risk, which are listed in Sections 3.1.1 and 3.1.2, respectively. For in-depth assessment of these benefit and risk issues, refer to Sections 6.3 and 7.7, respectively.

3.1.1. Key Review Issues Relevant to Evaluation of Benefit

3.1.1.1. Clinical Relevance of Patient-Reported Outcome (PRO) Endpoints

- Interpretation of clinical benefit based on PRO results (Pyruvate Kinase Deficiency Diary and Pyruvate Kinase Deficiency Impact Assessment)

3.1.1.2. Clinical Benefit in Patients With PKD Who Are Regularly Receiving Transfusions

- Interpretation of clinical benefit in patients who achieved the primary endpoint (i.e., a reduction in transfusion burden, defined as ≥33% reduction of RBC units) in a single-arm study

3.1.2. Key Review Issues Relevant to Evaluation of Risk

3.1.2.1. Long-Term Risk of Aromatase Inhibition With Chronic Administration

3.1.2.2. Understanding of the Dose Taper

3.2. Approach to the Review

The review of this NDA was jointly conducted. Xiaoyu Cai and Yeh-Fong Chen analyzed the data supporting efficacy; the Clinical Data Scientist team, Ling Cao, and Jinzhong Liu analyzed the data supporting safety; Hyon-Zu Lee and Tanya Wroblewski also analyzed and reviewed the data and information supporting safety and efficacy; Shaji Theodore, Frederica Basso, and Pedro DelValle reviewed the nonclinical toxicology studies; and Xiaomeng Xu and Doanh Tran reviewed the clinical pharmacology data.

The NDA clinical trials that support the benefit-risk assessment of mitapivat are summarized in Table 3. The review of safety and efficacy for the proposed indication focused on Studies AG-348-C-006 and AG-348-C-007 (Studies 006 and 007, respectively). Studies 006 and 007 are also referred to in this review as Studies 006 and 007, respectively.

Table 3. Clinical Trials Submitted in Support of Efficacy and/or Safety Determinations[1] for Mitapivat

Trial Identifier (NCT#)	Trial Population	Trial Design	Regimen (Number. Treated), Duration	Primary and Key Secondary Objectives	Number of Subjects Planned; Actual Randomized[2]	Number of Centers and Countries
AG-348-C-006 NCT03548220 Completed	Adults with PK deficiency who are not regularly transfused	Phase 3, randomized, double-blind, placebo-controlled study	Mitapivat 5 mg, 20 mg, or 50 mg BID Placebo: BID Duration: 26 weeks (including 2-week dose taper)	Primary endpoint: Hb response. Key secondary endpoints: Average change from baseline in Hb, bilirubin, LDH, haptoglobin, reticulocyte, PKDD, and PKDIA	Mitapivat: 40 subjects Placebo: 40 subjects	Centers: 36 Countries:14
AG-348-C-007 NCT03559699 Completed	Adults with PK deficiency who are regularly transfused	Phase 3, single-arm, open-label study	Mitapivat 5 mg, 20 mg, or 50 mg BID Duration: 42 weeks (including 3-week dose taper)	Primary endpoint: Incidence of reduction in transfusion burden. Key secondary endpoints: Annualized total number of RBC units transfused (Parts 1 and 2); number of transfusion episodes; and proportion of patients who become transfusion-free in Part 2; proportion of patients who have Hb in normal range at least once.	27 subjects	Centers: 17 Countries: 9

Trial Identifier (NCT#)	Trial Population	Trial Design	Regimen (Number. Treated), Duration	Primary and Key Secondary Objectives	Number of Subjects Planned; Actual Randomized[2]	Number of Centers and Countries
AG-348-C-003 NCT02476916 Ongoing (CSR data cutoff: 8/28/2020)	Adults with PK deficiency who are not regularly transfused	Phase 2, randomized, open-label, dose-ranging study (study initially started with capsules then transitioned to tablets)	Mitapivat 300 mg BID or 50 mg BID Duration: 99.5 months Core period: 24 weeks Extension: 90 months, dose taper: 4 weeks	Primary endpoint: Safety evaluation	52 subjects (50 mg BID: 27 subjects, 300 mg BID: 25 subjects)	Centers: 14 Countries: 6
AG-348-C-0010 NCT03692052 Ongoing (CSR: Core period only)	Adults with nontransfusion dependent thalassemia	Phase 2, single-arm, open-label study	Mitapivat 50 mg BID followed by optional dose increase to 100 mg BID Duration: 138 months Core period: 24 weeks Extension period: 516 weeks Dose taper: 2 weeks	Primary endpoint: Hb response Key secondary endpoints: Mean change from baseline in Hb, sustained Hb response, delayed Hb response, time to first ≥1.0 g/dL increase in Hb, change from baseline in markers of hemolysis and erythropoietic activity, safety, and PK endpoints.	20 subjects	Centers: 4 Countries: 3

Trial Identifier (NCT#)	Trial Population	Trial Design	Regimen (Number. Treated), Duration	Primary and Key Secondary Objectives	Number of Subjects Planned; Actual Randomized[2]	Number of Centers and Countries
AG-348-C-011 NCT03853798 Ongoing (CSR data cutoff: 11/12/2020)	Adults with PK deficiency previously treated in Studies AG-348-C-006 or AG-348-C-007	Phase 3, open-label, extension study	Mitapivat 5 mg, 20 mg, or 50 mg BID Duration: 194 weeks (including 2-week dose taper)	Primary endpoint: Safety evaluation. Key secondary endpoints: Hb response, average change from baseline in Hb, change from baseline in Hb, bilirubin, LDH, haptoglobin, reticulocyte, PKDD, PKDIA, number of transfusion events, number of RBC units transfused and PK endpoints.	90 subjects enrolled, 88 subjects initiated treatment. Of the 88 subjects, 36 received placebo in Study 006 (Cohort 1), 35 received mitapivat in Study 006 (Cohort 2) and 17 received mitapivat in Study 007 (Cohort 3).	Centers: 42 Countries: 16

Source: Reviewer.
[1] Includes all submitted clinical trials, even if not reviewed in-depth, except for Phase 1 and pharmacokinetic studies.
[2] If no randomization, then replace with "Actual Enrolled"
Abbreviations: BID, twice daily; DB, double-blind; LTE, long-term extension study; MC, multicenter; N, number of subjects; OL, open-label; PC, placebo-controlled; PG, parallel group; R, randomized, mos: months; Hb, hemoglobin; LDH, lactate dehydrogenase; PKDD, Pyruvate Kinase Deficiency Diary; PKDIA, Pyruvate Kinase Deficiency Impact Assessment

4. Patient Experience Data

The Applicant submitted a PRO assessment. Assessing the impact of mitapivat on quality of life was a secondary endpoint in Study 006. Two instruments were used to measure the impact of mitapivat: Pyruvate Kinase Deficiency Diary (PKDD) and Pyruvate Kinase Deficiency Impact Assessment (PKDIA).

Table 4. Patient Experience Data Submitted or Considered

Data Submitted in the Application		
Check if Submitted	**Type of Data**	**Section Where Discussed, if Applicable**
Clinical outcome assessment data submitted in the application		
☒	Patient-reported outcome	Sections 6.2.2.2. and 6.3.1.
☐	Observer-reported outcome	
☐	Clinician-reported outcome	
☐	Performance outcome	
Other patient experience data submitted in the application		
☐	Patient-focused drug development meeting summary	
☒	Qualitative studies (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel)	
☐	Observational survey studies	
☐	Natural history studies	
☐	Patient preference studies	
☐	Other: (please specify)	
☐	**If no patient experience data were submitted by Applicant, indicate here.**	
Data Considered in the Assessment (But Not Submitted by Applicant)		
Check if Considered	**Type of Data**	**Section Where Discussed, if Applicable**
☒	Perspectives shared at patient stakeholder meeting	
☐	Patient-focused drug development meeting summary report	
☐	Other stakeholder meeting summary report	
☐	Observational survey studies	
☐	Other: (please specify)	

5. Pharmacologic Activity, Pharmacokinetics, and Clinical Pharmacology

We comprehensively evaluated the clinical pharmacologic properties of mitapivat (Table 5).

Table 5. Summary of General Clinical Pharmacology and Pharmacokinetics

Characteristic	Drug Information
	Pharmacologic Activity
Established pharmacologic class (EPC)	Pyruvate Kinase Activator
Mechanism of action	Mitapivat allosterically activates pyruvate kinase enzyme in red blood cells (RBC) to elevate intracellular ATP levels and improve RBC energy homeostasis resulting in improved RBC longevity and reduced incidence of hemolysis.
Active moieties	Mitapivat
QT prolongation	At a dose sixfold the maximum recommended dose, mitapivat does not prolong the QT interval to any clinically relevant extent.
	General Information
Bioanalysis	Plasma mitapivat concentrations were measured using a validated liquid chromatography-mass spectrometry (LC-MS/MS) method.
Healthy subjects versus patients	Plasma pharmacokinetics of mitapivat are similar in healthy subjects and patients with PK deficiency, at steady-state following the maximum recommended dose of 50 mg twice daily.
Drug exposure at steady state following the therapeutic dosing regimen (or single dosage, if more relevant for the drug)	**Table 6. Mitapivat Exposure Pharmacokinetics Parameters** **Mitapivat Dosage** | **C_{max} (ng/mL)** | **C_{trough} (ng/mL)** | **AUC_{0-12} (ng*h/mL)** | **Accumulation Ratio** 5 mg twice daily | 101.2 (17%) | 10.1 (74%) | 450.4 (28%) | 1.2 20 mg twice daily | 389.9 (18%) | 32.3 (77%) | 1623.8 (28%) | 1.1 50 mg twice daily | 935.2 (18%) | 62.1 (80%) | 3591.4 (28%) | 1.0 Source: FDA Analysis. Pharmacokinetics parameters are presented as geometric mean (CV%). The simulations were performed until 100 days after first dose. The interval of the last 12 hours was selected for steady-state pharmacokinetics parameters calculation. Residual error was not included during simulation.
Range of effective dosage(s) or exposure	Pivotal trials evaluated mitapivat doses ranging from 5 to 50 mg twice daily. In the Phase 3 Study 006, after achieving the target maintenance dose of 5, 20, or 50 mg twice daily during the 3-month fixed-dose treatment period of mitapivat, 40% of patients with PK deficiency who were not regularly transfused were hemoglobin (Hb) responders, defined as a ≥1.5 g/dL increase in Hb concentration from baseline sustained at two or more scheduled assessments. In the placebo group, none of the patients with PK deficiency was a Hb responder. In the Phase 3 open-label Study 007, after achieving the target maintenance dose of 50 mg twice daily during the 6-month fixed-dose treatment period of mitapivat, 37% of patients with PK deficiency who were regularly transfused achieved a >33% reduction in the number of RBC units transfused.
Maximally tolerated dosage or exposure	The highest tested single dose administration was 2,500 mg under fasted condition in healthy subjects (Phase 1 Study AG-348-C-001). The highest multiple-dose administration was 700 mg twice daily for 14 days under fasted condition in healthy

Characteristic	Drug Information
	subjects (Phase 1 Study AG-348-C-002). Gastrointestinal adverse events (AEs) were observed following single doses ≥700 mg. In patients with PK deficiency, starting doses of 50 and 300 mg twice daily of mitapivat led to at least one dose modification in a majority (73.1%) of patients in Phase 2 Study 003, due to treatment-emergent AEs including insomnia, back pain, arthralgia, headache, gastrointestinal disorders, asthenia, chest discomfort, hemolysis, and palpitations.
Dosage proportionality	After single-dose administration of mitapivat, there was a near dose-proportional increase in mean plasma C_{max} and $AUC_{0\text{-last}}$ across the dose range of 30 to 700 mg. After repeated-dose administrations of mitapivat, there was a near dose-proportional increase in mean plasma C_{max} and $AUC_{0\text{-last}}$ across the dose range of 5 to 60 mg twice daily, and a less than dose-proportional increase across the dose range of 120 mg to 700 mg twice daily.
Accumulation	Little to no accumulation was observed with 5 to 50 mg twice daily doses in Phase 3 Study 006, and 50 mg twice daily doses in Phase 2 Study 003, as well as from 15 to 60 mg twice daily doses in Phase 1 Study 002.
Time to achieve steady-state	Based on the plasma trough values (C_{trough}) with twice daily doses of 15 to 60 mg mitapivat for 14 days, mitapivat appeared to reach steady-state by approximately 7 to 10 days.
Bridge between to-be-marketed and clinical trial formulations	The tablet formulation used in Phase 3 studies is the intended final commercial formulation, with the only change for the commercial market being the addition of a cosmetic unique product identifier of ink-printed characters. A bioequivalence study was not required to compare the Phase 3 drug product (plain-faced, film-coated tablets) with the proposed commercial drug product (imprinted, film-coated tablets), because comparative dissolution testing was conducted in three dissolution media across the physiologically relevant pH range, and similarity was shown for the plain-faced tablets used in the pivotal studies and the imprinted tablets intended for commercialization.
	Absorption
Bioavailability	Absolute bioavailability is 72.7%.
T_{max}	Median t_{max} values at steady-state were 0.5 to 1.0 hour postdose across the dose range of 5 to 50 mg twice daily.
Food effect (fed/fasted) Geometric least square mean and 90% CI	100 mg tablet taken with a high-fat, high-calorie breakfast: $AUC_{0\text{-}\infty}$ fed/fasted ratio (90% CI): 1.00 (0.94-1.05) C_{max} fed/fasted ratio (90% CI): 0.58 (0.52-0.65) t_{max} (median): 0.75 hours (fasted); 3.00 hours (fed) High-fat, high-calorie food did not change the exposure of mitapivat but reduced its absorption rate. Mitapivat can be taken with or without food.
	Distribution
Volume of distribution	The mean volume of distribution at steady-state (V_{ss}) is 42.5 L following intravenous administration of 100 µg mitapivat in healthy subjects.
Plasma protein binding	Mean: 97.7% Protein binding was not concentration dependent at tested concentrations of 0.2, 1.0, and 10µM. Mean RBC-to-plasma ratio: 0.37.
Drug as substrate of transporters	In vitro, mitapivat is a substrate of P-gp. In the human ADME Study 009, the fraction absorbed (f_a) × fraction escaping gut metabolism (f_g) was calculated to be >0.8, suggesting that the risk of clinically relevant drug interaction on mitapivat by P-gp inhibitors is low. Other in vitro studies showed that mitapivat is not a substrate of BCRP, OATP1B1, and OATP1B3. Mitapivat as a substrate of renal transporters was not evaluated. However, only 2.6% of the mitapivat dose was excreted unchanged in urine in the human ADME Study 009. Renal elimination is considered a minor pathway of mitapivat excretion.

Characteristic	Drug Information
	Elimination
Mass balance results	Following a single-dose administration of 120 mg mitapivat capsule containing approximately 100 µCi of [C^{14}] mitapivat, 50% of the dose was recovered in urine and 40% was recovered in feces. Unchanged mitapivat represented less than 1% of the administered dose in feces and 2.6% in urine (human ADME Study 009). A total of 17 pharmacologically inactive metabolites was found in plasma, with oxidative glucuronide-mitapivat being the most abundant circulating metabolite, accounting for less than 10% of the total circulating radioactivity.
Clearance	Population pharmacokinetics derived median apparent clearance (CL/F) at steady-state is 11.5, 12.7, and 14.4 L/h for the 5 mg twice daily, 20 mg twice daily, and 50 mg twice daily regimens, respectively. CL/F increased after repeated doses of mitapivat from 17.9 L/h to 38.4 L/h for 60 mg twice daily to 700 mg twice daily. Clearance after a single intravenous dose of 0.1 mg mitapivat is 9.53 L/h.
Half-life	The mean effective half-life of mitapivat ranged from 3 to 5 hours following multiple oral administrations of doses of 5 mg twice daily to 50 mg twice daily in patients with PK deficiency, calculated from accumulation ratios at steady-state.
Metabolic pathway(s)	In vitro studies demonstrated that CYP3A4/5 is responsible for the metabolism of mitapivat.
Primary excretion pathways (% dosage)	Hepatic metabolism followed by excretion of metabolites in feces and urine.
	Intrinsic Factors and Specific Populations
Body weight	Based on population pharmacokinetic analyses, body weight is not a statistically significant covariate on mitapivat clearance. Adjustment of dose based on body weight is not needed.
Age	Based on population pharmacokinetic analyses, age (18 to 78 years) was not a statistically significant covariate on mitapivat clearance. Clinical studies of mitapivat did not include sufficient numbers of subjects aged 65 years and over to determine whether elderly respond differently from younger subjects. Other reported clinical experience did not identify differences in response between the elderly and younger patients.
Renal impairment	A dedicated renal impairment study was not conducted. Renal elimination is not expected to be the major elimination pathway of mitapivat. Only 2.6% of radioactivity was recovered as unchanged mitapivat in urine in the human ADME Study 009. Population pharmacokinetic analyses showed no significant difference in systemic exposure of mitapivat between patients with mild renal impairment and normal renal function. Dose adjustment is currently not needed in patients with any degree of renal impairment. However, the dose recommendation for patients with severe renal impairment should be reassessed based on the results of the hepatic impairment study (issued as a PMR) due to the concern over uremic toxins affecting hepatic drug clearance.
Hepatic impairment	A dedicated hepatic impairment study was not conducted. Mitapivat undergoes extensive hepatic metabolism based on the results of the human ADME Study 009. Therefore, moderate and severe hepatic impairment is expected to increase the systemic exposure of mitapivat. Use of mitapivat should be avoided in patients with moderate and severe hepatic impairment. A PMR was issued for a reduced design hepatic impairment study to evaluate the effect of moderate hepatic impairment (Child-Pugh B) on the pharmacokinetics of mitapivat to inform proper dose adjustment of mitapivat in patients with hepatic impairment and to evaluate the necessity of enrolling subjects with mild (Child-Pugh A) and/or severe (Child-Pugh C) hepatic impairment.
	Drug Interaction Liability (Drug as Perpetrator)
Inhibition/induction of metabolism	A clinical drug-drug interaction (DDI) study of mitapivat on inhibition/induction of metabolism of other drugs was not conducted. In vitro studies showed that treatment of cultured human hepatocytes with mitapivat (up to 100µM) caused a

Characteristic	Drug Information
	concentration-dependent increase in CYP3A4/5, CYP2B6, CYP2C8, CYP2C9, and CYP2C19 enzyme activity and more than two-fold increase in mRNA expression of CYP3A4/5, CYP2B6, CYP2C8, CYP2C9, and UGT1A1. Autoinduction of mitapivat was observed in clinical studies with repeated dosing of mitapivat at >120 mg twice daily, probably due to the induction effect on CYP3A. The CYP3A induction effect of mitapivat was confirmed by physiological-based pharmacokinetic modeling and simulation using midazolam as a CYP3A probe, where AUC_{inf} and C_{max} of midazolam decreased by 21% and 19%, respectively, when coadministered with 5 mg mitapivat twice daily; 43% and 39%, respectively, with 20 mg mitapivat twice daily; and 57% and 52%, respectively, with 50 mg mitapivat twice daily. The physiological-based pharmacokinetic analysis is inadequate to evaluate the induction effect of mitapivat on the pharmacokinetics of CYP2C and CYP2B6 substrates. Use of mitapivat should be avoided with substrates of CYP3A, CYP2B6, or CYP2C that have narrow therapeutic index.
Inhibition/induction of transporter systems	A clinical DDI study of mitapivat on inhibition of transporter systems of other drugs was not conducted. In vitro studies showed that mitapivat inhibited human P-gp–mediated transport of digoxin across Caco-2 cells with an IC_{50} of 12.8µM. Use of mitapivat should be avoided with P-gp substrates that have a narrow therapeutic index.

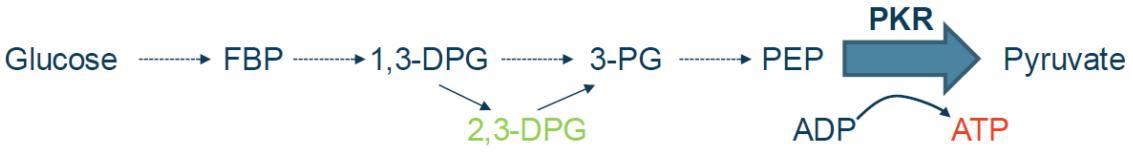
Source: FDA Analysis.
Abbreviations: ADME, absorption, distribution, metabolism, excretion; AE, adverse event; ATP, adenosine triphosphate; AUC, area under the concentration-time curve; BCRP, breast cancer resistance protein; CL/F, apparent clearance; C_{max}, maximum serum concentration; C_{trough}, predose trough concentration; DDI, drug-drug interaction; Hb, hemoglobin; OATP1B1, solute carrier organic anion transporter family member 1B1; OATP1B3, solute carrier organic anion transporter 1B3; P-gp, P-glycoprotein; PK, pyruvate kinase; PMR, postmarketing requirements; RBC, red blood cell

5.1. Nonclinical Assessment of Potential Effectiveness

Pharmacological Basis of Effectiveness

Mature RBCs rely on glucose as their only source of energy. Glucose is metabolized by the glycolytic pathway in RBCs to yield ATP, the energy carrier molecule. PK-R catalyzes the final step in glycolysis, converting phosphoenolpyruvate to pyruvate, with concomitant conversion of ADP to ATP. Patients with PKD, an autosomal recessive disease, are characterized by defective glycolysis in RBCs, deficiency in ATP levels, and consequent abnormalities in structure of RBCs resulting in increased hemolysis. Several mutant PK-R isoforms have been described in PKD, most of which disrupt the protein's catalytic activity, stability, or expression (Zanella et al. 2005; Zanella et al. 2007). Mitapivat (AG-348) is an allosteric activator of PK-R. Mitapivat also allosterically binds to and enhances the catalytic activity of two other isoforms, namely, M2-type pyruvate kinase (PKM2), which is expressed in lung, adipocytes, intestine, epithelial cells, and proliferating cells; and L-type pyruvate kinase (PKL), which is expressed in the liver and kidney. Mitapivat stabilized mutant PK-R isoforms that are susceptible to thermal denaturation; this stabilization may contribute to increased PK-R activity, leading to increased intracellular ATP levels and improved RBC function and longevity, thus reducing the incidence of hemolysis in patients with PKD.

Figure 1. Mechanism of Action Relevant to the Efficacy of Mitapivat



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

Source: Excerpted from the Applicant's background package.

Nonclinical proof of effectiveness was obtained from in vitro studies evaluating the effect of mitapivat on the stability and function of wild-type (WT) and mutant isoforms of PK-R, an ex vivo study for PK-R catalytic activity, and in vivo studies in healthy mice and in a $Hbb^{th3}/+$ mouse model of β-thalassemia, a model of hemolytic anemia.

In Vitro Evidence of Potential Effectiveness

Mitapivat induced significant (>2-fold) activation of WT PK-R and several mutant PK-R isoforms that were recombinantly expressed and purified. The half-maximal activation concentration (AC_{50}) was 0.013nM in wild-type human PK-R and ranged from 0.009 to 0.059nM in the mutant isoforms. Mitapivat also activated PKM2 and PKL (PK isoforms), with AC_{50} values of 0.038 and 0.037μM, respectively. Mitapivat stabilized several mutant PK-R isoforms (R510Q, G364D, and R532W) that are more sensitive to thermal denaturation. Mitapivat 0.539nM significantly improved the thermal stability of R510Q. At 53°C, calculated half-life values were 257.5 minutes for PK-R wild-type, 5.2 minutes for R510Q, and 176.9 minutes for

R510Q with mitapivat preincubation. These findings suggest that mitapivat increases PK-R activity by improving protein stabilization. Furthermore, mitapivat caused a potent and dose-dependent increase in cellular ATP (AC_{50}: 10.9±7nM) in healthy human RBCs.

Ex Vivo Evidence of Potential Effectiveness

The effect of mitapivat on PK-R flux, e.g., the rate of carbon flow through the PK-R enzyme reaction, was assessed in whole blood from mice treated with mitapivat using a stable glucose isotope strategy. Mitapivat increased carbon flux through PK-R by 80% in C57BL/6 mouse whole blood. This result shows that mitapivat bound and activated the PK-R enzyme and enhanced pathway activity in RBCs. In RBCs from four patients with PKD, mitapivat increased PK-R activity, decreased 2,3-bisphosphoglyceric acid (2,3-DPG) and phosphoenolpyruvate levels, and increased ATP levels after incubation for 3 to 24 hours. These data support the ability of mitapivat to activate the WT PK-R enzyme in normal mice and to activate mutant PK-R isoforms in patients with PKD, thus influencing RBC metabolism.

In Vivo Evidence of Potential Effectiveness

The Applicant stated that in vivo studies were conducted in normal C57BL/6 mice and in the murine $Hbb^{th3}/+$ model of β-thalassemia, another hemolytic anemia, because suitable animal models of PKD were not available during the early development of mitapivat.

The exposure-response relationship for mitapivat was evaluated in healthy female C57BL/6 mice administered mitapivat for 7 days at doses of 1 to 150 mg/kg. Systemic exposure to mitapivat increased with increasing dose, achieving systemic exposures (area under the concentration-time curve [AUC]) of 41.3 to 4076 ng*h/mL. Compared to the vehicle control, mice administered repeated doses of mitapivat exhibited a dose-dependent decrease in plasma 2,3-DPG and dose-dependent increases in plasma ATP and ATP/2,3-DPG ratio. The pharmacodynamic effects of mitapivat in mice occurred at systemic exposures (41.3 to 4076 ng*h/mL) that cover the steady-state clinical exposure of mitapivat at 5, 20, and 50 mg BID (AUC, 450.4, 1623.8, and 3591.4 ng*h/mL, respectively). The maximal pharmacodynamic response occurred at the high dose (150 mg/kg), with systemic exposure comparable to the steady-state mitapivat exposure at the maximum recommended human dose (MRHD) of 50 mg BID.

The effect of mitapivat on RBC structure, function and longevity was evaluated in an $Hbb^{th3}/+$ mouse model of β-thalassemia (Rab et al. 2019). This mouse model does not reflect a deficiency in PK activity but does reflect impaired RBC structure, function, and longevity, thus facilitating evaluation of parameters of anemia at the RBC level. Moreover, preliminary data from the Applicant have shown that PK activity and stability are compromised in patients with various forms of hereditary hemolytic anemia (Rab et al. 2019). In $Hbb^{th3}/+$ mice, characterized by dysfunctional β-globin chains, the Hb levels are markedly reduced, reticulocytes are increased, and RBCs exhibit abnormal morphology (Yang et al. 1995). Mitapivat was administered by oral gavage for 21 days at 50 mg/kg BID and through feed at 1200 ppm (equivalent to 100 mg/kg/day) for 56 days. The dose was selected based on the efficacy observed in WT mice treated with mitapivat up to 150 mg/kg/day and based on exposure (AUC) obtained in WT mice, which covers the mitapivat clinical therapeutic exposure. Mitapivat administration improved RBC morphology; increased total hemoglobin, mean corpuscular volume, and mean corpuscular hemoglobin; and significantly reduced reticulocyte counts in $Hbb^{th3}/+$ mice administered mitapivat compared to vehicle for 21 days (oral gavage) and in the 56-day (dietary) dosing. In

addition, there was a significant decrease in iron overload in duodenal enterocytes, hepatocytes, and Kupffer cells, accompanied by an increase in hepcidin (an iron-regulator protein).

Although a proof-of-concept from an in vivo model of PKD was not conducted, the beneficial effect of mitapivat on RBC in the mouse model of thalassemia, combined with the ex vivo studies with PK isoforms, and pharmacological rationale (i.e., allosteric activation of a deficient PK enzyme) provides sufficient evidence of potential effectiveness in treating PKD. For additional details of primary pharmacology studies, see Section III.13.1.1.1.

Mitapivat is a first-in-class new molecular entity that is an activator of PK. Therefore, there is no existing established pharmacological class for mitapivat. In addition to the RBC-specific isoform PK-R, mitapivat activates the isoforms PKM2 and PKL. The general established pharmacological class term “pyruvate kinase activator” is consistent with the in vitro and in vivo pharmacology of mitapivat and takes into consideration the nonspecific nature of PK activation by mitapivat.

In vitro studies assessing off-target activity showed that mitapivat inhibited aromatase activity in human recombinant insect BT1-TN-5B1-4 cells (half maximal inhibitory concentration [IC_{50}]: 0.92µM), human placental microsomes (IC_{50}: 2.05µM) and in rat ovarian microsomes (IC_{50}: 0.493µM). For context, the maximum plasma concentration (C_{max}) in humans is approximately 2.14µM, or 965 ng/mL. Toxicity findings in the reproductive organs, consistent with aromatase inhibition, were observed in the chronic rat study and reproductive and development studies; however, these effects were only observed at high multiples of the clinical exposure; for more details, see Section 7.

6. Assessment of Effectiveness

6.1. Dose and Dose Responsiveness

6.1.1. Applicant’s Proposed Dosing Regimen

The starting dose for mitapivat is 5 mg taken orally twice daily. To gradually increase Hb levels and maximize the effect, titrate mitapivat through sequential doses of 5 mg twice daily, 20 mg twice daily, and 50 mg twice daily, with sequential dose increases occurring every 4 weeks (Table 7). Assess Hb level before increasing to the next dose level, because some patients may reach and maintain a normal Hb level at 5 mg twice daily or 20 mg twice daily. The maximum dose is 50 mg twice daily.

Table 7. Dose Titration Schedule

(b) (4)

Source: Applicant's proposed labeling.
Abbreviations: Hb, hemoglobin

Selection of Dosing Regimen for the Phase 3 Trials

Mitapivat's preliminary evidence of effectiveness was demonstrated in the Phase 2 Study Study 003, as evidenced by improvements in Hb levels, markers of hemolysis, and erythropoietic markers. The planned doses in this Phase 2 study were 50 mg and 300 mg twice daily. However, due to treatment-emergent adverse events (TEAEs), the majority (73.1%) of patients underwent at least one dose modification, resulting in a broad range of doses administered from 5 mg three times weekly to 300 mg twice daily throughout the study. To assist with dose selection for Phase 3 Studies 006 and 007, an exposure-response (E-R) analysis was conducted using pharmacokinetic, efficacy, and safety data from Study 003. A preliminary pharmacokinetics/pharmacodynamics (pharmacokinetic-PD) model was developed using the increase in Hb as the efficacy variable, and a binary logistic regression approach was used for E-R analyses for the safety variables (e.g., alanine aminotransferase [ALT], aspartate aminotransferase [AST], total and free testosterone, estrone, estradiol, insomnia, and hot flush).

After model development, simulations were conducted to select three dose levels for evaluation in Phase 3 studies: (1) selection criterion for the starting dose was to identify the lowest dose at which patients were likely to have an Hb increase of ≥1.5 g/dL from baseline without exceeding the upper limit of normal (ULN) Hb (efficacy criterion), with a minimal probability of occurrence of Grade ≥1 insomnia (safety criterion) and (2) from the point of intrapatient dose escalation, the mid- and high-dose levels were selected such that they would result in an approximately 2- to 2.5-fold increase in exposure compared to the starting- and mid-dose levels, respectively.

Based on these criteria and simulations from the population pharmacokinetic-efficacy/safety analyses, doses of 5 mg BID, 20 mg BID, and 50 mg BID were selected for the Dose Optimization Period in Study 006. The Applicant is seeking approval of the dosing regimen that was evaluated in the Phase 3 trials.

6.1.2. Evaluation of the Proposed Dosing Regimen

Starting Dose of 5 mg BID

The proposed starting dose of 5 mg BID was supported by the decreased dose-modification incidents than at 50 mg BID and 300 mg BID in the Phase 2 Study 003.

In Study 003, 38 of 52 (73.1%) patients receiving 50 mg or 300 mg BID underwent dose modifications throughout the study. Even at the lower dose of 50 mg BID, 19% (5/27), 15% (4/27), and 15% (4/27) patients experienced TEAEs leading to dose reduction, TEAEs leading to dose interruption, and TEAEs leading to permanent discontinuation, respectively. The TEAEs leading to dose modification included insomnia and headache.

When the starting dose was decreased to 5 mg BID and a titration algorithm up to 50 mg BID was applied in the two Phase 3 studies, 006 and 007, no patient experienced dose modification due to TEAEs in Study 006, and only one (3.7%) patient experienced a TEAE leading to dose reduction in Study 007. Therefore, the proposed starting dose of 5 mg BID is acceptable based on improved tolerability and reduced dose modifications upon uptitration.

Individual Maintenance Doses of 5, 20, and 50 mg BID

E-R analysis for safety included one Phase 2 Study 003, Studies 006 and 007, and the Extension Study 001. The exposure-safety analyses showed that there were statistically significant but weak relationships between mitapivat average exposure up to the time of the event versus the incidence of all-grade insomnia, and all-grade hot flush. The predicted probability of all-grade insomnia was 19.9% for 5 mg BID, 22.1% for 20 mg BID, and 26.0% for 50 mg BID, whereas the predicted probability of all-grade hot flush was 3.4% for 5 mg BID, 4.0% for 20 mg BID, and 5.5% for 50 mg BID. Although greater estrone decrease and testosterone increase were observed with increasing mitapivat exposure, the changes were generally within the normal range. Weak E-R relationships were also observed for all-grade adverse events (AEs) of musculoskeletal and connective tissue disorders (nominal p=0.046) and AEs of endocrinological interest (nominal p=0.001). However, the predicted 95% confidence interval (CI) for the probability of AEs largely overlapped between placebo and all mitapivat doses. No statistically significant E-R relationships were observed for new or worsening all-grade laboratory abnormalities related to AST, ALT, and serum triglycerides, change from baseline in adjusted spine dual-energy x-ray absorptiometry (DXA) T-score, or change from baseline in femoral total DXA T-score. Refer to Section III.14.3.2.1 for more details.

E-R analysis for efficacy showed that there was no statistically significant E-R relationship for Hb response rate, defined as the proportion of patients with a ≥1.5 g/dL increase in Hb from baseline that was sustained at two or more scheduled assessments, in patients who were not regularly receiving blood transfusions in Studies 006 and Study 011. There was also no significant E-R relationship for transfusion burden reduction (defined as ≥33% reduction in the number of RBC units transfused through the 24 weeks of the Fixed-Dose Period compared with the historical transfusion burden standardized to 24 weeks), or transfusion free (defined as zero transfusions administered through the 24 weeks of the Fixed-Dose Period), in patients who were regularly receiving blood transfusions in Study 007. Refer to Section III.14.3.2.2 for more information.

Overall, the individual maintenance doses of 5, 20, and 50 mg BID were supported by the weak E-R relationships for safety measures and by the plateauing of E-R relationships for efficacy.

Dose Titration Algorithm

The Applicant proposed to titrate mitapivat from 5 mg BID to 20 mg, and up to 50 mg BID every 4 weeks. The Applicant also conducted population pharmacokinetic-PD modeling and simulations to evaluate various dose-titration schemes.

The Applicant first developed an indirect-effect pharmacokinetic-PD model to characterize the relationship between the Population pharmacokinetic model-predicted mitapivat concentration and Hgb measure using data from Studies 003, 006, and 011 (Cohort 1). The developed pharmacokinetic-PD model was then used for simulation. The Base Case Scenario mimics the proposed titration regimen. The Fast Response Scenario evaluated whether efficacy could be accelerated by increasing the dose every 2 weeks. The Super Aggressive Scenario evaluated whether patients could start at a higher fixed dose of 50 mg BID, and in so doing reduce the time to achieve the target Hb response. The downtitration margin was 2 g/dL below the ULN of Hb. The simulation results indicated that the proposed titration algorithm led to a similar probability of Hb response and lowest dose reduction due to Hb overshooting (Table 8). Although the Fast Response regimen and Super Aggressive Regimen may achieve an earlier Hb response (e.g., a higher Hb response at Week 4), the major drawback of the Fast Response Regimen was that it did not allow sufficient time for the individual Hgb level to achieve a plateau prior to uptitrating the dose to the next level. As a result, the probability of dose reductions due to Hb overshooting was estimated to be 1.7-fold that of the proposed dosing regimen. The Super Aggressive Regimen is also not an optimal dosing regimen since the probability of dose reductions due to Hb overshooting was estimated at 2.3-fold that of the proposed dosing regimen.

Overall, the Applicant's pharmacokinetic-PD analysis supported that the proposed dose titration algorithm would result in a lower risk of Hb overshooting and maintain a similar responder rate compared to more aggressive titration algorithms.

Table 8. Predicted Hemoglobin Response Metrics (Means [95% CI]) for Alternate Regimens With a Downtitration Margin of 2 g/dL Below the ULN

Scenario	Dose Reduction Due to Hgb Overshooting (%)	Hgb CFB ≥1.5 g/dL at Week 4 (%)	Probability of Response (%)
Base case	3.9	14.3	41.4
5→20→50 mg titrated Q4W	[1.5, 6.8]	[9.8, 18.7]	[33, 49.8]
Fast response	6.8	30.8	41.5
5→20→50 mg titrated Q2W	[3.0, 10.8]	[24.5, 38.0]	[32.8, 50.2]
Super aggressive	8.9	42.9	41.2
50 mg starting dose	[4.7, 13.8]	[34.9, 51.4]	[32.1, 49.6]

Source: Report ag348-pmx-002, Table 45.
Abbreviations: CFB, change from baseline; Hgb, hemoglobin; Q2W, every 2 weeks; Q4W, every 4 weeks; ULN, upper limit of normal

In summary, the Applicant's proposed dosing regimen of mitapivat is acceptable.

6.2. Clinical Trials Intended to Demonstrate Efficacy

In support of the proposed indication, the Applicant conducted Study 006 entitled, "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" and

Study 007 entitled, “An Open-Label Study to Evaluate the Efficacy and Safety of Mitapivat in Regularly Transfused Adult Subjects with Pyruvate Kinase Deficiency.” Relevant clinical trial landmarks and protocol amendments for Studies 006 and 007 are summarized in Section 15.1.

The review team did not conduct integrated analyses of efficacy due to differences in the design of Studies 006 and 007. See Sections 6.2.2 and 6.2.4 for analyses of individual trial results.

Data Sources

The NDA was submitted electronically. All submitted analysis datasets and tabulation datasets are in standardized format. The Applicant provided Statistical Analysis System programs for deriving efficacy and safety analysis datasets and for analysis of the efficacy endpoints.

Data Quality and Integrity

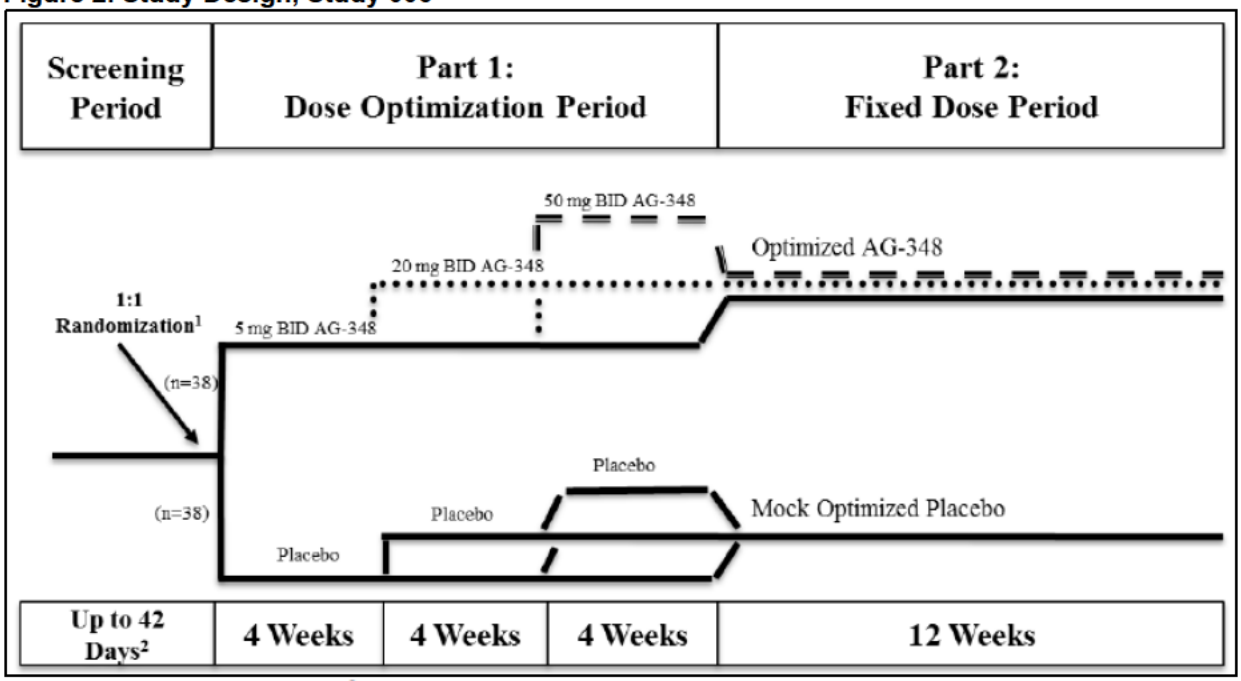
The quality and integrity of the submitted data generally appeared adequate to support the efficacy and safety of mitapivat for the proposed indication. No issues were identified. Refer to Section III.20 for a summary of the Office of Scientific Investigations review.

6.2.1. Study 006

6.2.1.1. Design, Study 006

Study 006 was a Phase 3, randomized (1:1), double-blind, placebo-controlled, multicenter trial to evaluate the efficacy and safety of orally administered mitapivat in patients with PKD who were not regularly receiving blood transfusions. The trial comprised a Dose Optimization Period (Part 1, a 12-week period starting on Day 1) followed by a Fixed-Dose Period (Part 2, a 12-week period following the Week 12 Visit through Week 24). Randomization was stratified by average baseline hemoglobin concentrations (<8.5 versus ≥8.5 g/dL [5.28 mmol/L]) and the PKLR gene variant category (missense/missense vs. missense/nonmissense). In cases in which the PKLR gene variant could not be categorized (e.g., if a patient harbored three variant PKLR alleles), the subject was assigned to the missense/nonmissense category.

Figure 2. Study Design, Study 006



[1]Stratified by average of screening Hb concentrations (Hb <8.5 g/dL vs Hb ≥8.5 g/dL [5.28 mmol/L]) and the *PKLR* gene mutation category (missense/missense vs missense/non-missense)
[2] A subject's Screening Period duration may be extended beyond 42 days upon the Medical Monitor's, or designee's, approval.
Source: Study 006 protocol.
Abbreviations: BID, twice daily; Hb, hemoglobin; PKLR, pyruvate kinase liver and red blood cell

During Part 1, patients received the scheduled dose increases depending on their response to study treatment (safety and efficacy). The goal of Part 1 was to maximize a subject's increase in Hb while maintaining an acceptable safety profile. All subjects randomized to mitapivat received an initial dose of 5 mg BID of study treatment with a potential dose level increase in two sequential steps (i.e., from 5 to 20 mg BID and from 20 to 50 mg BID; no increase beyond 50 mg BID was allowed). Patients were assessed for safety and efficacy (as defined by hemoglobin increase) every 4 weeks to determine if their dose should be increased, maintained at the current level, or decreased. At the Week 4 and Week 8 Visits, study treatment dose was increased to the next dose level (or mock titrated if on placebo) if the patient tolerated the study treatment; and the Hb concentration was lower than 2.5 g/dL (1.55 mmol/L) below the ULN. Dose re-escalation or reintroduction was to be avoided after the Week 8 Visit but was permitted. At the Week 12 Visit, if the subject had tolerated the study treatment, the subject remained at their current dose level. If it was deemed necessary to reduce the study treatment for safety reasons, the dose could be reduced to one of the two available lower dose levels (i.e., 5 mg BID, 20 mg BID). If the patient was already receiving 5 mg BID and/or could not tolerate BID dosing, another regimen (i.e., once daily dosing) was allowed.

Following Part 1, patients proceeded to Part 2 and continued study treatment on their individually optimized dose. For the purposes of dosing during Part 2, the dose that the patient received at the Week 12 Visit was considered their optimized dose and the patient received that dose during Part 2. All patients who remained on study treatment during Part 2 through the Week 24 Visit were eligible for an open-label extension study (Study 011), in which all patients were to receive mitapivat.

Patients who discontinued or interrupted study treatment during the study were to undergo the recommended dose taper (Table 126) due to the risk of withdrawal hemolysis, unless an emergency situation justified discontinuing or interrupting the study treatment. Whether or not the recommended dose taper was performed, subjects who discontinued or interrupted study treatment were monitored for signs of withdrawal hemolysis and worsening of anemia.

The maximum total duration that a subject could receive study treatment was 24 weeks (not including the recommended dose taper). Patients received an eDiary at screening to document dosing, PRO assessments, and menstrual cycle (for menstruating women) throughout the study.

Subjects who continued study treatment through the Week 24 Visit but did not continue on into an extension study, underwent the recommended dose taper and attended a Follow-up Visit 28±4 days after the last dose of study treatment. An independent data-monitoring committee reviewed the safety data of the study.

6.2.1.2. Objectives and Endpoints, Study 006

Objectives

The primary objective was to evaluate the efficacy of mitapivat compared with placebo in increasing Hgb concentrations.

The secondary objectives included safety evaluation of mitapivat; assessment of the effect on markers of hemolysis, hematopoietic activity, and other indicators of clinical activity; the effect on PROs; and pharmacokinetics.

Endpoints

The primary endpoint was the Hgb response, defined as a ≥1.5 g/dL (0.93 mmol/L) increase in Hb concentration from baseline that was sustained at two or more scheduled assessments in the Fixed-Dose Period. Patients who discontinued the study before having at least two Hb laboratory assessments during the Fixed-Dose Period were to be considered not achieving an Hb response.

The Applicant proposed an increase in Hb from baseline of ≥1.5 g/dL for two or more scheduled assessments at Weeks 16, 20, and 24. The increase in Hb from baseline of > 1.5 g/dL in this disease represents a change that will likely reflect meaningful clinical outcomes such as decrease in transfusion burden and improvement in how a patient functions and feels. Patients had to meet this threshold for at least 2 assessments to to accommodate acute instances of worsening hemolysis, which can occur after infections or other stressors. Intercurrent stressors can cause or precipitate further drops in baseline hemolsyis and ensuring assessment over at least two assessments helps to demonstrate stability of response. The Applicant also supported the selection of this cutpoint by data from Study AG-348-C-003. The Applicant's definition of Hb responders is intended to account for assessment variation and for any potential acute hemolytic events. The Division accepted the Applicant's rationale and agreed to the response threshold.

The key secondary endpoint was the average change from baseline in Hb concentration at Weeks 16, 20, and 24. Other major secondary endpoints were the following:

- Average change from baseline at Weeks 16, 20, and 24 in markers of hemolysis: Indirect bilirubin, lactate dehydrogenase (LDH), and haptoglobin levels.

- Average change from baseline at Weeks 16, 20, and 24 in a marker of hematopoietic activity, the reticulocyte percentage.
- Change from baseline in PRO scores: PKDD and PKDIA. See Sections 6.3.1 and III.16.4 for a detailed discussion of these instruments.

6.2.1.3. Eligibility Criteria, Study 006

The study enrolled adults ≥18 years of age with clinical laboratory confirmation of PKD (defined as documented presence of at least two variant alleles in the PKLR gene, of which at least one is a missense variant, as determined per the genotyping performed by the central genotyping laboratory); a hemoglobin concentration ≤10.0 g/dL (6.21 mmol/L); considered not regularly transfused (defined as no more than four transfusion episodes in the 12-month period up to the first day of study treatment and no transfusions in the 3 months prior to the first day of study treatment); and adequate organ function. The dates of transfusions for the 12-month period (prior to enrollment) were required for subject eligibility confirmation. Patients who were homozygous for the R479H variant or had two nonmissense variants (without the presence of another missense variant) in the PKLR gene or had splenectomy within 12 months were excluded. Other inclusion/exclusion criteria of the study are presented in Section 15.2.

According to Bianchi 2020, patients with nonmissense/nonmissense variants have a more severe phenotype, with lower hemoglobin levels after splenectomy, a higher number of transfusions throughout their lifetime, a higher rate of iron overload, and a higher rate of splenectomy, when compared with patients with missense/missense or missense/nonmissense PKLR variants (Bianchi and Fermo 2020). The majority of patients with PKD are compound heterozygous for two missense variants. In Study 006, patients with two nonmissense variants (without the presence of another missense variant) in the PKLR gene were excluded. According to the Applicant, a genotype-response analysis in a prior study with mitapivat (Study DRIVE-PK) demonstrated that all 10 subjects with two nonmissense alleles did not respond to treatment with mitapivat.

The nonmissense variant category, in general, can encompass a wide variety of different types of variants (coding, promoter, intronic variants; deletions, insertions, nonsense, etc.) with a wide range of effects on the protein. The Hb changes do not appear to be consistently predictable based on the nonmissense/nonmissense status alone in the Phase 2 study; however, conducting a trial involving patients with nonmissense/nonmissense variants is likely not feasible. Therefore, the clinical team does not recommend exclusion of these patients from the indication statement. It is reasonable to consider an initial trial for this group to evaluate responsiveness.

Five subjects with the R479H/R479H genotype (drawn from the U.S. Pennsylvania Amish community) in the DRIVE-PK study were nonresponders. These patients had a very low PK protein level at baseline and minimal to no change in Hb. This response is expected because those with no protein will not have a response. It is reasonable to consider an initial trial for this group to evaluate responsiveness. The clinical team recommends including information on the subgroups in Section 14 or in the special populations section of the label.

6.2.1.4. Statistical Analysis Plan, Study 006

Only subjects who signed the informed consent form were enrolled in the study and considered in the following analysis sets defined as follows:

- The full analysis set (FAS) included all subjects who were randomized. Subjects were classified according to the randomized treatment arm.
- The safety analysis set included all subjects who received at least one dose of study treatment. Subjects were classified according to the treatment actually received. If a subject randomized to placebo received at least one dose of mitapivat then the subject was classified to the mitapivat arm.
- The per-protocol set was a subset of the FAS. Subjects who met any of the following criteria were excluded from the per-protocol set:
  - Did not receive at least one dose of the randomized treatment.
  - Did not have Hb assessments at Weeks 16, 20, and 24 during the Fixed-Dose Period.

Analysis of the Primary Endpoint

The primary endpoint was Hb response, defined as a ≥15 g/L (1.5 g/dL) increase in Hb concentration from baseline that was sustained at two or more scheduled assessments at Weeks 16, 20, and 24 during the Fixed-Dose Period. The primary analysis of Hb response was a logistic regression model, including treatment as an independent variable and adjusted for the randomization stratification factors, which included the average screening Hb concentration (<8.5, ≥8.5 g/dL [5.28 mmol/L]) and the PKLR gene mutation category (missense/missense, missense/nonmissense). According to the statistical analysis plan (SAP), both a logistic regression model and Cochran-Mantel-Haenszel would be used to analyze the data depending on whether the logistic regression model converged. The estimated odds ratio between the mitapivat arm and the placebo arm with the corresponding 95% CI and the two-sided p-value was planned to be provided. However, because the logistic regression model failed to converge due to quasi-complete separation (there were no Hb responders in the placebo arm), the nonparametric Cochran-Mantel-Haenszel test was conducted to demonstrate the drug's efficacy. Because the odds ratio was not estimable, the adjusted difference in Hb response rates between the mitapivat arm and the placebo arm with the corresponding 95% CI and the two-sided p-value were provided based on the Mantel-Haenszel stratum weighted method adjusting for the randomization stratification factors.

Analysis of the Key Secondary Endpoint

The key secondary endpoint was the average change from baseline in Hb concentration at Weeks 16, 20, and 24 during the Fixed-Dose Period. The average change from baseline in Hb concentration at Weeks 16, 20, and 24 was compared between the mitapivat arm and the placebo arm using the mixed-effect model repeat measurement (MMRM) method, which included change from baseline as the dependent variable; baseline as a covariate; 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. The estimated treatment difference between the mitapivat arm and the placebo arm in the average change from

baseline at Weeks 16, 20, and 24 based on the least square means was provided with the 95% CI and two-sided p-value.

Analyses of Other Major Secondary Endpoints Under Testing Hierarchy

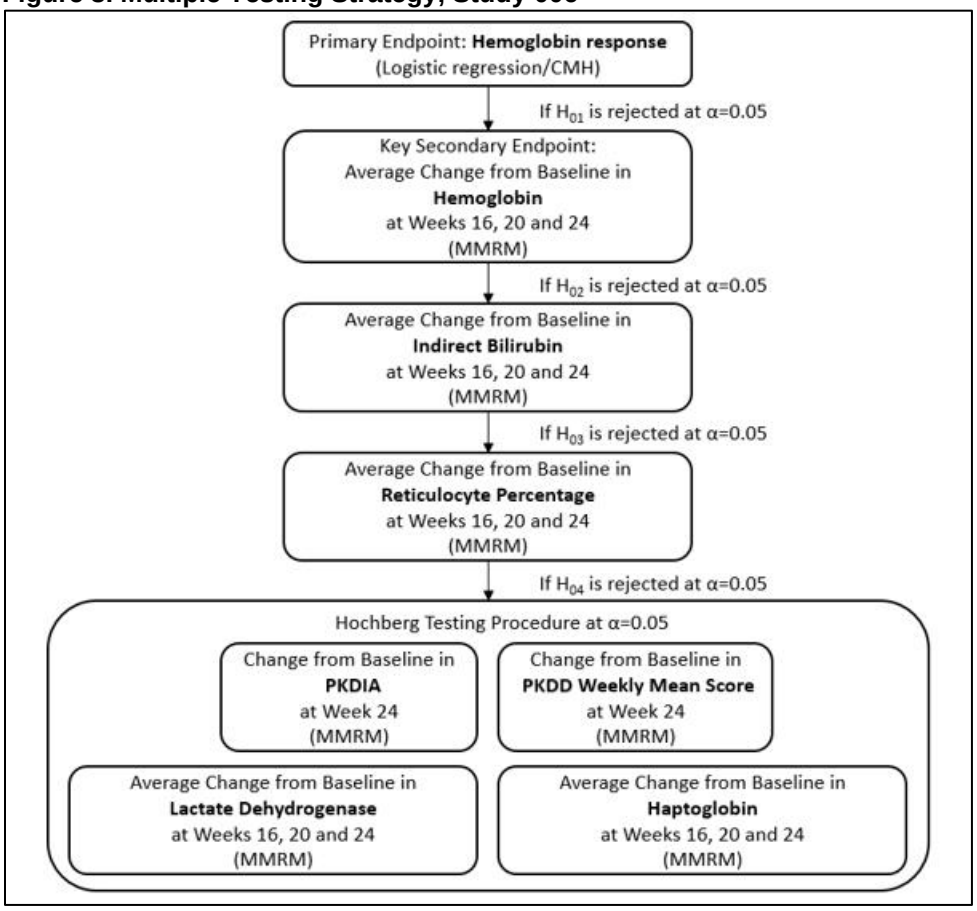
The remaining secondary endpoints that are part of the formal statistical testing strategy were assessed as described below, following a similar methodology to that outlined for the key secondary endpoint.

Multiple Testing Strategy

To control the overall type I error in the study at the two-sided 5% level, the fixed-sequence testing procedure was used to adjust for multiple statistical testing of the primary and secondary efficacy endpoints. These endpoints were assessed following the testing strategy in Figure 3.

For PKDD, PKDIA, LDH, and haptoglobin endpoints, the Hochberg testing procedure was used with two-sided α=0.05 if all four endpoints higher in the hierarchy achieved statistical significance. Four two-sided p-values for PKDD, PKDIA, LDH, and haptoglobin endpoints were ordered from largest to smallest and compared to a set of critical values. The comparison was conducted sequentially by comparing the largest p-value to α, then the second-largest p-value to $\alpha/2$, then the third largest p-value to $\alpha/3$, and the fourth largest p-value to $\alpha/4$, until a p-value for an endpoint is smaller than the corresponding critical value, whereupon the Hochberg procedure provides a conclusion of a statistically significant effect of mitapivat compared to placebo for that endpoint and all endpoints with smaller p-values.

Figure 3. Multiple Testing Strategy, Study 006



Source: Study 006 Clinical Study Report, Figure 2 (p. 30).
Abbreviations: CMH, Cochran-Mantel-Haenszel test; MMRM, mixed-effect model repeated measure; PKDD, Pyruvate Kinase Deficiency Diary; PKDIA, Pyruvate Kinase Deficiency Impact Assessment

Handling of Missing Data

In the analyses of the primary endpoint, subjects with missing Hb assessment(s) over Weeks 16, 20, and 24 and who did not have at least two assessments with a ≥15 g/L (1.5 g/dL) increase from baseline were considered as nonresponders. For all (key) secondary endpoints, the MMRM assuming missing at random (MAR) was the primary analysis.

6.2.2. Results of Analyses, Study 006

6.2.2.1. Patient Disposition, Baseline Demographics and Disease Characteristics, Study 006

Patient Disposition

Study 006 randomized a total of 80 patients (mitapivat: 40, placebo: 40). Of the randomized patients, one patient in the placebo arm did not receive treatment and was lost to follow-up. The safety analysis set comprised 79 patients (mitapivat: 40, placebo: 39); of them, 71 patients (mitapivat arm: 35 patients, placebo arm: 36 patients) continued in the Extension Study 011.

Table 9. Analysis Populations and Patient Disposition, Study 006

Analysis Population	Mitapivat (n=40)	Placebo (n=40)	Total (n=80)
SAT population	40 (100%)	39 (97.5%)*	79 (98.8%)
PP population	33 (82.5%)	29 (72.5%)	62 (77.5%)
FAS population	40 (100%)	40 (100%)	80 (100%)
Completed study	40 (100%)	39 (97.5%)	79 (98.8%)
Discontinued	0	1 (2.5%)*	1 (1.3%)
Lost to follow-up	0	1 (2.5%)*	1 (1.3%)

Source: FDA analysis based on ADSLSUB.xpt.
*One patient in the placebo arm did not receive study treatment and was lost to follow-up.
Abbreviations: FAS, full analysis set; PP, per protocol; SAT, safety analysis set

Baseline Demographics and Disease Characteristics

Patient demographics were generally balanced between the two treatment arms in Study 006. Overall, 60% of patients were females, the median age was 32.5 years (range: 18, 78 years), 75% were white, and the majority was in Western Europe (48.8%) and North America (38.8%).

Table 10. Baseline Demographics, Full Analysis Set, Study 006

Demographic	Mitapivat (n=40)	Placebo (n=40)	Total (n=80)
Gender			
Male	16 (40.0%)	16 (40.0%)	32 (40.0%)
Female	24 (60.0%)	24 (60.0%)	48 (60.0%)
Childbearing potential*	17 (70.8%)	20 (83.3%)	37 (77.1%)
Age (years)			
Median	31.5	35.5	32.5
Range	18, 70	19, 78	18, 78
Age category (years)			
<65	38 (95.0%)	38 (95.0%)	76 (95.0%)
≥65	2 (5.0%)	2 (5.0%)	4 (5.0%)
Race			
White	28 (70.0%)	32 (80.0%)	60 (75.0%)
Asian	5 (12.5%)	3 (7.5%)	8 (10.0%)
Native Hawaiian or Other Pacific Islander	1 (2.5%)	0	1 (1.3%)
Black or African American	0	0	0
Other	0	1 (2.5%)	1 (1.3%)
Not reported	6 (15.0%)	4 (10.0%)	10 (12.5%)
Weight (kg)			
Median	64.3	67.0	65.9
Range	41.4, 106.3	40.0, 132.9	40.0, 132.9
Region			
Western Europe	19 (47.5%)	20 (50.0%)	39 (48.8%)
North America	15 (37.5%)	16 (40.0%)	31 (38.8%)
Asia	5 (12.5%)	3 (7.5%)	8 (10.0%)
Rest of the World	1 (2.5%)	1 (2.5%)	2 (5.0%)

Source: ADSL.xpt, ADSLSUB.xpt.
* The denominator used to calculate percentages is the number of female subjects in the Full Analysis Set within each treatment arm.

Baseline disease characteristics were generally similar between the treatment arms, as were the clinical characteristics of patients with PKD. The median baseline hemoglobin was 8.5 g/dL (range: 6.4, 10.2 g/dL), 68.8% of patients had missense/missense PKLR gene variants, the median ferritin was elevated at 479 µg/L (range: 21, 5890 µg/mL), the majority of patients had

zero (73.8%) or one (18.8%) transfusion during the prior 52-week period, splenectomy and cholecystectomy each were performed in 72.5% of patients, and 18.8% of patients had chelation therapy during the prior 52 weeks.

In Study 006, three patients (mitapivat: two patients, placebo: one patient) started to receive chelation therapy postbaseline. These three patients had a history of iron overload and prior chelation therapy (at any time other than the 12 months before the start of study treatment), had high ferritin levels at baseline, and started chelation therapy during the study.

Table 11. Baseline Disease Characteristics, Full Analysis Set, Study 006

Disease Characteristic	Mitapivat (n=40)	Placebo (n=40)	Total (n=80)
Hemoglobin (g/dL), n	40	40	80
Mean (SD)	8.6 (1.0)	8.5 (0.8)	8.6 (0.9)
Median	8.7	8.5	8.5
Range	6.4, 10.2	6.4, 10.0	6.4, 10.2
Hemoglobin category (g/dL), n	40	40	80
<8.5	19 (47.5%)	21 (52.5%)	40 (50.0%)
≥8.5	21 (52.5%)	19 (47.5%)	40 (50.0%)
PKLR gene variant, n	40	40	80
Missense/missense	28 (70.0%)	27 (67.5%)	55 (68.8%)
Missense/nonmissense	12 (30.0%)	13 (32.5%)	25 (31.3%)
Ferritin (mcg/L), n	39	38	77
Mean (SD)	748 (1116)	688 (605)	718 (896)
Median	383	511	479
Range	21, 5890	76, 2357	21, 5890
Reticulocyte (fraction of 1), n	40	40	80
Mean (SD)	0.37 (0.24)	0.40 (0.22)	0.39 (0.23)
Median	0.36	0.44	0.40
Range	0.06, 0.83	0.04, 0.78	0.04, 0.83
Indirect bilirubin (mg/dL), n	37	39	76
Mean (SD)	4.8 (3.6)	5.2 (3.6)	5.0 (3.6)
Median	4.0	4.9	4.4
Range	0.8, 17.2	0.6, 16.4	0.6, 17.2
LDH (U/L), n	39	40	79
Mean (SD)	348 (276)	260 (140)	303 (221)
Median	236	217	224
Range	148, 1191	101, 838	101, 1191
Haptoglobin (mg/dL), n	40	40	80
Mean (SD)	8.2 (10.7)	8.3 (13.8)	8.0 (12.0)
Median	3.0	3.0	3.0
Range	3.0, 42.7	3.0, 69.7	3.0, 69.7
Prior transfusion episodes*			
0	29 (72.5%)	30 (75.0%)	59 (73.8%)
1	8 (20.0%)	7 (17.5%)	15 (18.8%)
2	0	1 (2.5%)	1 (1.3%)
3	3 (7.5%)	1 (2.5%)	4 (5.0%)
≥4	0	1 (2.5%)	1 (2.5%)

Disease Characteristic	Mitapivat (n=40)	Placebo (n=40)	Total (n=80)
Prior splenectomy, n	40	40	80
Yes	28 (70.0%)	30 (75.0%)	58 (72.5%)
Prior cholecystectomy, n	40	40	80
Yes	28 (70.0%)	30 (75.0%)	58 (72.5%)
Prior chelation*, n	40	40	80
Yes	5 (12.5%)	10 (25.0%)	15 (18.8%)

Source: ADSLSUB.xpt.
* Within 52 weeks (364 days) before the first dose of study treatment.
Abbreviations: LDH, lactate dehydrogenase; PKLR, pyruvate kinase liver and red blood cell gene; SD, standard deviation

The baseline disease demographics represent a population that is not regularly transfused, as evidenced by >70% of patients in the placebo and mitapivat arms not having had a transfusion episode in the prior 52 weeks and less than one quarter of patients requiring chelation therapy in the prior 52 weeks. This is consistent with intended population for Study 006.

Table 12 summarizes the baseline bone characteristics of patients in Study 006. The median T-scores for femoral neck and lumbar spine bone mineral density (BMD) were -1.02 (range: -3.43, 2.10) and -1.67 (range: -3.78, 1.94). Overall, 6.3% of patients had a femoral neck T-score in the osteoporotic range (≤-2.5) and 13.8% of patients had a lumbar spine T-score in the osteoporotic range. Overall, 45% of patients had a femoral neck T-score in the osteopenia range (between -1.0 and -2.5) and 57.5% had a lumbar spine T-score in the osteopenia range.

Table 12. Baseline Bone Characteristics, Full Analysis Set, Study 006

Bone Characteristic*	Mitapivat (N=40)	Placebo (N=40)	Total (N=80)
Femoral neck total			
Bone mineral density (g/cm^2)			
Median	0.82	0.88	0.85
Range	0.60, 1.17	0.61, 1.26	0.60, 1.26
T-Score			
Median	-1.10	-1.00	-1.02
Range	-3.43, 1.29	-3.00, 2.10	-3.43, 2.10
T-Score category			
≤-2.5	3 (7.5%)	2 (5.0%)	5 (6.3%)
>-2.5 to <-1.0	18 (45.0%)	18 (45.0%)	36 (45.0%)
≥-1.0	18 (45.0%)	20 (50.0%)	38 (47.5%)
Missing	1 (2.5%)	0	1 (1.3%)
Adjusted spine			
Bone mineral density (g/cm^2)			
Median	0.91	0.96	0.94
Range	0.65, 1.33	0.78, 1.41	0.65, 1.41
T-Score			
Median	-1.69	-1.53	-1.67
Range	-3.78, 1.23	-3.00, 1.94	-3.78, 1.94
T-Score category			
≤-2.5	9 (22.5%)	2 (5.0%)	11 (13.8%)
>-2.5 to <-1.0	23 (57.5%)	23 (57.5%)	46 (57.5%)
≥-1.0	7 (17.5%)	15 (37.5%)	22 (27.5%)
Missing	1 (2.5%)	0	1 (1.3%)

Source: ADSLSUB.xpt.
* Measured by dual-energy X-ray absorptiometry scan.
Abbreviations: N, number of subjects in each category

Baseline symptoms as assessed by PKDD and PKDIA were generally similar between the two arms. As shown in Table 13 and Table 14, Study 006 enrolled patients who had mild to moderate symptoms. See Section 6.3.1 and Section III.16.4 for a detailed discussion of these instruments.

According to Al-Samkari 2020, patients with PKD who are regularly transfused or have severe disease are mostly diagnosed in the neonatal period or in early childhood (Al-Samkari et al. 2020). Patients who have never or rarely received transfusions are generally diagnosed in adulthood and typically have mild to moderate anemia. According to Grace 2020, approximately 70% of all patients with PKD undergo splenectomy between the ages of 5 and 18 years either to reduce the need for transfusion (in patients who are regularly receiving transfusions) or to increase hemoglobin (in patients who are not regularly receiving transfusions but who tolerate their anemia poorly) (Grace and Barcellini 2020). In Study 006, the mean and median ages at splenectomy were 7.8 years (standard deviation: 6.9 years) and 5 years (range: 1, 29 years), respectively. Based on age at splenectomy, the population enrolled in Study 006 was diagnosed in childhood and perhaps the reason for less transfusions is splenectomy.

The PKDD is a seven-item PRO instrument designed to assess the signs and symptoms of pyruvate kinase deficiency. Items are rated on multiple response scales. Six items are rated using an 11-point numeric rating scale ranging from 0 (“No or not at all sign/symptom”) to 10 (“Extremely or worst possible or high sign/symptom). One item (Item 3, jaundice) is rated on a five-point verbal rating scale that ranges from 0 (“No yellow eyes and/or skin") to 4 (“Very severe yellow eyes and/or skin"). Item 4 (bone pain) also contains a “Not experience” response option for participants who have never experienced this symptom. Item 5 (shortness of breath) has a “Not applicable” response option, as well as an option for those participants who avoided the activity due to the inability to perform moderate physical activity. The recall period is “today (from the time you woke up this morning to the time you are completing this questionnaire).” A copy of the instrument is provided in Figure 51. The PKDD generates a weekly average T-score based on a raw score to T-score transformation table. The T-score ranges from 25 to 76, with higher scores indicating greater symptom severity. Additional details regarding the scoring algorithm can be found in Figure 54.

The PKDIA is an eight-item PRO instrument designed to assess the impacts of pyruvate kinase deficiency. Seven items are rated on an 11-point numeric rating scale ranging from 0 (“None of the time” or “Not at all difficult”) to 10 (“All of the time” or “Extremely difficult”). One item (Item 8, need for additional rest or sleep) uses a five-point verbal rating scale that ranges from 0 (“No additional rest or sleep”) to 4 (“A lot of additional rest or sleep”). The recall period is the previous 7 days. A copy of the instrument is provided in Figure 53. The PKDIA generates a T-score based on a raw sum score to T-score transformation table. The T-score ranges from 30 to 76, with higher scores indicating greater negative impact. Additional details regarding the scoring algorithm can be found in Figure 54.

Table 13. PKDD: Median Baseline by Weekly Mean Raw Score, Full Analysis Set, Study 006

PKDD Category	Mitapivat (N=40)	Placebo (N=40)
All items* (n)	39	38
Median (range)	50.6 (27.0, 65.1)	48.8 (27.0, 58.1)
Tired worst (0-10)	6.3 (0, 9)	5.3 (0, 9)
Daily activities (0-10)	5.7 (0.2, 10)	5.0 (0, 9)
Jaundice (0-4)	1.5 (0, 3)	1.7 (0, 3)
Bone pain worst (0-10)	0.1 (0, 8)	0.7 (0, 6)
Shortness of breath (0-10)	2.6 (0, 8)	3.8 (0, 9)
Energy level beginning of day (0-10)	5.0 (1, 9.4)	5.2 (1.7, 10)
Energy level end of day (0-10)	4.3 (0, 9.2)	4.4 (2, 10)

Source: adpkdd.xpt.
* T-score derived as the sum of all items in the PKDD.
The scores were based on patient's experience with PKD on the day (from the time of waking up the morning to the time of completing the questionnaire).
Tired worst (tiredness at its worst of today), daily activities (tiredness after finishing daily activities [e.g., work, social, leisure, physical or household activities] today), jaundice (how yellow the eyes and/or skin appeared when looking in the mirror today), bone pain (at its worst today), shortness of breath during moderate (e.g., walking on an incline or upstairs) physical activity today, energy level at the beginning of the day (after being awake for one hour), energy level at the end of the day (right now).
Abbreviations: N, total number of subjects; n, number of subjects in each category; PKD, pyruvate kinase deficiency; PKDD, Pyruvate Kinase Deficiency Diary

Table 14. PKDIA: Median Baseline by Raw Score for Select Items, Full Analysis Set, Study 006

PKDIA Category	Mitapivat (N=40)	Placebo (N=40)
All items*(n)	39	39
Median (range)	51.0 (30, 67)	51.0 (30, 66)
Tired finish things (0-10)	6.0 (0, 10)	4.0 (0, 9)
Interfere with household activities (0-10)	3.0 (0, 9)	3.0 (0, 9)
Interfere with social activities (0-10)	3.0 (0, 8)	4.0 (0, 9)
Interfere with leisure activities (0-10)	4.0 (0, 8)	4.0 (0, 9)
Relationship negatively affected (0-10)	2.0 (0, 8)	3.0 (0, 10)
Difficulty concentrating (0-10)	4.0 (0, 9)	3.0 (0, 10)
Difficulty physical activity (0-10)	3.0 (0, 10)	3.5 (0, 9)

Source: Clinical Study Report and adpkdia.xpt.
* T-score derived as the sum of select items in the PKDIA.
Abbreviation: PKDIA, Pyruvate Kinase Deficiency Impact Assessment

6.2.2.2. Analysis of the Primary and Secondary Endpoints, Study 006

Analysis of the Primary Endpoint

The primary endpoint was Hb response, defined as a ≥15 g/L (1.5 g/dL) increase in Hb concentration from baseline that was sustained at two or more scheduled assessments at Weeks 16, 20, and 24 during the fixed-dose period. The Applicant's primary efficacy results were confirmed by the FDA statistical reviewer.

Sixteen patients (40.0%) in the mitapivat arm and no patient in the placebo arm achieved the primary endpoint. In patients (n=16) who achieved the primary endpoint in the mitapivat arm, prohibited concomitant medications such as hematopoietic stimulating agents were not administered, no patients received blood transfusions on study, and there were no major protocol violations.

Because there was zero Hb response in the placebo arm, the maximum-likelihood estimate for the odds ratio did not exist due to quasi-complete separation and the primary analyses based on

logistic regression model failed to converge. The two-sided p-value from the exact Cochran-Mantel-Haenszel test for the comparison of Hb response rate between the two treatment arms was <0.0001, which is statistically significant.

To provide an estimate of the mitapivat effect on Hb response rate compared with placebo, an additional sensitivity analysis was conducted using the Mantel-Haenszel stratum weighted method adjusting for the randomization stratification factors; the adjusted difference in Hb response rate between the mitapivat arm and the placebo arm with the corresponding 95% CI and the two-sided p-value were provided for the Full Analysis Set in Table 15. The associated two-sided p-value was <0.0001, consistent with that observed for the primary analysis.

Table 15. Sensitivity Analysis of Hemoglobin Response—Mantel-Haenszel Stratum Weighted Method, Full Analysis Set, Study 006

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

Source: Study 006 Clinical Study Report, Table 8 (p. 46); Statistical Reviewer's analysis.
Hb responders: subjects who obtained ≥15 g/L (1.5 g/dL) increase in Hb concentration from baseline at two 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.
Abbreviations: CI, confidence interval; Hb, hemoglobin; N, total number of subjects; n, number of subjects in each category

Analysis of the Key Secondary Endpoint

The key secondary endpoint was the average change from baseline in Hb concentration at Weeks 16, 20, and 24 during the Fixed-Dose Period. The Applicant's primary efficacy results for the key secondary endpoint based on the MMRM method were confirmed by the FDA statistical reviewer. We agree that mitapivat demonstrated a statistically significant (two-sided p<0.0001) improvement in the average change from baseline in Hb concentrations across Weeks 16, 20, and 24 compared with placebo (Table 16).

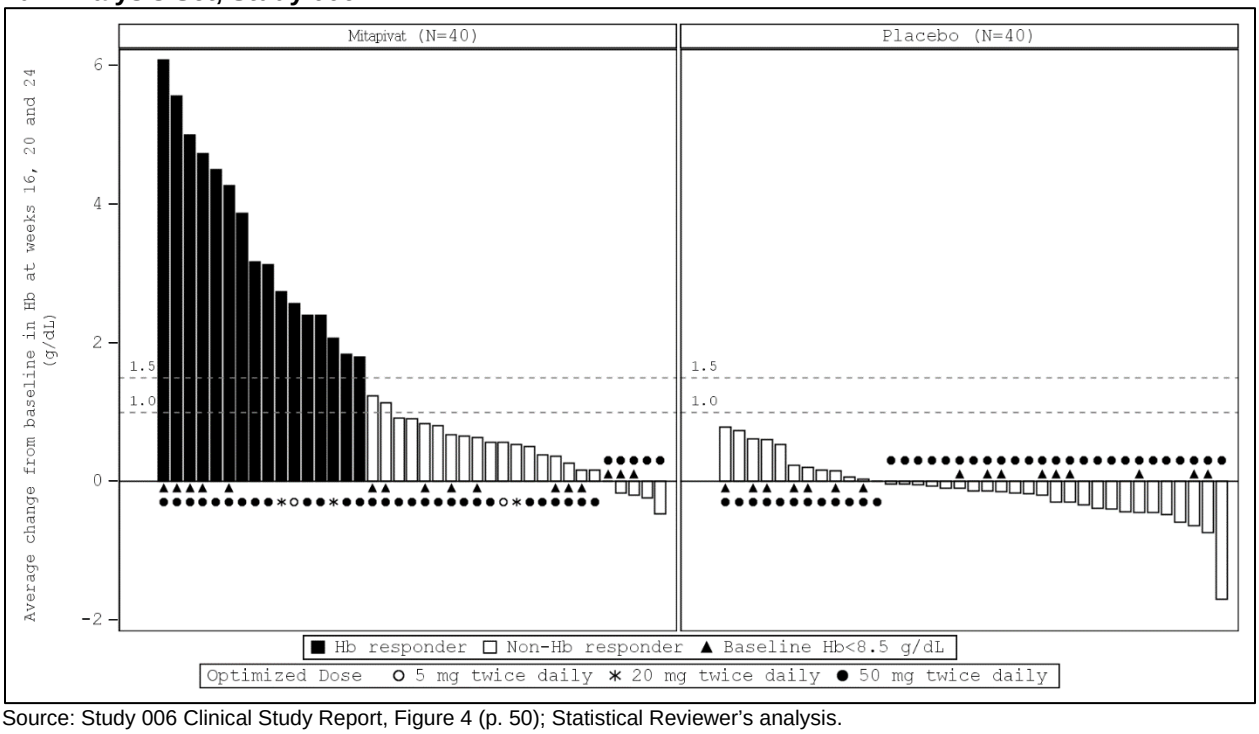
Table 16. Analysis of Average Change From Baseline in Hemoglobin (g/dL) at Weeks 16, 20, and 24 by MMRM, Full Analysis Set, Study 006

Visit	Mitapivat N=40	Placebo N=40
Baseline		
n	40	40
Mean (SD)	8.6 (1.0)	8.5 (0.8)
Average of Weeks 16, 20, and 24		
Change from baseline[1]		
LS mean (SE)	1.7 (0.2)	-0.1 (0.2)
95% CI	(1.3, 2.1)	(-0.6, 0.3)
Difference in LS mean (SE) (mitapivat-placebo)		1.8 (0.3)
95% CI		(1.2, 2.4)
Two-sided p-value		<0.0001

Source: Study 006 Clinical Study Report, Table 9 (p. 47); Statistical Reviewer's analysis.
Note: 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.
[1] 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.
Abbreviations: CI, confidence interval; LS, least square; MMRM, mixed-effect model repeated measure; N, total number of subjects; n, number of subjects in each category; SD, standard deviation; SE, standard error

Most subjects in the mitapivat arm experienced some level of increase in Hb (with almost all responders having ≥2 g/dL increase in Hb and six (38%) responders having a >4 g/dL increase in Hb), whereas most subjects in the placebo arm experienced a decrease in Hb as measured by average change from baseline at Weeks 16, 20, and 24 (Figure 4). The decrease in Hb in the placebo arm would be expected in a patient population with no intervention or therapy and reflects the natural history of the disease.

Figure 4. Waterfall Plot of Average Change From Baseline in Hemoglobin at Weeks 16, 20, and 24, Full Analysis Set, Study 006



Source: Study 006 Clinical Study Report, Figure 4 (p. 50); Statistical Reviewer's analysis.
Abbreviations: Avg, average; BID, twice daily; Hb, hemoglobin

Note that the MMRM assuming MAR is the primary analysis. To evaluate the robustness of the results, the Applicant conducted a sensitivity analysis by analysis of covariance (ANCOVA) but with observed values (i.e., subjects with missing baseline Hb value or missing Hb value at Week 16, 20 or 24 were excluded from the analysis). The model used the average change from baseline in Hb at Weeks 16, 20 and 24 as the response variable; fixed effects included baseline Hb concentration, treatment arm and the randomization stratification factors. The results (Section III.16.3) of this ANCOVA were similar to those based on the key secondary endpoint, with a two-sided p<0.0001.

Analyses of Other Major Secondary Endpoints Under Testing Hierarchy

The six other major secondary endpoints included in the Applicant's statistical testing hierarchy (Figure 3) are as follows:

- Average change from baseline in indirect bilirubin at Weeks 16, 20, and 24;
- Average change from baseline in reticulocyte percentage at Weeks 16, 20, and 24;
- Average change from baseline in lactate dehydrogenase at Weeks 16, 20, and 24;
- Average change from baseline in haptoglobin at Weeks 16, 20, and 24;
- Change from baseline in PKDD weekly mean score at Week 24; and
- Change from baseline in PKDIA score at Week 24.

The Applicant's primary efficacy results for those six other major secondary endpoints under a testing hierarchy based on the MMRM method (summarized in Table 17 to Table 20) were confirmed by the FDA Statistical Reviewer. Mitapivat demonstrated a statistically significant improvement in all six other major secondary endpoints under the testing hierarchy. However, the FDA review team had concerns regarding the clinical relevance of the two PRO endpoints, i.e., PKDD and PKDIA. See the detailed explanation in Section 6.3.1 and Section III.16.4. In addition, the Division of Clinical Outcome Assessment was consulted regarding the two PRO instruments. See Section III.16.4 for additional discussion.

Indirect Bilirubin (mg/dL)

Table 17. Average Change From Baseline in Indirect Bilirubin (mg/dL) at Weeks 16, 20, and 24 by MMRM, Full Analysis Set, Study 006

Visit	Mitapivat N=40	Placebo N=40
Baseline		
n	37	39
Mean (SD)	4.8 (3.6)	5.2 (3.6)
Average of Weeks 16, 20, and 24		
Change from baseline		
LS mean (SE)	-1.2 (0.2)	0.3 (0.2)
95% CI	(-1.7, -0.7)	(-0.2, 0.8)
Difference in LS mean (SE) (mitapivat-placebo)		-1.5 (0.3)
95% CI		(-2.2, -0.9)
Two-sided p-value		<0.0001

Source: Study 006 Clinical Study Report, Table 10 (p. 51); Statistical Reviewer's analysis.
Note: Same as Table 16 of this report.
Abbreviations: CI, confidence interval; LS, least square; MMRM, mixed-effect model repeated measure; N, total number of subjects; n, number of subjects in each category; SD, standard deviation; SE, standard error

Reticulocyte (Fraction of One)

Table 18. Analysis of Average Change From Baseline in Reticulocyte Percentage (Fraction of One) at Weeks 16, 20, and 24 by MMRM, Full Analysis Set, Study 006

Visit	Mitapivat N=40	Placebo N=40
Baseline		
n	40	40
Mean (SD)	0.37 (0.24)	0.40 (0.22)
Average of Weeks 16, 20, and 24		
Change from baseline		
LS mean (SE)	-0.10 (0.01)	0.00 (0.01)
95% CI	(-0.13, -0.07)	(-0.02, 0.03)
Difference in LS mean (SE) (mitapivat-placebo)		-0.10 (0.02)
95% CI		(-0.14, -0.06)
Two-sided p-value		<0.0001

Source: Study 006 Clinical Study Report, Table 11 (p. 56); Statistical Reviewer's analysis.
Note: Same as Table 16 of this report.
Abbreviations: CI, confidence interval; LS, least square; MMRM, mixed-effect model repeated measure; N, total number of subjects; n, number of subjects in each category; SD, standard deviation; SE, standard error

Lactate Dehydrogenase (U/L)

Table 19. Analysis of Average Change From Baseline in Lactate Dehydrogenase (U/L) at Weeks 16, 20, and 24 by MMRM, Full Analysis Set, Study 006

Visit	Mitapivat N=40	Placebo N=40
Baseline		
n	39	40
Mean (SD)	348 (276)	260 (140)
Average of Weeks 16, 20, and 24		
Change from baseline		
LS mean (SE)	-92 (16)	-21 (16)
95% CI	(-124, -60)	(-53, 11)
Difference in LS mean (SE) (mitapivat-placebo)		-71 (22)
95% CI		(-116, -26)
Two-sided p-value		0.003

Source: Study 006 Clinical Study Report, Table 10 (p. 52); Statistical Reviewer's analysis.
Note: Same as Table 16 of this report.
Abbreviations: CI, confidence interval; LS, least square; MMRM, mixed-effect model repeated measure; N, total number of subjects; n, number of subjects in each category; SD, standard deviation; SE, standard error

Haptoglobin (g/dL)

Table 20. Analysis of Average Change From Baseline in Haptoglobin (mg/dL) at Weeks 16, 20, and 24 by MMRM, Full Analysis Set, Study 006

Visit	Mitapivat N=40	Placebo N=40
Baseline		
n	40	40
Mean (SD)	8.2 (10.7)	8.3 (13.8)
Average of Weeks 16, 20, and 24		
Change from baseline		
LS mean (SE)	16.9 (4.1)	1.2 (4.1)
95% CI	(8.8, 25.1)	(-7.0, 9.4)
Difference in LS mean (SE) (mitapivat-placebo)		15.8 (5.8)
95% CI		(4.3, 27.3)
Two-sided p-value		0.0079

Source: Study 006 Clinical Study Report, Table 10 (p. 52); Statistical Reviewer's analysis.
Note: Same as Table 16 of this report.
Abbreviations: CI, confidence interval; LS, least square; MMRM, mixed-effect model repeated measure; N, total number of subjects; n, number of subjects in each category; SD, standard deviation; SE, standard error

In summary, the improvement in the laboratory markers of hemolysis (LDH, bilirubin, and haptoglobin) compared to the placebo group reflects a treatment effect of mitapivat in improving the chronic hemolysis that is part of PKD.

The section below shows the results for the PKDD and PKDIA Instruments. See Section 6.3.1 and Section III.16.4 for further details and our overall assessment of these findings.

PKDD

Table 21. Analysis of Change From Baseline in PKDD Weekly Mean T-Score at Week 24 by MMRM, Full Analysis Set, Study 006

Visit	Mitapivat N=40	Placebo N=40
Baseline		
n	37	36
Mean (SD)	50.5 (7.3)	47.0 (8.1)
Week 24		
Change from baseline[1]		
LS mean (SE)	-5.2 (1.0)	-2.1 (1.0)
95% CI	(-7.1, -3.3)	(-4.0, -0.1)
Difference in LS mean (SE) (mitapivat-placebo)		-3.1 (1.4)
95% CI		(-5.8, -0.4)
Two-sided p-value		0.025

Source: Study 006 Clinical Study Report, Table 12 (p. 59); Statistical Reviewer's analysis.
Note: 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.
[1] 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.
Abbreviations: CI, confidence interval; LS, least square; MMRM, mixed-effect model repeated measure; N, total number of subjects; n, number of subjects in each category; SD, standard deviation; SE, standard error

PKDIA

Table 22. Analysis of Change From Baseline in PKDIA T-Score at Week 24 by MMRM, Full Analysis Set, Study 006

Visit	Mitapivat N=40	Placebo N=40
Baseline		
n	39	39
Mean (SD)	49.2 (9.0)	48.5 (9.2)
Week 24		
Change from baseline		
LS mean (SE)	-4.7 (1.1)	-1.4 (1.2)
95% CI	(-6.9, -2.4)	(-3.7, 0.9)
Difference in LS mean (SE) (mitapivat-placebo)		-3.3 (1.6)
95% CI		(-6.4, -0.1)
Two-sided p-value		0.042

Source: Study 006 Clinical Study Report, Table 13 (p. 60); Statistical Reviewer's analysis.
Note: Same as Table 21 of this report.
Abbreviations: CI, confidence interval; LS, least square; MMRM, mixed-effect model repeated measure; N, total number of subjects; n, number of subjects in each category; SD, standard deviation; SE, standard error

MMRM assuming MAR was used when analyzing these six major secondary endpoints under the testing hierarchy, including the two PRO endpoints, PKDD and PKDIA. Because of concerns regarding the scoring algorithm and to evaluate the robustness of the results, sensitivity analyses using ANCOVA were also performed for these endpoints. The results of sensitivity analyses by ANCOVA based on observed data (i.e., subjects with missing data were excluded from the analysis) are summarized in Section III.16.3. The analyses show that the two-sided p-values were no longer significant for PKDD and PKDIA endpoints if the ANCOVA method was only applied to the observed data.

However, the ANCOVA analyses with only observed values can be biased due to the relatively small sample size of the study and missing data. To further evaluate the robustness of the results based on the primary MMRM analysis, sensitivity analyses using ANCOVA with multiple imputation (ANCOVA-MI) using control-based pattern-mixture model were performed by the Applicant and the statistical reviewer for PKDD and PKDIA, which assumes missing not at random for the missing data. The results are summarized in Table 133 in Section III.16.3. In brief, the values using ANCOVA-MI imputation were also nominally statistically significant and the MMRM and ANCOVA-MI results are consistent. However, due to concerns with the scoring method used for the PKDD instrument, further analyses using the raw scores were performed. See Section 6.3.1 for further details.

The review team had concerns about the clinical relevance of the items comprising PKDIA because the mean difference between the two arms is too small to be clinically meaningful and the items in the PKDIA are more distal from the core signs and symptoms of the disease. (b) (4) .
Refer to Section 6.3.1 for further discussion regarding the PRO endpoint issues.

6.2.2.3. Subgroup Analysis of Primary Endpoints, Study 006

Subgroup analyses were conducted to assess the potential for differences in the treatment effect for various demographic and clinical characteristics groups and are presented in Section III.16.4. Overall, the treatment effect of mitapivat compared to placebo appeared consistent across baseline demographics and disease characteristics subgroups of average of screening Hb, PKLR

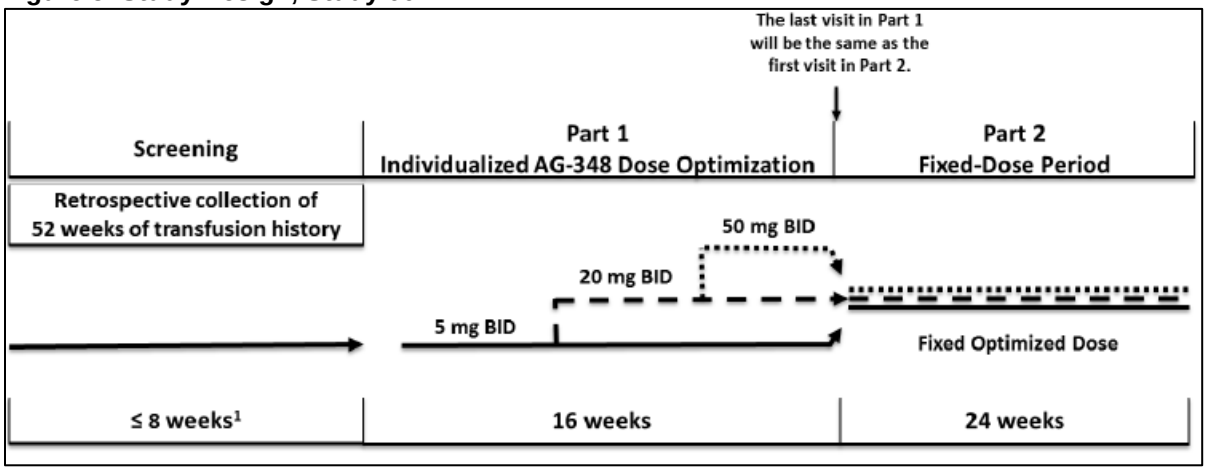
gene mutation, baseline Hb, age at screening (years), sex, race, geographic region, prior splenectomy status, prior cholecystectomy status and prior chelation status. Of note, these subgroup analyses are limited by the small numbers of patients, and so the results should be interpreted with caution.

6.2.3. Study 007

6.2.3.1. Design, Study 007

Study 007 was a Phase 3, open-label, multicenter, two-part study comprising a Dose Optimization Period (Part 1, a 16-week period starting on Day 1) followed by a Fixed-Dose period (Part 2, a 24-week period following the Week 16 Visit through Week 40) to evaluate the efficacy and safety of mitapivat in adult patients with PKD who are regularly receiving blood transfusions. At screening, transfusion history information (see eligibility criteria in Section III.15) for the preceding 52 weeks was collected as historical rate data in the analysis.

Figure 5. Study Design, Study 007



Abbreviation: BID = twice daily.
[1] Screening may be extended beyond 8 weeks if there is a delay in obtaining a subject's complete transfusion history or to ensure that the first dose of study drug can be administered 2-7 days after the most recent transfusion, upon approval by the Medical Monitor (or designee).

Source: Study 007 protocol.

In Part 1, all patients started on a mitapivat dose of 5 mg BID. Each patient's dose level could be increased two times beyond the starting dose of 5 mg BID (i.e., from 5 to 20 mg BID and from 20 to 50 mg BID). In Part 2, patients received mitapivat at the optimized dose. The goal of Part 1 was to maximize the increase in hemoglobin while maintaining an acceptable safety profile. All patients received an initial dose of 5 mg BID of mitapivat and the dose was increased consistent with Study 006. The hemoglobin was monitored throughout the study to evaluate the need for a transfusion. The following rules were applied:

- At the Week 4 Visit, the dose of study drug treatment was increased to the next dose level (i.e., from 5 mg BID to 20 mg BID) if the patient had not experienced an adverse reaction.
- The timing of the dose increase was based on the individual transfusion trigger (TT), which is the mean of a subject's collected historical pretransfusion Hb concentrations. For each historical transfusion, only the hemoglobin concentration within 1 week prior to and closest to the transfusion was included in this calculation. The dose was increased

either at the Week 10 visit if the patient had reached the individual TT (and transfused) between Week 8 and Week 10, or when the patient reached the Week 12 visit (without having reached the individual TT between Week 8 and Week 12). In either case, the study drug dose was increased to the next dose level if the subject did not experience an adverse reaction.

The study drug dose was not increased at any of these visits if hemoglobin from the previous visits (per the central laboratory analysis) was ≥14.5 g/dL (9.0 mmol/L) in males or ≥13.0 g/dL (8.07 mmol/L) in females. If it was deemed necessary to reduce the study drug dose for safety reasons, the dose could be reduced to one of the two available lower dose levels (i.e., 5 mg BID or 20 mg BID). If the patient was already receiving 5 mg BID and/or could not tolerate BID dosing, once daily dosing was allowed. Dose escalation or reintroduction was generally to be avoided after the Week 12 visit through the end of the study but permitted for patients who had their dose reduced or suspended for reasons related to safety. At any time during the study, the dose could be reduced or interrupted for reasons related to safety.

The scheduled hematology assessments in Part 1 were used to determine whether a patient reached the individual TT, in which case the patient was transfused with his/her mean number of blood units of packed RBCs. The mean number of blood units was the sum of the number of units transfused at each transfusion during the 52-week period prior to informed consent divided by the number of transfusions during this period. Patients, in particular those with a historical mean transfusion frequency of every 3 or 4 weeks, had additional hematology assessments (Hb) if the patient's individual TT was not reached at a scheduled visit.

In Part 2, patients received their optimized dose of mitapivat for 24 weeks. Patients continued to be transfused when the Hb decreased to their individual TT.

An abrupt interruption or discontinuation of mitapivat may result in withdrawal hemolysis and compound the existing baseline hemolysis due to underlying PKD, resulting in an acute drop in Hb. Therefore, patients were advised not to abruptly interrupt or discontinue dosing without first speaking with the treating Investigator except in case of medical emergency. A study drug taper would help to prevent a precipitous drop in Hb. Patients who discontinued study drug at any time during the study were to undergo a dose taper unless an emergency situation justified discontinuing the study drug abruptly. Whether the dose taper was performed or not, patients discontinuing mitapivat were monitored for signs of hemolysis and worsening of anemia. Patients were instructed to use the eDiary to record responses to each of the PRO assessments for the relevant study visit.

The maximum total duration of mitapivat treatment in this study was 40 weeks (not including the dose taper). All patients who remained on study during Part 2 through the Week 24 Visit were eligible for an open-label extension study (i.e., Study 011) with mitapivat.

No interim analysis was planned or performed. There was no independent data monitoring committee for this study.

6.2.3.2. Objectives and Endpoints, Study 007

Objectives

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

Endpoints

The primary endpoint was the proportion of subjects who achieve 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 standardized to 24 weeks (Standardized Control Period). Patients who completed at least 12 weeks of treatment in Part 2 were to be evaluated for their transfusion reduction status based on change in transfused RBC units. Patients who discontinued the study before completing 12 weeks of treatment in Part 2 were to be considered nonresponders. The Applicant prespecified that efficacy would be established if the lower bound of the associated two-sided 95% confidence interval for transfusion responders was >10%.

The review team requested the Applicant to provide justification for testing the primary endpoint with a 33% reduction threshold and how a 33% reduction represents clinical benefit. The Applicant provided justification in their response to an FDA information request based on clinical evidence.

The Applicant stated that the 33% reduction in number of RBC units transfused was chosen as the threshold to demonstrate clinical benefit based on discussions with clinical experts in PKD. To be eligible for Study 007, patients had to have a minimum of six transfusion episodes in the prior 52-week period and patients with transfusions occurring on average more frequently than once every 3 weeks (i.e., 18 transfusions) during the 52 weeks were excluded. A 33% reduction in transfusion episodes is equivalent to approximately two to six fewer transfusions per year, depending on baseline transfusion burden. In Study 007, the lowest transfusion burden was every 8 weeks and the highest was every 3 weeks. Furthermore, the Applicant stated that reducing the transfusion burden from 6 to 36 RBC units (based on transfusion of one to two RBC units every 3 to 8 weeks) to 4 to 24 RBC units over 12 months could reduce transfusional-iron intake by approximately 400 to 2400 mg/year based on an estimated 200 mg iron/RBC unit, thus helping to prevent iron accumulation and subsequent organ damage. The review team accepted the Applicant's justification for the 33% reduction threshold for the proposed population in Study 007.

In addition to the 33% reduction threshold for determining individual patients' clinical benefit, the Agency also asked the sponsor to provide rationale for the threshold of the 10% improvement rate for the primary endpoint for the efficacy assessment. The Applicant's rationale for the chosen 10% for the statistical assessment is that without an effective therapy, a transfusion response rate >10% is highly unlikely to be achieved for patients receiving greater than or equal to six transfusions annually. They emphasized this decision rule is based on discussions with experts in the field. The review team had concerns with the choice of 10% based on expert opinion rather than on scientific evidence, as well as the small open label study design, however this threshold was considerably exceeded and the actual results provide compelling evidence for clinical benefit as described in the efficacy section below.

The secondary endpoints comprised the following:

- Annualized 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 Standardized Control Period.

- Proportion of subjects who become transfusion-free, defined as zero transfusions administered during Part 2.
- Proportion of subjects who achieve Hb in the normal range at least once, 8 weeks or more after a transfusion in Part 2.

6.2.3.3. Eligibility Criteria, Study 007

Study 007 enrolled adults ≥18 years of age with a laboratory confirmation of PKD (defined as documented presence of at least two variant alleles in the PKLR gene, of which at least one is a missense variant, as determined by the genotyping performed by the central genotyping laboratory), history of a minimum of six transfusion episodes in the 52-week period prior to screening, complete records of transfusion history and received at least 0.8 mg oral folic acid daily for at least 21 days prior to the first dose of study drug, to be continued daily during the study. Patients with chronic hemolysis consume folate due to continued turnover of cells. Consumption of folate may lead to megaloblastosis, therefore, folic acid supplementation is standard therapy for patients with chronic hemolytic anemia. Patients who were homozygous for the R479H variant or had two nonmissense variants (without the presence of another missense variant) in the PKLR gene; or diagnosis of any other congenital or acquired blood disorder or any other hemolytic process, except mild alloimmunization, as a consequence of transfusion therapy; or had splenectomy within 12 months were excluded. Other inclusion/exclusion criteria of the study are presented in Section 15.2.

6.2.3.4. Statistical Analysis Plan, Study 007

Only subjects who signed the informed consent form and were screened were enrolled and considered in the analysis sets described below.

- The FAS included all subjects who received at least one dose of study treatment.
- The Safety Analysis Set included all subjects who received at least one dose of study treatment. In this nonrandomized study, the FAS and the Safety Analysis Set were identical.
- The per-protocol set was a subset of the FAS and included all subjects who completed 12 weeks of treatment in the fixed-dose period (i.e., end date of the fixed-dose period − start date of the fixed-dose period +1 ≥84).

Analysis of the Primary Endpoint

The primary endpoint was the proportion of subjects who achieve a reduction in transfusion burden, 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. The following formulas were used:

- Historical transfusion burden standardized to 24 weeks (units/24 weeks)=total number of transfused RBC units during the 52 weeks before IC × 24 ÷ 52.
- On-study (fixed-dose period) transfusion burden standardized to 24 weeks=24 × total number of transfused RBC units in the Fixed-Dose Period ÷ (total number of days in the fixed-dose period ÷ 7).

The assessment of the primary endpoint was based on the percentage reduction in transfusion burden standardized to 24 weeks:

- Percentage reduction in transfusion burden standardized to 24 weeks = [Historical transfusion burden standardized to 24 weeks – On-study (Fixed-Dose Period) transfusion burden standardized to 24 weeks] ÷ Historical transfusion burden standardized to 24 weeks.

Subjects who completed at least 12 weeks of treatment in the Fixed-Dose Period (i.e., end date of the fixed-dose period − start date of the Fixed-Dose Period +1≥84) were considered responders if the percentage reduction in transfusion burden was ≥33%.

The frequency of subjects who were responders of the primary endpoint was summarized based on the FAS along with the two-sided 95% exact CI using the Clopper-Pearson method (exact CI for a binomial proportion as computed by default by the FREQ procedure using the EXACT option). For testing the hypothesis, H0: transfusion reduction response rate ≤10% versus H1: transfusion reduction response rate >10%, efficacy was to be demonstrated if the lower bound of the two-sided 95% CI was >10%.

Historical transfusion burden standardized to 24 weeks, on-study (Fixed-Dose Period) transfusion burden standardized to 24 weeks and its reduction and percentage reduction from historical transfusion burden standardized to 24 weeks were also summarized.

The frequencies of subjects with different categories of percentage reduction were also provided (<0, 0 to <20, 20 to <33, 33 to <50, and ≥50%).

Analysis of the Secondary Endpoint

The analysis of the secondary endpoint, the proportion of subjects who became transfusion-free (defined as zero transfusions during the Fixed-Dose Period) was included in this report. The frequency of subjects who became transfusion-free in the Fixed-Dose Period was summarized along with the 95% exact CI using the Clopper-Pearson method. Subjects who did not complete 12 weeks of treatment in the Fixed-Dose Period were not considered transfusion free.

Multiple Testing Strategy

No formal type I error control strategy for multiple testing was proposed for this study because the secondary endpoints were not statistically tested.

Handling of Missing Data

In the analyses of the primary endpoint, subjects who discontinued the study before completing 12 weeks of treatment in the Fixed-Dose Period were considered nonresponders.

6.2.4. Results of Analyses, Study 007

6.2.4.1. Patient Disposition, Baseline Demographics and Disease Characteristics, Study 007

Patient Disposition

In Study 007, a total of 27 patients was enrolled and received treatment with mitapivat. Of the 27 patients, 23 (85.2%) were included in the per-protocol set. A total of 20 patients (74.1%) completed the study. The most common reason for discontinuing study prematurely was withdrawal by subject (six patients, 22.2%); the reasons were 'subject and principal investigator did not see benefits' (n=2), 'subject did not see' benefits (n=1), 'subject did not see benefits and long travel was also a reason' (n=1), 'subject expressed that they are always very tired after travelling to and from the site' (n=1), and 'subject withdrew consent due to health situation' (n=1, this is also the patient who experienced Grade 3 joint swelling and Grade 3 back pain that required dose reduction of mitapivat. See Section 7.6.4).

Table 23. Analysis Populations and Patient Disposition, Study 007

Analysis Population	Mitapivat (N=27)
SAT population	27 (100%)
PP population	23 (85.2%)
FAS population	27 (100%)
Completed study	20 (74.1%)
Discontinued	7 (25.9%)
Withdrawal by patient	6 (22.2%)
Lost to follow-up	1 (3.7%)

Source: ADSLSUB.xpt.
Abbreviations: FAS, full analysis set; N, total number of subjects; PP, per protocol; SAT, safety analysis set

In summary, more patients in Study 007 discontinued the study than in Study 006. However, although the studies enrolled patients with PKD, Study 007 enrolled a population with more frequent transfusion requirements. Four patients discontinued due to lack of benefit, one withdrew due to a Grade 3 adverse event of joint swelling and back pain and one for fatigue with traveling. In the patients who completed the study, there was a clear benefit in those able to reduce their transfusion burden.

Baseline Demographics and Disease Characteristics

In Study 007, 74% of the patients were females, the median age was 36 years (range: 18, 68 years), 74% were white, and most were in Western Europe (77.8%).

Table 24. Baseline Demographics, Full Analysis Set, Study 007

Demographic	Mitapivat (N=27)
Gender	
Male	7 (25.9%)
Female	20 (74.1%)
Childbearing potential*	15 (75.0%)
Age (years)	
Median	36
Range	18, 68

Demographic	Mitapivat (N=27)
Age category (years)	
<65	26 (96.3%)
≥65	1 (3.7%)
Race	
White	20 (74.1%)
Asian	3 (11.1%)
Black or African American	0
Other	4 (14.8%)
Weight (kg)	
Median	67.1
Range	43.1, 91.8
Region	
Western Europe	21 (77.8%)
North America	4 (14.8%)
Asia	2 (7.4%)

Source: ADSLSUB.xpt.
* The denominator used to calculate percentages is the number of female subjects in the Full Analysis Set in each treatment arm.
Abbreviations: N, total number of subjects

Table 25 summarizes the baseline disease characteristics of the patients in Study 007. The baseline disease characteristics were consistent with a population with PKD receiving regular transfusions. The median baseline hemoglobin was 9.1 g/dL (range: 7.4, 10.9 g/dL); 74.1% of patients had missense/missense PKLR gene variants; the median ferritin was high at 1324 µg/L (range: 163, 5357 µg/L); and the majority of patients had prior splenectomy (77.8%), cholecystectomy (85.2%), and chelation (88.9%). The median number of transfusion episodes in the 52 weeks before trial entry was 9 (range: 6, 17). Among the patients who received treatment with iron chelation during the study, all were on chelation therapy at baseline.

Table 25. Baseline Disease Characteristics, Full Analysis Set, Study 007

Disease Characteristic	Mitapivat (N=27)
Hemoglobin (g/dL)	
Median	9.1
Range	7.4, 10.9
PKLR gene variant	
Missense/missense	20 (74.1%)
Missense/nonmissense	7 (25.9%)
Ferritin (µg/L)	
Median	1324
Range	163, 5357
Transfusion burden	
Number of transfusion episodes in 52 weeks	
Median	9.0
Range	6, 17
Number of RBC units transfused standardized to 24 weeks	
Median	6.9
Range	2.8, 20.3

Disease Characteristic	Mitapivat (N=27)
Prior splenectomy	
Yes	21 (77.8%)
Prior cholecystectomy	
Yes	23 (85.2%)
Prior chelation*	
Yes	24 (88.9%)

Source: ADSLSUB.xpt.
* Within 52 weeks (364 days) before the first dose of study treatment.
Abbreviations: N, total number of subjects; PKLR, pyruvate kinase liver and red blood cell gene; RBC, red blood cell

The population in Study 007 had higher baseline Hb than the population in Study 006, likely due to regular transfusions. The population in Study 007 is representative of patients with more severe disease, as evidenced by higher baseline median ferritin and 89% requiring chelation, whereas in Study 006 only about 25% of patients required chelation therapy.

In Study 007, the median T-scores for femoral neck and lumbar spine bone mineral density were -1.2 (range: -3.4, 0.3) and -1.3 (range: -4.9, 0.6), respectively. Overall, 3.7% and 11.1% of patients had femoral neck and lumbar spine T-scores of ≤-2.5, respectively, reflecting osteoporosis, and 55.6% and 51.9% of patients had femoral neck and lumbar spine T-scores between <-1.0 and >-2.5, respectively, reflecting osteopenia.

Table 26. Baseline Bone Characteristics, Full Analysis Set, Study 007

Bone Characteristic*	Mitapivat (N=40)
Femoral total	
Bone mineral density (g/cm^2)	
Median	0.865
Range	0.528, 1.144
T-Score	
Median	-1.2
Range	-3.4, 0.3
T-Score category	
≤-2.5	1 (3.7%)
>-2.5 to <-1.0	15 (55.6%)
≥-1.0	10 (37.0%)
Missing	1 (3.7%)
Adjusted spine	
Bone mineral density (g/cm^2)	
Median	0.949
Range	0.51, 1.29
T-Score	
Median	-1.3
Range	-4.9, 0.6
T-Score category	
≤ -2.5	3 (11.1%)
>-2.5 to <-1.0	14 (51.9%)
≥ -1.0	9 (33.3%)
Missing	1 (3.7%)

Source: ADSLSUB.xpt
* Measured by dual-energy X-ray absorptiometry scan.
Abbreviations: N, total number of subjects

6.2.4.2. Analysis of the Primary and Secondary Endpoints, Study 007

Analysis of the Primary Endpoint

The primary endpoint was the proportion of subjects who achieve a reduction in transfusion burden, defined as a ≥33% reduction in the 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.

The Applicant's primary efficacy results (Table 27) were confirmed by the statistical reviewer. The study demonstrated a statistically significant result for reduction in transfusion burden. The lower bound (19.4%) of the two-sided 95% CI is >10%, and 10 (37%) of the subjects achieved the primary endpoint, demonstrating that treatment with mitapivat reduces the need for transfusions in subjects with PK deficiency who are regularly receiving transfusions. All of the 10 transfusion-reduction responders completed the study.

Table 27. Summary of Transfusion Reduction Response, Full Analysis Set, Study 007

Transfusion Reduction Response	Total N=27
Transfusion reduction responders, n (%)	10 (37.0)
95% CI	(19.4, 57.6)

Source: Study 007 Clinical Study Report, Table 8 (p. 42); Statistical Reviewer's analysis.
Note: The primary endpoint (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. CI is based on the Clopper-Pearson method.
Efficacy is demonstrated if the lower bound of the two-sided 95% CI for the transfusion reduction response rate is >10%.
Abbreviations: CI, confidence interval; N, total number of subjects; n, number of subjects in each category; RBC, red blood cell

Ten patients (37%) achieved the primary endpoint. Of the 10 patients, 1 had a reduction in transfusion burden of 33% to 50%; the remaining 9 had a ≥50% reduction. In addition, one patient achieved a 100% reduction in transfusion burden, however, this patient was considered a nonresponder because the patient received <12 weeks of treatment with the study drug during the Fixed-Dose Period. Among the 10 patients who achieved the primary endpoint, none received prohibited concomitant medications (such as hematopoietic stimulating agents) during the study and there were no major protocol violations.

Historical transfusion burden standardized to 24 weeks, on-study (Fixed-Dose Period) transfusion burden standardized to 24 weeks and its reduction and percentage reduction from historical transfusion burden standardized to 24 weeks, and the frequency of subjects with different categories of percentage reduction (<0, 0 to <20, 20 to <33, 33 to <50, ≥50%) are summarized in Table 28.

In Study 007, the median historical RBC units transfused (standardized to 24 weeks during the Fixed-Dose Period) in the 27 patients was 6.9 units (range: 2.8, 20.3 units). The median reduction from historical RBC units transfused during the Fixed-Dose Period (standardized to 24 weeks during the Fixed-Dose Period) was 1.6 units (range: -3.5, 8.8 units) and the median percentage reduction from historical RBC units transfused (standardized to 24 weeks during the Fixed-Dose Period) was 18.8% (range: -46.8, 100%). There were six out of the 27 patients (22%) who received more transfusions compared to their historical RBC units transfused. The median RBC unit and percentage increase (in RBC unit) standardized to 24 weeks in these six patients was 1.0 unit (range: 3.5, 0.4 units) and 12.2% in RBC units (range: 46.8, 4.0%), respectively.

For the 27 patients in Study 007, based on the datasets, the median annualized historical RBC units transfusion preceding the trial entry was 15 units (range: 6, 44 units) which translates to approximately 3000 mg of iron (range: 1200, 8800 mg). During the Dose Optimization Period (the initial 16 weeks), the median number of RBC units transfused in the 27 patients was 4 units (range: 0, 11 units) which approximates to an annualized RBC transfusion of 13 units (range: 0, 36 units) which is approximately 2600 mg of iron (range: 0, 7200 mg). The difference between 3000 mg and 2600 mg may reflect variability and not a true difference during the Dose Optimization Period.

Table 28. Summary of RBC Units Transfused Standardized to 24 Weeks, Full Analysis Set, Study 007

RBC Units Transfused	Total N=27
Historical RBC units transfused	
n	27
Mean (SD)	7.7 (4.0)
Median (Q1, Q3)	6.9 (5.1, 9.7)
Min, max	2.8, 20.3
RBC units transfused during the Fixed-Dose Period	
n	26
Mean (SD)	5.4 (5.7)
Median (Q1, Q3)	4.5 (0.0, 7.8)
Min, max	0.0, 23.7
Reduction from historical RBC units transfused	
n	26
Mean (SD)	2.1 (3.3)
Median (Q1, Q3)	1.6 (0.1, 4.6)
Min, max	-3.5, 8.8
Percentage reduction from historical RBC units transfused	
n	26
Mean (SD)	37.1 (46.8)
Median (Q1, Q3)	18.8 (2.1, 100.0)
Min, max	-46.8, 100.0
Percentage 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: Study 007 Clinical Study Report, Table 9 (p. 43); Statistical Reviewer's analysis.
Both the historical and RBC units transfused during the Fixed-Dose Period are standardized to 24 weeks.
* One subject achieved a ≥50% reduction but was considered a nonresponder because the subject received <12 weeks of treatment during the Fixed-Dose Period.
** One subject discontinued before the Fixed-Dose Period and was therefore not evaluable for RBC units transfused during the Fixed-Dose Period.
Abbreviations: max, maximum; min, minimum; N, total number of subjects; n, number of subjects in each category; Q1, first quartile; Q3, third quartile; RBC, red blood cell; SD, standard deviation

A request for information was sent to the Applicant to provide the mean (and median) changes in Hb from baseline observed in Study 007 and to provide an explanation of the difference from those in Study 006. The Applicant responded as follows:

"The interpretation of changes in Hb from baseline is not comparable between Studies 006 and 007. While in a nonregularly transfused patients, an increase in Hb ≥15 g/L (1.5 g/dL) can be used to assess a treatment response, this is not the case for regularly transfused patients. In a

regularly-transfused population with pyruvate kinase deficiency (PK deficiency; such as subjects included in Study 007), an increase in Hb is not considered a valuable assessment of response because of the impact of transfusions on Hb concentration. In Study 007, there are eight subjects with Hb increase of ≥15 g/L at least once from baseline where baseline Hb is the last Hb measurement before start of study treatment; this measurement is impacted by the subject's most recent transfusion and thus does not reflect their nadir. Therefore, increase in Hb ≥15 g/L from baseline is not considered a valuable assessment of response to mitapivat in these subjects and a hemoglobin increase as a treatment response cannot be used as it is impacted by transfusions. Hence, for this population, benefit needs to be assessed by reduction in transfusion burden, which is the primary endpoint chosen for Study 007."

Table 29 summarizes the change in hemoglobin from baseline at Week 24 of Part 2 (fixed-dose period). The mean increase in hemoglobin observed in patients who received mitapivat and were not regularly receiving transfusions in Study 006 (See Table 16) was not observed in patients who received mitapivat and were regularly receiving transfusions. The mean change in hemoglobin was -1.0 (SD 1.7) in Study 007.

Table 29. Change in Hemoglobin From Baseline to Part 2, Week 24, Full Analysis Set, Study 007

Hemoglobin Level	Mitapivat (N=27)
Baseline hemoglobin (g/dL), n	27
Mean (SD)	9.2 (1.0)
Median	9.1
Range	7.4, 10.9
Part 2 Week 24 hemoglobin (g/dL), n	24
Mean (SD)	-1.0 (1.7)
Median	-1.2
Range	-2.9, 3.5

Source: ad b.xpt.
Abbreviation: N, total number of subjects; n, number of subjects in each category; SD, standard deviation

Analysis of the Secondary Endpoint

The analysis results of the secondary endpoint, the proportion of subjects who became transfusion-free (defined as zero transfusions administered during the Fixed-Dose Period) based on the FAS are summarized in Table 30. There were six transfusion-free responders. The frequency of subjects who became transfusion-free in the Fixed-Dose period was 22.2% (95% exact CI 8.6%, 42.3%) using the Clopper-Pearson method. All subjects who became transfusion free during the Fixed-Dose Period completed treatment during the Fixed-Dose Period.

For the six patients who became transfusion-free responders, the number of transfusions in the historical period standardized per 24 weeks is provided below:

- AG-348-C-007-[redacted] (b) (6) 7.8
- AG-348-C-007-[redacted] 4.6
- AG-348-C-007-[redacted] 8.8
- AG-348-C-007-[redacted] 5.1
- AG-348-C-007-[redacted] 5.1
- AG-348-C-007-[redacted] 5.1

Table 30. Summary of Transfusion-Free Responders, Full Analysis Set, Study 007

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

Source: Study 007 Clinical Study Report, Table 10 (p. 44); Statistical Reviewer's analysis.
Transfusion-free responders: Subjects transfusion-free in the Fixed-Dose Period.
CI is based on the Clopper-Pearson method.
Abbreviation: N, total number of subjects; n, number of subjects in each category; CI, confidence interval

During the Fixed-Dose Period, Hb values were generally obtained during the scheduled visits (i.e., Weeks 4, 8, 12, 18 and 24). The Hb measurements occurred at appropriate time intervals in the trial allowing for appropriate detection of the individual transfusion trigger. In Study 007, a total of 20 patients (74.1%) reached their individual transfusion trigger during the Fixed-Dose period. Among these patients, there were 15 patients (55.6%) (at 33 time points) who missed transfusion(s) after reaching their individual transfusion trigger. Table 31 summarizes transfusion compliance during the Fixed-Dose period.

Table 31. Summary of Transfusion, Fixed-Dose Period (Part 2), Full Analysis Set, Study 007

Transfusion Summary	Total N=27
Subjects who reached individual TT, n (%)	20 (74.1%)
Number of time points individual TT was reached	94
Transfusion administered	61 (64.9%)
No transfusion administered	33 (35.1%)
Reason for transfusion not administered	
Clinically asymptomatic	8
Scheduling challenges	8
Patient decision	6
Waiting for additional assessments	5
*Other	6

Source: Applicant's response to information request, adtc.xpt and adtfr.xpt
*Includes: "The Hb assessment was not associated with a transfusion because the subject received a transfusion on the same day that was associated with a previous Hb assessment", "Hb value was too high for a transfusion" and "The decision not to transfuse was due to blood shortage resulting from COVID-19 and the subject's history of alloimmunization".
Abbreviations: TT, transfusion trigger

Among the ten responders of the primary endpoint, there were a total of three patients (patient IDs: (b) (6) and (b) (6)) who reached their individual transfusion trigger, but did not receive transfusion(s) during the Fixed-Dose period. Among the three patients, patient (b) (6) also was a transfusion-free responder.

Patient (b) (6) reached the transfusion trigger at three time points (Study Days 155, 281, and 324) during the Fixed-Dose Period. Transfusion was not administered at two of those time points (Study Days 281 and 324) because the subject was clinically asymptomatic. The patient's Hb for these time points was 9.3 g/dL (Day 281) and 9.1 g/dL (Day 324).

Patient (b) (6) reached the transfusion trigger at two time points (Study Days 197 and 281) during the Fixed-Dose Period. Transfusion was not administered at one of these time points (Study Day 281) because of scheduling challenges (transfusion trigger was reached on the last study day). The subject's Hb for this time point was 7.0 g/dL.

Patient (b) (6) reached the transfusion trigger at the start of the Fixed-Dose Period (Study Day 114). Transfusion was not administered at this time point because the Investigator considered the Hb to be too high for transfusion. The patient's Hb for this time point was 9.8

g/dL, which was within the transfusion trigger range but closer to the upper bound and also above the lower range of the transfusion trigger and above most of the patient's historical pretransfusion Hb values.

A request for information was sent to the Applicant to provide the percentage of RBC unit reduction standardized to 24 weeks after imputing transfusions for the missed transfusions in the Fixed-dose Period in these three patients.

After imputation for the missed transfusions, the 24-Week percentages of RBC unit reduction in the three patients (b) (6)) compared with the historical transfusion burden (standardized to 24 weeks) was an increase of 29.2% and reduction of 27.3% and 78.1%, respectively. However, transfusions in the two patients (b) (6) and (b) (6) were clinically not indicated at the transfusion trigger time points because the patients were either asymptomatic or the Hb was too high for transfusion as described above. This is consistent with the standard clinical approach to transfusions and reasons for the missed transfusions in the two patients are justified.

With regard to patient (b) (6) who missed transfusion due to scheduling challenges after reaching the transfusion trigger time point, the missed transfusion should be counted as a needed transfusion in the assessment of the primary endpoint. After this imputation for the missed transfusion, the 24-Week percentage of RBC unit reduction compared with the historical transfusion burden (standardized to 24 weeks) was a reduction of 27.3% (compared to the 63.7% RBC unit reduction without imputation). Therefore, this patient should not be considered a responder of the primary endpoint.

Based on the above findings, we conclude:

- A total of nine patients (33.3%, 95% CI: 16.5%, 54.0%) had at least 33% reduction in the number of RBC units transfused during the 24 weeks of the Fixed-Dose period compared with the historical transfusion burden (standardized to 24 weeks).
- A total of six patients (22.2%, 95% CI: 8.6%, 42.3%) became transfusion free during the Fixed-Dose period.

6.2.4.3. Subgroup Analysis of Primary Endpoint, Study 007

Subgroup analyses were conducted to assess potential differences in treatment effect for various demographic and clinical characteristics and are presented in Section III.16.6. Transfusion reduction responders were observed in all prespecified subgroups across baseline demographic and disease characteristic subgroups of age at screening (years), sex, race, PK-R genotype, baseline individual transfusion trigger, historical transfusion episodes during the 52 weeks before informed consent standardized to 24 weeks, number of RBC units transfused during the 52 weeks before informed consent standardized to 24 weeks, and splenectomy at baseline. Of note, these subgroup analyses are limited by the small numbers of patients, and so the results should be interpreted with caution.

6.3. Key Review Issues Relevant to Evaluation of Benefit

6.3.1. Clinical Relevance of Patient-Reported Outcome (PRO) Endpoints

Issue and Background

Study 006 met the primary and all secondary endpoints under the prespecified testing hierarchy, including the PRO endpoints (PKDD and PKDIA) (Section 6.2.2.2). Demonstration of clinical benefit was imperative in Study 006 because an increase in Hb is neither a direct measure of clinical benefit nor has it been established as a validated surrogate endpoint in this setting. The Applicant elected to demonstrate clinical benefit by showing improvement in signs and symptoms of the disease using PROs. The review team conducted sensitivity analyses and consulted the Division of Clinical Outcome Assessment (DCOA) regarding the PRO endpoints to ensure that the clinical impacts of the PRO instruments are clinically relevant (b) (4)

DCOA Analysis of PRO Endpoints

DCOA was consulted to review the adequacy of the PKDD and PKDIA (see Section III.16.4 for details). As summarized by DCOA, both instruments measure some important aspects of PKD symptoms and associated functional impacts. However, DCOA had concerns regarding the relevancy of some of the instrument items based on the observed skewness in the PKDD and PKDIA item-response distributions at baseline (i.e., high endorsement of the least severe category responses). To address the observed skewness, the Applicant adopted multiple score transformations (i.e., collapsing response options, converting raw scores to T-scores). The Applicant has not provided adequate justification for this scoring approach, including limited details on the exact score transformation. Specifically, DCOA has concern that the rescaling of the numeric and verbal rating scales (i.e., collapsing response options) is arbitrary and the small sample size does not support item response theory (IRT) scoring. Further, the scoring approach makes it difficult to interpret the final score. As such, the reliability and validity of the T-scores created from the PKDD and PKDIA are unable to be verified.

Additional sensitivity analyses using the raw scores of the individual items of instruments were conducted (discussed below and in Section III.16.3) for the PKDD instrument. DCOA recommends that if PRO data are labeled from the PKDD, a description of the items that are driving the total score should be included to avoid inclusion of misleading claims as well as to note important limitations regarding the interpretability of the data.

DCOA also raised concerns with the PKDIA instrument because it includes concepts that might be influenced by factors other than treatment (e.g., interference with leisure activities, negative impact on relationships, difficulty with physical activity, interference with household activities), and may not accurately describe clinical benefit. (b) (4)

Assessment

Sensitivity Analyses

The PRO endpoints for PKDD and PKDIA using the prespecified SAP analysis method of MMRM for the T-scores demonstrated statistical significance. The T-scores are based on an average of the individual scores. To evaluate the robustness of the results, sensitivity analyses using the ANCOVA method were also performed for the PKDD and PKDIA endpoints. The results of sensitivity analyses by ANCOVA based on observed data (i.e., subjects with missing data were excluded from the analysis) using the T-scores are summarized in Section III.16.3. The analyses results show that the two-sided p-values were no longer significant for PKDD and PKDIA endpoints if the ANCOVA method was applied only to the observed data.

ANCOVAs with only observed values can be biased due to the small sample size of the study and missing data. To further evaluate the robustness of the results based on the primary MMRM analysis, sensitivity analyses by ANCOVA with multiple imputation (ANCOVA-MI) using a control-based pattern-mixture model were performed by the Applicant and the statistical reviewer for PKDD and PKDIA, which assumes missing not at random for missing data. See Section III.16.3 for the results. The results of PKDD and PKDIA analyzed by ANCOVA-MI using T-scores are nominally statistically significant, consistent with the primary analysis results by MMRM, which ensures the robustness of the analysis results for PKDD and PKDIA.

However, DCOA had concerns regarding the assessment of the psychometric properties of the PKDD and PKDIA because of the Applicant's proposed scoring approaches. Based on the skewness observed in the PKDD item-response distributions, the Applicant collapsed response options for the six PKDD items and seven PKDIA items rated on a 0-10 response scale to result in a 0-4 response scale for all PKDD and PKDIA items in order to sum the items into a daily sum score. The PKDD daily sum score and PKDIA weekly score were subsequently converted to a T-score. Per DCOA, the Applicant has not provided adequate justification for their scoring approaches. The psychometric analyses were subsequently performed using this scoring approach. As such, the reliability and validity of the T-scores created from PKDD and PKDIA are unable to be verified because of the limitations of the scoring approach.

Because results from the MMRM and the ANCOVA based on observed cases reached different conclusions and because of concerns with the scoring algorithms used in PKDD (i.e., arbitrary rescaling of the numeric and verbal rating scales, small sample size does not support item response theory scoring), we requested the Applicant to provide the changes at Week 24 from baseline of the individual items of the PKDD instrument (based on weekly average raw scores) analyzed by both the MMRM and ANCOVA with multiple imputation (ANCOVA-MI) with the control-based pattern-mixture model (see Section III.16.3) using the raw scores. As shown in Table 32, although the differences in least squares mean (mitapivat − placebo) were small (by MMRM analysis), there was a general tendency of a greater improvement of the PKDD items in the mitapivat arm compared to those in the placebo arm. The results using the ANCOVA-MI method were generally consistent with the MMRM results for the raw scores.

Table 32. MMRM Analysis of Change From Baseline in PKDD Weekly Raw Score at Week 24 Based on Average Daily Raw Total Scores for Each Individual Item, Full Analysis Set, Study 006

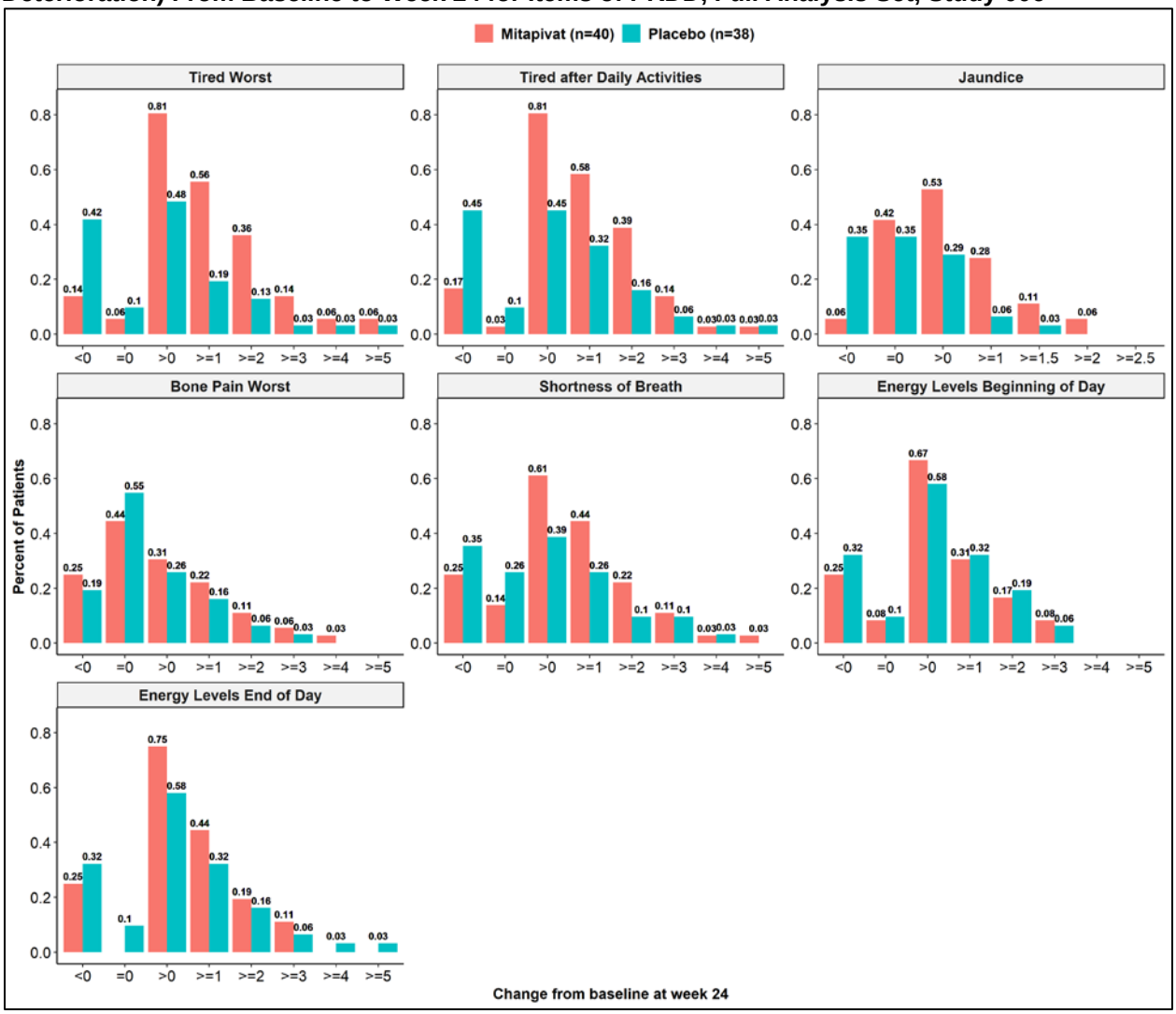
PKDD Category	Mitapivat LS Mean (SE)	Placebo LS Mean (SE)	Difference in LS Mean (SE) (Mitapivat-Placebo)
Tired worst (0-10)	-1.3 (0.3)	-0.2 (0.3)	-1.1 (0.4)
Tired after daily activities (0-10)	-1.2 (0.3)	-0.3 (0.3)	-0.9 (0.4)
Jaundice (0-4)	-0.5 (0.1)	0.0 (0.1)	-0.4 (0.1)
Bone pain worst (0-10)	-0.1 (0.2)	0.1 (0.2)	0.1 (0.2)
Shortness of breath (0-10)	-0.9 (0.2)	-0.5 (0.2)	-0.3 (0.3)
Energy levels beginning of day (0-10)	-0.6 (0.2)	-0.6 (0.2)	0.0 (0.3)
Energy levels end of day (0-10)	-0.8 (0.2)	-0.5 (0.3)	-0.3 (0.4)

Source: Applicant's response to information request dated December 15, 2021; Statistical Reviewer's analysis.
Results are based on a mixed-effects model for repeated measures method which includes change from baseline for the individual PKDD item as the dependent variable, baseline PKDD item level as a covariate, and treatment group, visit, treatment-by-visit interaction, and the randomization stratification factors as fixed factors, and subject as the random effect.
Baseline for each individual PKDD item 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.
The scores were based on patient's experience with PKD on the day (from the time of waking up the morning to the time of completing the questionnaire).
Tired worst (tiredness at its worst of today), Tired after daily activities (tiredness after finishing daily activities [e.g., work, social, leisure, physical or household activities] today), jaundice (how yellow the eyes and/or skin appeared when looking in the mirror today), bone pain (at its worst today), shortness of breath during moderate (e.g., wa king on an incline or upstairs) physical activity today, energy level at the beginning of the day (after being awake for 1 hour), energy level at the end of the day (right now).
Abbreviations: LS, least squares; MMRM, mixed-effect model for repeated measures; PKDD, Pyruvate Kinase Deficiency Diary; SE, standard error.

The items with the greatest improvement were tired worst, tiredness after finishing daily activities, jaundice and shortness of breath, but the mean changes in these scores were small and it is unclear whether the mean changes demonstrated in these items are meaningful to patients. Anchor-based methods are the primary methods used to interpret meaningful within-patient score changes in COA endpoints. However, the external anchor selected by the Applicant is inadequate (i.e., the measurement concept of the external anchor is the global impact of pyruvate kinase deficiency ["PK deficiency affected me"] and not adequately aligned with the concepts [severity of PKD symptoms] measured in the PKDD) and limits interpretation of anchor-based analyses.

Even though the four items with the greatest improvement had small mean changes, a numerically greater proportion of mitapivat-treated patients had larger responses compared to placebo-treated patients on these items (e.g., as shown in the figure below, 36% vs. 13% with ≥2-point improvement in tired worst, 14% vs. 3% with ≥3-point improvement in tired worst, 39% vs. 16% with ≥2-point improvement in tired after daily activities, 14% vs. 6% with ≥3-point improvement in tired after daily activities, 6% vs. 0% with ≥2-point improvement in jaundice, 22% vs. 10% with ≥2-point improvement in shortness of breath.) These data support that some patients can have more sizeable improvements in these symptoms and signs with mitapivat.

Figure 6. Percentage of Patients Who Experienced Various Levels of Change (Improvement and Deterioration) From Baseline to Week 24 for Items of PKDD, Full Analysis Set, Study 006



Source: FDA analysis.
The bar-plots are based on the observed data without any imputation. The percent of patients is calculated using the number of non-missing responses in each treatment arm.
Jaundice is measured on a 0-4 verbal rating scale. All other items are measured on a 0-10 numeric rating scale.
Abbreviations: PKDD, Pyruvate Kinase Deficiency Diary

Conclusion

PKDD

Study 006 enrolled patients with PKD who were not regularly transfused (required less frequent transfusions), however, they had symptoms and signs of PKD at baseline that affected how they function and feel. Although mean improvements in tiredness, shortness of breath, and jaundice in the treatment group compared to the control group were small, some patients had more sizeable improvements in these symptoms and signs with mitapivat.

PKDIA

The PKDIA results are statistically significant based on the prespecified analysis in the protocol, however, the review team cannot conclude that the items included in the PKDIA are clinically

meaningful. Furthermore, these concepts (interference in leisure activities, negative impact on relationships) are distal to the core signs and symptoms of PKD and may be impacted by factors other than treatment with mitapivat. (b) (4)

Conclusion

The results of the PRO endpoint for PKDD showed that there was a small mean improvement of some of the symptoms and signs and disease burden in the mitapivat arm compared with the placebo arm, with some patients having more sizeable improvements with mitapivat. Only the PKDD PRO endpoint will be described in the labeling. To ensure PRO data are communicated in a way that is accurate, interpretable and not misleading, the raw scores using the MMRM analysis method for the items that had the most significant effects will be described in the labeling.

6.3.2. Clinical Benefit in Patients With PKD Who Are Regularly Receiving Transfusions

Issue and Background

Study 007 was a single-arm study in patients with PKD who were regularly receiving blood transfusions. The primary endpoint was the proportion of patients who achieved a reduction in transfusion burden, defined as at least a 33% reduction in the number of RBC units transfused during the 24 weeks of the Fixed-Dose Period compared with the historical transfusion burden (standardized to 24 weeks). The review team further assessed the clinical meaningfulness of at least a 33% reduction in transfusion burden in this population.

Assessment

In Study 007, each patient had a patient-specific transfusion trigger that was used to more objectively determine the need for transfusion based on the patient's historical Hb trigger for transfusions. As discussed above, upon review of the transfusion trigger data, about one-third of the transfusion triggers did not lead to transfusion during the treatment period. After imputation for the missed transfusions, a total of 9 patients (33.3%) had at least 33% reduction in transfusion burden (primary efficacy endpoint) and 6 patients (22.2%) remained transfusion free. Another patient achieved a 100% reduction in transfusion burden, however, this patient was considered a nonresponder because the patient received <12 weeks of treatment with the study drug during the Fixed-Dose Period. Due to the open-label study design, there always exists potential bias, but use of the objective individual patient trigger criteria reduces the impact of subjective assessments of the need for transfusion. Even in a worst-case scenario analysis where we stringently classify all missed transfusions as inappropriate and count those instances as if a transfusion occurred, the responder rate (proportion of patients with ≥33% reduction in transfusion) would still be 26% and 19% of the patients would still be classified as transfusion-free. This sensitivity analysis confirms that a meaningful proportion of patients can have improvements in their transfusion burden.

In patients who achieved the primary endpoint with imputation (n=9), the median decrease of RBC unit transfusion during the Fixed-Dose Period (standardized per 24 weeks) was 5.1 units (range: 2.5 to 8.8 units) which translates to annualized median RBC reduction of 11.1 units

(range: 5.4 to 19.1 units). Because one unit of RBCs contains approximately 200 mg of iron, this translates to a median annualized decrease of approximately 2220 mg (range: 1080 to 3820 mg) of iron. The baseline median annualized RBC unit transfusion in this subset of patients was 12.5 units (range: 6 to 23 units), which translates to approximately 2500 mg of iron (range: 1200 to 4600 mg). The reduction in the need for RBC transfusion decreases the risk of iron accumulation and complications of subsequent organ damage. In children, risk of iron overload occurs after receipt of 10 units of RBCs and in adults 15 units, which is equivalent to 2000 mg and 3000 mg of iron, respectively. Patients with PKD have a risk of developing iron overload due to chronic transfusions and ineffective erythropoiesis and risk develops early in life, therefore a reduction of approximately 2220 mg of iron annually represents a benefit.

Conclusion

The review team concluded that there is a significant clinical benefit in patients who achieved the primary endpoint (i.e., ≥33% reduction in the number of RBC units) in Study 007. After imputation for the missed transfusion, 33% of patients achieved a ≥33% reduction in transfusion burden. There were six patients (22.2%) who clearly became transfusion free during the 24-week Fixed-Dose period.

7. Risk and Risk Management

7.1. Potential Risks or Safety Concerns Based on Nonclinical Data

The potential safety concerns for mitapivat were evaluated in nonclinical studies that included secondary pharmacology studies, safety pharmacology studies in rats and monkeys, 6-month and 9-month repeat-dose toxicity studies in rats and monkeys, respectively, genetic toxicology studies (Ames, in vitro chromosomal aberration and in vivo rat micronucleus assays), a full battery of reproductive toxicity studies in rats and rabbits, and 2-year rat and 6-month Tg.rasH2 mouse carcinogenicity studies. There were no outstanding nonclinical safety issues of significant concern at clinically relevant exposures as assessed by the nonclinical toxicology studies. Therefore, Nonclinical Pharmacology-Toxicology supports approval of this NDA. Safety margins were calculated based on clinical AUC of 3591.4 ng*h/mL at the MRHD of 50 mg BID (Section III.13.1.1.6).

Reproductive Toxicity

Noteworthy nonclinical findings were observed in the reproductive organs in the rats and were likely due to mitapivat-mediated aromatase (also known as cytochrome P450 [CYP19]) inhibition (IC_{50} of 2.05µM for human CYP19, and IC_{50} of 0.49µM for rat CYP19). Aromatase inhibition is an off-target activity of mitapivat and neither necessary for nor contributory to its therapeutic mechanism of action of increasing PK activity. Based on the IC_{50} values, mitapivat is a less potent inhibitor of aromatase than known aromatase inhibitors such as anastrozole, exemestane, fadrozole, and letrozole (IC_{50} 8.29 to 397nM). Because aromatase is a key enzyme in estrogen synthesis, inhibition of aromatase could affect the levels of reproductive hormones, particularly estrogen, leading to adverse effects secondary to reduced estrogen (increased risk of fracture and effects on reproductive performance). The inhibition of human aromatase with an

IC_{50} of 2.05µM is particularly relevant because the aromatase inhibition in vitro occurred at a concentration comparable to the C_{max} in human (2.14µM or 965 ng/mL) at the clinical dose. Tubular degeneration in the testes, spermatid retention in the epididymis, abnormal sperm morphology, reduced sperm density, and incomplete corpora lutea and luteinized follicles in the ovaries occurred in the chronic rat study at >7× MRHD. All findings were reversible or trended towards reversibility following dose cessation. Progesterone, testosterone, estradiol, and prolactin were measured, but the results were inconclusive due to high interindividual variability. The no observed adverse effect level for the reproductive findings in the 6-month rat study was identified at 7× and 18× MRHD in male and female rats, respectively. In a fertility study in rats, male and female fertility was not adversely affected up to the highest dose tested (45× MRHD). No effects on reproductive organs were observed in the chronic monkey study up to the highest dose tested (6× MRHD). Despite the exposure margins identified in the rat toxicology studies, decreases in estrone, estradiol, and testosterone were observed in the clinical studies (Section 7.6), which may be reasonably attributable to aromatase inhibition by mitapivat.

Liver Toxicity

In general-toxicology studies conducted in rats and monkeys, increase in liver weight associated with diffuse hepatocellular hypertrophy was observed at doses >7× MRHD in rats, and at the clinical exposure in monkeys, based on AUC. In monkeys, at doses >2.5× MRHD, hepatocellular hypertrophy was significant enough to cause subcapsular inflammation and necrosis, likely secondary to increased pressure from hypertrophy and was considered adverse. Hepatocellular findings were reversible, did not have microscopic and clinical correlates indicative of liver injury in rats at any dose or in monkeys at clinical exposure, and were considered an adaptive change secondary to induction of hepatic metabolic enzymes (cytochrome p450, CYP450). The metabolism in monkey is comparable to that in humans since the predominant CYP450 isoform involved is CYP3A4. The half-life in monkey is comparable to the effective half-life (3 to 5 hours) in human. Mitapivat is extensively metabolized by the hepatic CYP450 enzymes and there is potential for interaction with strong CYP450 inducers or inhibitors (see Section 8.2).

Carcinogenicity

Mitapivat was not carcinogenic in a 2-year rat study at doses up to 150 mg/kg/BID in males and 100 mg/kg/BID in females (47× and >100× MRHD, respectively), or in transgenic ras H2 mice up to the highest doses tested at 500 mg/kg/day in males and 250 mg/kg/day in females when given orally for 26 weeks. The genotoxicity battery did not indicate mutagenic or clastogenic potential for mitapivat.

Histamine Receptor 3 Antagonism

Secondary pharmacology studies identified an antagonistic (IC_{50} 0.102µM) or inverse agonistic effect (half-maximal effective concentration of 0.012µM) of mitapivat on human histamine H3 receptors in vitro at clinically relevant concentrations. Histamine H3 is an autoreceptor in the brain that negatively regulates histamine release from neurons through a feedback loop and thus favors sleep. Therefore, antagonism of H3 would be expected to promote insomnia. However, there were no central nervous system (CNS) findings in a safety pharmacology study in the rat or general toxicology studies in rats and monkeys. The lack of clinical signs related to H3 inhibition

in vivo in animals could be attributed to poor distribution of mitapivat in the brain since the brain/plasma ratio ranged from 0.06 to 0.09 (6% to 9% in brain compared to in plasma). Insomnia was observed in clinical studies (Section 7.6).

Table 33. Safety Margins for Pivotal Chronic Toxicology Studies

Study	NOAEL (mg/kg/BID)	Nonclinical Exposure (ng.hr/mL)	Safety Margins* (Dose Multiples)
Six-month rat	(M) 30	(M) 23,900	(M) 7×
	(F) 25	(F) 66,000	(F) 18×
Nine-month monkey	(M) 25	(M) 4370	(M) 1×
	(F) 50	(F) 8040	(F) 2×

Source: Nonclinical Reviewer
* Safety margins were based on the simulated population pharmacokinetic data generated by the clinical pharmacology reviewer simulated exposure every 12 hours (dosing interval) AUC0-12h at steady-state with a geometric mean of 3591.4 ng*h/mL (CV% 28%) for the MRHD of 50 mg BID
Abbreviations: AUC, area under the concentration-time curve; BID, twice daily; CV, coefficient of variation; F, female; M, male; MRHD, maximum recommended human dose; NOAEL, no observed adverse effect level

7.2. Potential Risks or Safety Concerns Based on Drug Class or Other Drug-Specific Factors

Not applicable. Mitapivat is a first-in-class drug.

7.3. Potential Safety Concerns Identified Through Postmarket Experience

Mitapivat is not yet approved in the United States or any foreign market for any indication.

7.4. FDA Approach to the Safety Review

7.4.1. Sources of Data for Clinical Safety Assessment.

The NDA submission contained the following four clinical studies of mitapivat in patients with PK deficiency:

- Study 006: A randomized, double-blind, placebo-controlled trial in adult patients with PKD who are not regularly transfused. For a summary of the study design, see Section 6.2.1.
- Study 007: A single-arm, open-label trial in adult patients with PKD who are regularly transfused. For a summary of the study design, see Section 6.2.3.
- Study 011: A Phase 3, open-label, extension trial for patients treated with the study drug in Studies 006 and 007. For a summary of the study design, see Section 16.1. The duration of mitapivat treatment was a maximum of 192 weeks (~3.7 years).
- Study 003: A Phase 2, randomized, open-label, dose-ranging trial in adult patients with PKD who are not regularly transfused. Patients in this study were randomized (1:1) to Arm 1 (300 mg BID, n=25) or Arm 2 (50 mg BID, n=27). The study comprised a 24-week Core Period and an 8-year Extension Period (following the end of the Core Period).

The NDA submission also contained a Phase 2, single-arm, clinical study (AG-348-C-010) of mitapivat in patients with nontransfusion dependent thalassemia. The safety information of this study was reviewed when applicable.

The safety evaluation of mitapivat is primarily based on the analyses of the two Phase 3 studies in patients with PK deficiency (Studies 006 and 007). Safety data from the two supportive Studies 011 and 003 were also reviewed.

7.4.2. Safety Analysis Plan and Definitions

The prespecified safety analysis plan and definitions were reviewed during the protocol development and were acceptable to the clinical review team.

TEAE was defined as from the first dose of study drug to 28 days after the last dose of study drug. Medical Dictionary for Regulatory Activities (MedDRA) terminology version 23.1 was used to categorize AEs. Adverse events were graded according to the National Cancer Institute Common Technology Criteria for Adverse Events version 4.03 coding system. Grade mapping of the verbatim AE terms to MedDRA preferred term and system organ class (SOC) was acceptable. The coding of the preferred terms was reviewed and was acceptable. Adverse events were analyzed by MedDRA preferred term and by pooling similar AEs (referred to as the FDA MedDRA Query [FMQ]). The FMQ analysis is similar to a customized MedDRA query.

7.4.3. Reviewer's Approach to Safety Evaluation

Clinical trial data were independently analyzed using JMP software. All safety assessments and conclusions are those of the clinical review team unless otherwise specified. The review team did not identify any major data quality or integrity issues that precluded performing a thorough safety review. No major issues were identified with respect to coding, recording, and categorizing AEs.

The safety review included the following:

- Electronic submissions of the clinical study reports and other relevant portions of the NDA were reviewed;
- Safety data were audited or reproduced;
- Pooled safety analyses were conducted when appropriate;
- Applicant's responses to FDA information requests;
- Relevant published literature; and
- The 120-day safety update.

Pooling of Data Within and Across Trials

Safety data from patients who received mitapivat in Studies 006 and 007 were pooled when appropriate for a total pooled safety set of 67 patients.

7.5. Adequacy of Clinical Safety Database

The safety database is adequate for comprehensive safety assessment of mitapivat for the proposed indication, patient population, dosage regimen, and duration. The NDA included safety information of mitapivat from a total of 400 subjects (PKD: 155, nontransfusion dependent thalassemia: 20, healthy subjects: 225) across 12 clinical trials. Since the dose regimens of mitapivat in these trials were not consistent (i.e., ranged from 5 mg once daily to 700 mg BID) and PKD is the proposed indication, the safety review was primarily based on the trials that enrolled patients with PKD.

In the above four clinical trials that studied mitapivat in patients with PKD, a total of 155 patients (Study 006, 40 patients; Study 007, 27 patients; Study 003, 52 patients; and Study 011, 36 patients who initially received placebo in Study 006) received treatment with mitapivat. Although the safety database is small, considering the rarity of the disease and that the safety analysis includes a double-blind, placebo-controlled study (Study 006), the size of the safety database is acceptable.

In Studies 006 and 007, the median duration of exposure to mitapivat was 24.1 weeks (range: 23.6, 27.4) and 40.3 weeks (range: 16.3, 46.3), respectively. In Studies 006 and 007 combined, a total of 54 patients (80.6%) received mitapivat longer than 24 weeks. In Study 007, most of the patients (20 patients [74.1%]) received mitapivat for >40 weeks.

Table 34. Duration of Exposure, Safety Analysis Set, Studies 006 and 007

	006		007	Pooled
Variable	Mitapivat N=40 n (%)	Placebo N=39 n (%)	Mitapivat N=27 n (%)	Mitapivat N=67 n (%)
Duration of exposure, weeks				
Mean (SD)	24.6 (1)	24.4 (0.7)	38 (7.2)	30 (8)
Median (Q1, Q3)	24.1 (24.1, 24.5)	24.1 (24.1, 24.3)	40.3 (40.1, 41.2)	24.6 (24.1, 40.2)
Min, max	23.6, 27.4	23.6, 27.4	16.3, 46.3	16.3, 46.3
Total exposure (person years)	19	18	20	39
Patients treated by duration, n (%)				
≤16 weeks	0	0	0	0
>16 to ≤20 weeks	0	0	1 (3.7)	1 (1.5)
>20 to ≤24 weeks	10 (25.0)	9 (23.1)	2 (7.4)	12 (17.9)
>24 to ≤36 weeks	30 (75.0)	30 (76.9)	2 (7.4)	32 (47.8)
>36 to ≤40 weeks	0	0	2 (7.4)	2 (3.0)
>40 weeks	0	0	20 (74.1)	20 (29.9)

Source: adex.xpt; software, R.
Abbreviations: N, number of subjects in treatment arm; n, number of subjects with given treatment duration; SD, standard deviation; Q1, first quartile; Q3, third quartile

Among patients who received treatment with mitapivat in Studies 006 and 007 and continued in the extension Study 011 (Study 006/Cohort 2 [n=35], Study 007/Cohort 3 [n=17]), 18 patients and 14 patients, respectively, received the study treatment for >1 year; and 12 patients and 9 patients, respectively, for >1.6 years (Table 146).

Treatment compliance in Studies 006 and 007 was assessed by drug accountability (i.e., percentage of tablets taken). In Study 006, the mean compliance rate was 98.0% in the mitapivat arm (n=38) and 98.8% in the placebo arm (n=22). In Study 007, the mean compliance rate of patients taking mitapivat (n=25) was 98.1%.

The optimized dose regimen was 50 mg BID in the majority of patients who received mitapivat in Studies 006 (87.5%) and 007 (92.6%). Patients who discontinued or interrupted study treatment during the study were to undergo the recommended dose taper regimen due to risk of withdrawal hemolysis (Section 15.3). In Studies 006 and 007, eight patients (mitapivat: six, placebo: two) and nine patients, respectively, had dose taper of the study treatment. In Studies 006 and 007, the median dose taper period in patients who had dose taper of the study treatment was 3 weeks.

Table 35. Summary of Exposure, Safety Analysis Set, Studies 006 and 007

	006		007
	Mitapivat N=40	Placebo N=39	Mitapivat N=27
Dose Exposure	n (%)	n (%)	n (%)
Optimized dose			
50 mg BID	35 (87.5)	39 (100)	25 (92.6)
20 mg BID	3 (7.5)	0	1 (3.7)
5 mg BID	2 (5.0)	0	1 (3.7)
Duration of exposure (weeks)			
Dose optimization period			
N	40	39	27
Median	12.1	12.1	16.3
Range	11.7, 13.9	11.9, 13.3	14.3, 22.1
Fixed-dose period			
N	40	39	26
Median	12.0	12.0	24.0
Range	9.7, 13.1	11.6, 12.9	3.4, 25.1
Dose taper period			
N	6	2	9
Median	3.0	3.0	3.0
Range	2.0, 3.0	3.0, 3.0	1.0, 3.1

Source: adexsum.xpt.
Abbreviation: BID, twice daily; N, total number of subjects; n, number of subjects in each category

7.6. Safety Findings and Concerns Based on Review of Clinical Safety Database

Overall Summary

The overall safety profile of mitapivat at the starting dose of 5 mg orally twice daily and gradual titration through sequential doses to 20 mg and 50 mg orally twice daily for the treatment of adult patients with PKD is acceptable.

In Study 006, the most common (≥10%) adverse reactions of mitapivat (occurring at a higher frequency than the placebo arm) including laboratory abnormalities were estrone level decreased (males), increased urate, back pain, estradiol level decreased (males), and arthralgia. Although the incidence of insomnia in the mitapivat arm (17.5%) was similar to the placebo arm (17.9%) in Study 006, insomnia has been associated with aromatase inhibitors and its incidence was correlated with higher mitapivat doses in the dose-ranging Study 003. Other adverse events of special interest for mitapivat include fractures (due to aromatase inhibition) and acute hemolysis (with abrupt interruption or discontinuation of mitapivat). Patients with PKD have significantly higher lifetime rates of osteoporosis, liver cirrhosis, and pulmonary hypertension compared to a matched population (Boscoe et al. 2021). Thus, the risk of fractures may be compounded by underlying disease and use of a drug that inhibits aromatase.

No deaths were reported in Study 006 or 007. Serious adverse reactions of mitapivat observed in Studies 006 and 007 were single events of atrial fibrillation, gastroenteritis, rib fracture, musculoskeletal pain, ovarian cyst, renal colic, and blood triglycerides increased. No TEAEs were reported in Studies 006 and 007 that led to discontinuation of mitapivat.

The 120-Day Safety Update Report provided 3 and 6 months of additional safety information from the ongoing extension Study 011 and the Extension Period of Study 003, respectively. The overall safety profile of mitapivat in the 120-Day Safety Update Report was generally consistent with the pivotal Studies 006 and 007. To assess the long-term safety of mitapivat in patients with PKD, PMRs will be requested from the Applicant to provide safety follow-up data from the ongoing Studies 003 and 011.

7.6.1. Overall Treatment-Emergent Adverse Event Summary, Studies 006 and 007

Table 36 summarizes the overall safety results in Studies 006 and 007. In Study 006, the incidence of TEAEs was similar between the two arms (mitapivat: 87.5%, placebo: 89.7%). Most of the TEAEs were Grade 1 or 2 in severity (mitapivat: 62.5%, placebo: 76.9%). There were no Grade 4 or fatal TEAEs in Study 006. Serious TEAEs occurred more often in the mitapivat arm (10.0%) compared to the placebo arm (5.1%).

In Study 007, the incidences of TEAEs (100%), Grade 1 or 2 TEAEs (70.4%) and serious TEAEs (11.1%) of mitapivat were consistent with those in Study 006. Interpretation of the safety results of Study 007 is limited by the single-arm (there was no comparator arm) trial design.

Table 36. Treatment-Emergent Adverse Events, Safety Analysis Set, Studies 006 and 007

Event Category	006 Mitapivat N=40 n (%)	006 Placebo N=39 n (%)	006 Mitapivat vs. Placebo (%) (95% CI)	007 Mitapivat N=27 n (%)	Pooled Mitapivat N=67 n (%)
Deaths	0	0	0 (0, 0)	0	0
Serious TEAEs	4 (10.0)	2 (5.1)	4.9 (-6.7, 16.5)	3 (11.1)	7 (10.4)
Study treatment-related	1 (2.5)	0	2.5 (-2.3, 7.3)	0	1 (1.5)
Any TEAEs	35 (87.5)	35 (89.7)	-2.2 (-16.2, 11.7)	27 (100)	62 (92.5)
Grade 4	0	0	0 (0, 0)	1 (3.7)	1 (1.5)
Grade 3	10 (25.0)	5 (12.8)	12.2 (-4.9, 29.2)	7 (25.9)	17 (25.4)
Grade 2	11 (27.5)	14 (35.9)	-8.4 (-28.8, 12.1)	15 (55.6)	26 (38.8)
Grade 1	14 (35.0)	16 (41.0)	-6.0 (-27.4, 15.3)	4 (14.8)	18 (26.9)
Any TEAEs study treatment-related	23 (57.5)	14 (35.9)	21.6 (0.1, 43.1)	18 (66.7)	41 (61.2)
TEAE leading to permanent discontinuation of study drug	0	0	0 (0, 0)	0	0
TEAE leading to dose modification of study drug	0	2 (5.1)	-5.1 (-12.1, 1.8)	1 (3.7)	1 (1.5)
AE leading to interruption of study drug	0	2 (5.1)	-5.1 (-12.1, 1.8)	0	0
AE leading to dose reduction of study drug	0	0	0 (0, 0)	1 (3.7)	1 (1.5)

Source: adae.xpt; software, R.
Treatment-emergent adverse events defined as adverse event onset was on or after the initiation of any study therapy to 28 days post last dose of any study therapy. Duration 26 weeks for Study 006 and 42 weeks for Study 007.
Risk difference column shows difference (with 95% confidence interval) between total treatment and comparator.
Abbreviations: AE, adverse event; CI, confidence interval; N, number of subjects in treatment arm; n, number of subjects with at least one event; SAE, serious adverse event; TEAE, treatment-emergent adverse event

7.6.2. Deaths, Studies 006 and 007

No deaths were reported in Studies 006 and 007 up to the clinical cut-off date for the Safety Update.

7.6.3. Serious Adverse Events, Studies 006 and 007

In Study 006, SAEs occurred in four patients (10.0%) (atrial fibrillation, gastroenteritis, rib fracture, musculoskeletal pain, one event each) in the mitapivat arm and two patients (5.1%) (metapneumovirus infection, obstructive pancreatitis, one event each) in the placebo arm. In Study 007, a total of three patients (11.1%) experienced SAEs (ovarian cyst, renal colic, blood triglycerides increased, one event each). All SAEs, except the patient who experienced serious musculoskeletal pain in Study 006, were reported as resolved without dose modification of the study treatment. These isolated SAEs do not raise concerns.

Table 37. Serious Adverse Events by System Organ Class and Preferred Term, Safety Analysis Set, Studies 006 and 007

System Organ Class Preferred Term	006 Mitapivat N=40 n (%)	006 Placebo N=39 n (%)	006 Mitapivat vs. Placebo (%) (95% CI)	007 Mitapivat N=27 n (%)	Pooled Mitapivat N=67 n (%)
All	4 (10.0)	2 (5.1)	4.9 (-6.7, 16.5)	3 (11.1)	7 (10.4)
Cardiac disorders	1 (2.5)	0	2.5 (-2.3, 7.3)	0	1 (1.5)
Atrial fibrillation	1 (2.5)	0	2.5 (-2.3, 7.3)	0	1 (1.5)
Gastrointestinal disorders	0	1 (2.6)	-2.6 (-7.5, 2.4)	0	0
Obstructive pancreatitis	0	1 (2.6)	-2.6 (-7.5, 2.4)	0	0
Infections and infestations	1 (2.5)	1 (2.6)	-0.1 (-7.0, 6.9)	0	1 (1.5)
Gastroenteritis	1 (2.5)	0	2.5 (-2.3, 7.3)	0	1 (1.5)
Metapneumovirus infection	0	1 (2.6)	-2.6 (-7.5, 2.4)	0	0
Injury, poisoning and procedural complications	1 (2.5)	0	2.5 (-2.3, 7.3)	0	1 (1.5)
Rib fracture	1 (2.5)	0	2.5 (-2.3, 7.3)	0	1 (1.5)
Investigations	0	0	0 (0, 0)	1 (3.7)	1 (1.5)
Blood triglycerides increased	0	0	0 (0, 0)	1 (3.7)	1 (1.5)
Musculoskeletal and connective tissue disorders	1 (2.5)	0	2.5 (-2.3, 7.3)	0	1 (1.5)
Musculoskeletal pain	1 (2.5)	0	2.5 (-2.3, 7.3)	0	1 (1.5)
Renal and urinary disorders	0	0	0 (0, 0)	1 (3.7)	1 (1.5)
Renal colic	0	0	0 (0, 0)	1 (3.7)	1 (1.5)
Reproductive system and breast disorders	0	0	0 (0, 0)	1 (3.7)	1 (1.5)
Ovarian cyst	0	0	0 (0, 0)	1 (3.7)	1 (1.5)

Source: adae.xpt; software, R.
Treatment-emergent adverse events defined as adverse event onset was on or after the initiation of any study therapy through 28 days post last dose of any study therapy.
Duration is 26 weeks for Study 006 and 42 weeks for Study 007.
Risk difference column shows difference (with 95% confidence interval) between total treatment and comparator.
Abbreviations: CI, confidence interval; N, number of subjects in treatment arm; n, number of subjects with adverse event

7.6.4. Dropouts and/or Discontinuations Due to Adverse Events, Studies 006 and 007

In Studies 006 and 007, no TEAEs were reported that led to discontinuation of the study treatment. In Study 006, there were no TEAEs that led to dose reduction or interruption of the study treatment in the mitapivat arm. In the placebo arm, two patients experienced TEAEs (Grade 3 obstructive pancreatitis [severe pain; vomiting; medical intervention indicated] and Grade 3 tonsilitis [severe; limiting self-care activities of daily living (ADL)]) that resulted in study treatment interruption.

In Study 007, no patient experienced a TEAE that resulted in interruption of the study treatment. One patient (ID: (b) (6)) experienced Grade 3 joint swelling (severe; limiting self-care ADL) and Grade 3 back pain (severe pain; limiting self-care ADL) that required dose reduction of mitapivat; the dose of mitapivat was reduced from 20 mg BID to 20 mg once daily (on Study Day 66) and further reduced to 5 mg BID (on Study Day 67). This patient discontinued during the Dose Optimization Period and did not enter the fixed-dose period due to subject withdrawal. The joint swelling was considered resolved on Study Day 59; the back pain was not resolved at the end of the study.

Joint stiffness and pain and joint swelling are common adverse reactions reported with aromatase inhibitors. Overall, there were few dose reductions or dose modifications of mitapivat.

7.6.5. Significant Adverse Events, Studies 006 and 007

In Study 006, the incidence of Grade 3 TEAEs was higher in the mitapivat arm compared with the placebo arm (mitapivat: 10 patients [25.0%], placebo: 5 patients [12.8%]). The Grade 3 TEAEs reported in more than one patient in the mitapivat arm were hypertriglyceridemia and hypertension. For the two patients who had hypertension in the mitapivat arm, it was reported that one patient had an ongoing medical history of hypertension and the other patient had a risk factor for hypertension (sleep apnea). No Grade 4 TEAEs were reported in Study 006.

In Study 007, eight patients (29.6%) had Grade 3 or 4 TEAEs. Grade 4 TEAE was reported for one patient (ID: (b) (6)) who experienced Grade 4 blood triglycerides increased (>1000 mg/dL). This patient was on mitapivat 50 mg BID; the event was resolved 6 days later with no treatment reported for the event or action taken with the study treatment due to the event.

Increases in circulating cholesterol and triglycerides are reported with the use of aromatase inhibitors. Although mitapivat is a weaker aromatase inhibitor than approved aromatase inhibitors such as anastrozole or letrozole, given the life-long use of the drug, monitoring of blood lipid levels during treatment will be an important consideration.

Table 38 summarizes the Grade 3 or 4 TEAEs that occurred in Studies 006 and 007. When the TEAEs that occurred in patients receiving mitapivat were pooled from the two studies, the Grade ≥3 TEAEs reported by more than one patient were hypertriglyceridemia, hypertension gastroenteritis, musculoskeletal pain, and syncope.

Table 38. Grade 3 or Higher TEAEs, Safety Analysis Set, Studies 006 and 007

Preferred Term	006 Mitapivat N=40 n (%)	006 Placebo N=39 n (%)	007 Mitapivat N=27 n (%)	Pooled Mitapivat N=67 n (%)
All	10 (25.0)	5 (12.8)	8 (29.6)	18 (26.9)
Hypertriglyceridemia[1]	2 (5.0)	0	1 (3.7)	3 (4.5)
Hypertension	2 (5.0)	0	0	2 (3.0)
Gastroenteritis[2]	1 (2.5)	0	1 (3.7)	2 (3.0)
Musculoskeletal pain[3]	1 (2.5)	0	1 (3.7)	2 (3.0)
Syncope	1 (2.5)	0	1 (3.7)	2 (3.0)
Lymphopenia	1 (2.5)	0	0	1 (1.5)
Neutropenia	1 (2.5)	0	0	1 (1.5)
Rib fracture	1 (2.5)	0	0	1 (1.5)
Diarrhea	0	0	1 (3.7)	1 (1.5)
Joint swelling	0	0	1 (3.7)	1 (1.5)
AST increased	0	1 (2.6)	1 (3.7)	1 (1.5)
Jugular vein occlusion	0	0	1 (3.7)	1 (1.5)
Ovarian cyst	0	0	1 (3.7)	1 (1.5)
Anemia	0	2 (5.1)	0	0
Obstructive pancreatitis	0	1 (2.6)	0	0
Tonsilitis	0	1 (2.6)	0	0

Source: adae.xpt.
[1] Hypertriglyceridemia includes hypertriglyceridemia and blood triglycerides increased.
[2] Gastroenteritis includes gastroenteritis and *Campylobacter* gastroenteritis.
[3] Musculoskeletal pain includes musculoskeletal pain and back pain.
Abbreviations: AST, aspartate aminotransferase; N, number of subjects in treatment arm; n, number of subjects with adverse event; TEAE, treatment emergent adverse event

7.6.6. Treatment-Emergent Adverse Events, Studies 006 and 007

In Study 006, the overall incidences of TEAEs were similar between the two treatment arms (mitapivat: 87.5%, placebo: 89.7%). The most frequent TEAEs (>10%) that occurred in the mitapivat arm with a ≥5% greater incidence compared with the placebo arm by SOC were musculoskeletal and connective tissue disorders (35.0% versus 25.6%); injury, poisoning, and procedural complications (12.5% versus 5.1%); vascular disorders (12.5% versus 0); and metabolism and nutrition disorders (10.0% versus 0).

In Study 007, all patients (100%) reported TEAEs. The most frequent TEAEs (>30%) by SOC were infections and infestations (66.7%); gastrointestinal disorders (55.6%); nervous system disorders (51.9%); investigations (48.1%); respiratory, thoracic, and mediastinal disorders (37.0%); and general disorders and administration site conditions (33.3%).

Table 39. TEAEs by System Organ Class Reported in ≥5% of Patients Treated With Mitapivat, Safety Analysis Set, Studies 006 and 007

System Organ Class	006 Mitapivat N=40 n (%)	006 Placebo N=39 n (%)	007 Mitapivat N=27 n (%)	Pooled Mitapivat N=67 n (%)
All	35 (87.5)	35 (89.7)	27 (100)	62 (92.5)
Gastrointestinal disorders	15 (37.5)	20 (51.3)	15 (55.6)	30 (44.8)
Infections and infestations	14 (35.0)	20 (51.3)	18 (66.7)	32 (47.8)
Musculoskeletal and connective tissue disorders	14 (35.0)	10 (25.6)	7 (25.9)	21 (31.3)

System Organ Class	006 Mitapivat N=40 n (%)	006 Placebo N=39 n (%)	007 Mitapivat N=27 n (%)	Pooled Mitapivat N=67 n (%)
Respiratory, thoracic, and mediastinal disorders	11 (27.5)	14 (35.9)	10 (37.0)	21 (31.3)
General disorders and administration site conditions	11 (27.5)	9 (23.1)	9 (33.3)	20 (29.9)
Nervous system disorders	10 (25.0)	14 (35.9)	14 (51.9)	24 (35.8)
Psychiatric disorders	9 (22.5)	10 (25.6)	8 (29.6)	17 (25.4)
Skin and subcutaneous tissue disorders	7 (17.5)	10 (25.6)	6 (22.2)	13 (19.4)
Reproductive system and breast disorders	5 (12.5)	3 (7.7)	6 (22.2)	11 (16.4)
Injury, poisoning, and procedural complications	5 (12.5)	2 (5.1)	3 (11.1)	8 (11.9)
Vascular disorders	5 (12.5)	0	2 (7.4)	7 (10.4)
Metabolism and nutrition disorders	4 (10.0)	0	4 (14.8)	8 (11.9)
Blood and lymphatic system disorders	2 (5.0)	2 (5.1)	2 (7.4)	4 (6.0)
Cardiac disorders	2 (5.0)	1 (2.6)	2 (7.4)	4 (6.0)
Eye disorders	2 (5.0)	0	1 (3.7)	3 (4.5)
Investigations	1 (2.5)	8 (20.5)	13 (48.1)	14 (20.9)
Renal and urinary disorders	1 (2.5)	1 (2.6)	2 (7.4)	3 (4.5)

Source: adae.xpt.
Abbreviations: N, number of subjects in treatment arm; n, number of subjects with adverse event; TEAE, treatment-emergent adverse event

In Study 006, the TEAEs that occurred at >5% in the mitapivat arm with a >4% higher incidence compared with the placebo arm were back pain, arthralgia, gastroenteritis, hypertriglyceridemia, and hot flush. Most patients experienced the first occurrence of a TEAE while on the 5 mg BID dose (mitapivat: 50%, placebo: 66.7%).

In addition, although not reported as TEAEs in Study 006, the incidence of the laboratory abnormality high urate was higher in the mitapivat arm (15.0%) compared with the placebo arm (7.7%). See Section 7.6.8 for more information.

In Study 007, the most common TEAEs (≥15%) were headache, upper respiratory tract infection, hepatic injury, nasopharyngitis, fatigue, insomnia, hemorrhage, dyspnea, nausea, vomiting, and back pain.

As in Study 007, the incidence of the laboratory abnormality high urate was 22.2%, although not reported as a TEAE. See Section 7.6.8 for more information.

Table 40. TEAEs That Occurred in More Than One Patient Among Patients Who Received Mitapivat in the Pooled Analysis by FDA Medical Query (Narrow), Safety Analysis Set, Studies 006 and 007

System Organ Class FMQ (Narrow)	006 Mitapivat N=40 n (%)	006 Placebo N=39 n (%)	006 Mitapivat vs. Placebo (%) (95% CI)	007 Mitapivat N=27 n (%)	Pooled Mitapivat N=67 n (%)
Any AEs	35 (87.5)	35 (89.7)	-2.2 (-16.2, 11.7)	27 (100)	62 (92.5)
Blood and lymphatic system disorders					
Leukopenia	2 (5.0)	0	5.0 (-1.8, 11.8)	0	2 (3.0)

System Organ Class FMQ (Narrow)	006 Mitapivat N=40 n (%)	006 Placebo N=39 n (%)	006 Mitapivat vs. Placebo (%) (95% CI)	007 Mitapivat N=27 n (%)	Pooled Mitapivat N=67 n (%)
Cardiac disorders					
Arrhythmia	2 (5.0)	0	5.0 (-1.8, 11.8)	2 (7.4)	4 (6.0)
Systemic hypertension	2 (5.0)	0	5.0 (-1.8, 11.8)	0	2 (3.0)
Gastrointestinal disorders					
Constipation	2 (5.0)	0	5.0 (-1.8, 11.8)	1 (3.7)	3 (4.5)
Dry mouth	2 (5.0)	0	5.0 (-1.8, 11.8)	1 (3.7)	3 (4.5)
Vomiting	1 (2.5)	1 (2.6)	-0.1 (-7.0, 6.9)	5 (18.5)	6 (9.0)
Nausea	7 (17.5)	9 (23.1)	-5.6 (-23.3, 12.1)	5 (18.5)	12 (17.9)
Abdominal pain*	5 (12.5)	7 (17.9)	-5.4 (-21.3, 10.4)	4 (14.8)	9 (13.4)
Diarrhea	4 (10.0)	7 (17.9)	-7.9 (-23.2, 7.3)	3 (11.1)	7 (10.4)
Dyspepsia	1 (2.5)	5 (12.8)	-10.3 (-21.9, 1.2)	3 (11.1)	4 (6.0)
General disorders and administration site conditions					
Decreased appetite	0	0	0 (0, 0)	2 (7.4)	2 (3.0)
Fatigue	6 (15.0)	6 (15.4)	-0.4 (-16.2, 15.4)	6 (22.2)	12 (17.9)
Peripheral edema	0	1 (2.6)	-2.6 (-7.5, 2.4)	2 (7.4)	2 (3.0)
Hepatobiliary disorders					
Hepatic injury	1 (2.5)	6 (15.4)	-12.9 (-25.2, -0.6)	10 (37.0)	11 (16.4)
Infections and infestations					
Nasopharyngitis	7 (17.5)	10 (25.6)	-8.1 (-26.2, 9.9)	7 (25.9)	14 (20.9)
Upper respiratory tract infection	4 (10.0)	6 (15.4)	-5.4 (-20.0, 9.3)	10 (37.0)	14 (20.9)
Gastroenteritis	3 (7.5)	0	7.5 (-0.7, 15.7)	1 (3.7)	4 (6.0)
Otitis media	1 (2.5)	0	2.5 (-2.3, 7.3)	1 (3.7)	2 (3.0)
Metabolism and nutrition disorder					
Hypertriglyceridemia	3 (7.5)	1 (2.6)	4.9 (-4.6, 14.5)	3 (11.1)	6 (9.0)
Musculoskeletal and connective tissue disorders					
Pain in extremity	2 (5.0)	3 (7.7)	-2.7 (-13.4, 8.1)	0	2 (3.0)
Osteopenia	1 (2.5)	0	2.5 (-2.3, 7.3)	1 (3.7)	2 (3.0)
Musculoskeletal pain	2 (5.0)	3 (7.7)	-2.7 (-13.4, 8.1)	0	2 (3.0)
Arthralgia	4 (10.0)	2 (5.1)	4.9 (-6.7, 16.5)	2 (7.4)	6 (9.0)
Back pain	6 (15.0)	3 (7.7)	7.3 (-6.6, 21.2)	5 (18.5)	11 (16.4)
Nervous system disorders					
Paresthesia	2 (5.0)	0	5.0 (-1.8, 11.8)	0	2 (3.0)
Syncope	1 (2.5)	0	2.5 (-2.3, 7.3)	1 (3.7)	2 (3.0)
Dizziness	5 (12.5)	5 (12.8)	-0.3 (-15.0, 14.3)	2 (7.4)	7 (10.4)
Somnolence	0	1 (2.6)	-2.6 (-7.5, 2.4)	2 (7.4)	2 (3.0)
Headache	6 (15.0)	13 (33.3)	-18.3 (-36.8, 0.1)	11 (40.7)	17 (25.4)
Psychiatric disorders					
Parasomnia	0	0	0 (0, 0)	2 (7.4)	2 (3.0)
Insomnia	7 (17.5)	7 (17.9)	-0.4 (-17.3, 16.4)	6 (22.2)	13 (19.4)
Reproductive system and breast disorders					
Dysmenorrhea	1 (2.5)	3 (7.7)	-5.2 (-14.9, 4.5)	1 (3.7)	2 (3.0)
Abnormal uterine bleeding	0	0	0 (0, 0)	3 (11.1)	3 (4.5)
Breast discomfort	2 (5.0)	0	5.0 (-1.8, 11.8)	0	2 (3.0)

System Organ Class FMQ (Narrow)	006 Mitapivat N=40 n (%)	006 Placebo N=39 n (%)	006 Mitapivat vs. Placebo (%) (95% CI)	007 Mitapivat N=27 n (%)	Pooled Mitapivat N=67 n (%)
Respiratory, thoracic, and mediastinal disorders					
Nasal congestion	2 (5.0)	3 (7.7)	-2.7 (-13.4, 8.1)	0	2 (3.0)
Oropharyngeal pain	3 (7.5)	2 (5.1)	2.4 (-8.3, 13.1)	3 (11.1)	6 (9.0)
Cough	4 (10.0)	4 (10.3)	-0.3 (-13.6, 13.1)	1 (3.7)	5 (7.5)
Dyspnea	3 (7.5)	4 (10.3)	-2.8 (-15.3, 9.8)	5 (18.5)	8 (11.9)
Skin and subcutaneous tissue disorders					
Rash	1 (2.5)	5 (12.8)	-10.3 (-21.9, 1.2)	3 (11.1)	4 (6.0)
Hyperhidrosis	1 (2.5)	0	2.5 (-2.3, 7.3)	1 (3.7)	2 (3.0)
Vascular disorders					
Hemorrhage	3 (7.5)	4 (10.3)	-2.8 (-15.3, 9.8)	6 (22.2)	9 (13.4)
Hot flush	3 (7.5)	0	7.5 (-0.7, 15.7)	1 (3.7)	4 (6.0)

Source: adae.xpt; software, R.
Grouped Terms by FDA Medical Query (FMQ).
*Abdominal pain includes abdominal pain, abdominal pain lower, abdominal pain upper, abdominal distension and abdominal discomfort.
Abnormal uterine bleeding includes menstruation irregular and vaginal hemorrhage.
Arrhythmia includes arrhythmia, tachycardia, heart rate increased and atrial f brillation.
Arthralgia includes arthralgia and joint swelling.
Back pain includes back pain, sciatica, and flank pain.
Cough includes cough, productive cough, and upper-airway cough syndrome.
Dizziness includes dizziness, presyncope and vertigo.
Dry mouth includes dry mouth and lip dry.
Dyspepsia includes dyspepsia and abdominal pain upper.
Dyspnea includes dyspnea and dyspnea exertional.
Fatigue includes fatigue, asthenia, and lethargy.
Headache includes headache and procedural headache.
Hemorrhage includes contusion, epistaxis, hematochezia, hematemesis, petechiae and vaginal hemorrhage.
Hepatic injury includes ALT increased and AST increased.
Hypertriglyceridemia includes hypertriglyceridemia and blood triglycerides increased.
Insomnia includes insomnia, initial insomnia, middle insomnia, and terminal insomnia.
Nasopharyngitis includes nasopharyngitis, pharyngitis, rhinitis allergic, viral pharyngitis, pharyngitis streptococcal and rhinitis.
Leukopenia includes lymphopenia and neutropenia.
Musculoskeletal pain includes musculoskeletal pain, muscle spasm, musculoskeletal chest pain and myalgia.
Parasomnia includes abnormal dreams and nightmare.
Peripheral edema includes peripheral edema and peripheral swelling.
Rash includes rash, blister, rash macular and rash pustular.
Somnolence includes somnolence and lethargy.
Systemic hypertension includes hypertension.
Upper respiratory tract infection includes upper respiratory tract infection, viral upper respiratory tract infection, metapneumovirus infection, influenza, and influenza like illness.
Vomiting includes vomiting and hematemesis.
Treatment-emergent adverse events defined as adverse event onset was on or after the initiation of any study therapy through 28 days post last dose of any study therapy.
Duration is 26 weeks for Study 006 and 42 weeks for Study 007.
Risk difference column shows difference (with 95% confidence interval) between total treatment and comparator.
For specific preferred terms under each FMQ, see the table “Adverse Events by System Organ Class, FDA Medical Query (Narrow) and Preferred Term…”
Abbreviations: AE, adverse event; ALT, alanine transaminase; AST, aspartate aminotransferase; CI, confidence interval; FMQ, FDA medical query; N, number of subjects in treatment arm; n, number of subjects with adverse event

The most common adverse events (≥10%) were insomnia, back pain, and arthralgia. However, insomnia did not occur at a higher incidence compared to placebo in Study 006. Back pain and arthralgia did occur at higher incidences in the mitapavit arm compared to the placebo arm. Back pain and arthralgia are known adverse reactions with drugs that inhibit aromatase. These adverse reactions will be presented in the labeling.

7.6.7. Adverse Events of Special Interest, Studies 006 and 007

The purpose of this section is to provide a detailed discussion regarding safety signals that occur at a high frequency and/or are associated with serious outcomes. Based on nonclinical studies and prior clinical experience with mitapivat, adverse events of special interest were the following: transaminase increase, insomnia, acute hemolysis, aromatase inhibition, gastrointestinal events, hypertriglyceridemia, and hypersensitivity.

7.6.7.1. Transaminase Increase

Transaminase increase was defined as increases of >2.5× baseline ALT or AST or an increase in ALT or AST to Grade ≥2 (whichever is lower). In Study 006, the incidence of transaminase increase was higher in the placebo arm compared with the mitapivat arm (mitapivat: one patient [2.5%], placebo: six patients [15.4%]), possibly due to untreated hemolysis associated with PKD. One patient (ID: (b) (6)) in the mitapivat arm had Grade 1 ALT and Grade 1 AST increase, which resolved without dose modification of the study treatment. None of the events led to discontinuation of study treatment.

According to Murakami, in hemolytic anemia, jaundice and hepatosplenomegaly are often seen mimicking liver diseases causing elevated serum AST and bilirubin levels (Murakami and Shimizu 2013). Liver dysfunction can also be caused by blood transfusion for anemia.

In Study 007, a total of 10 patients (37.0%) experienced an increase in transaminase. The incidence was higher than in the mitapivat arm in Study 006, possibly because patients in Study 007 were regularly receiving transfusions (i.e., higher risk for iron overload) and a larger proportion of patients were on chelation therapy (i.e., deferoxamine, deferasirox, or deferiprone, all of which can cause increased transaminases) than patients in Study 006. In all but one patient (ID: (b) (6)) who had Grade 1 ALT increased, the events were reported as resolved. One patient (ID: (b) (6)) had Grade 3 AST increased, one patient (ID: (b) (6)) had Grade 2 AST increased, and one patient ((b) (6)) had Grade 2 AST and Grade 2 ALT increased. The remaining patients had Grade 1 ALT/AST increased.

Table 41. Transaminase Increased, Safety Population, Studies 006 and 007

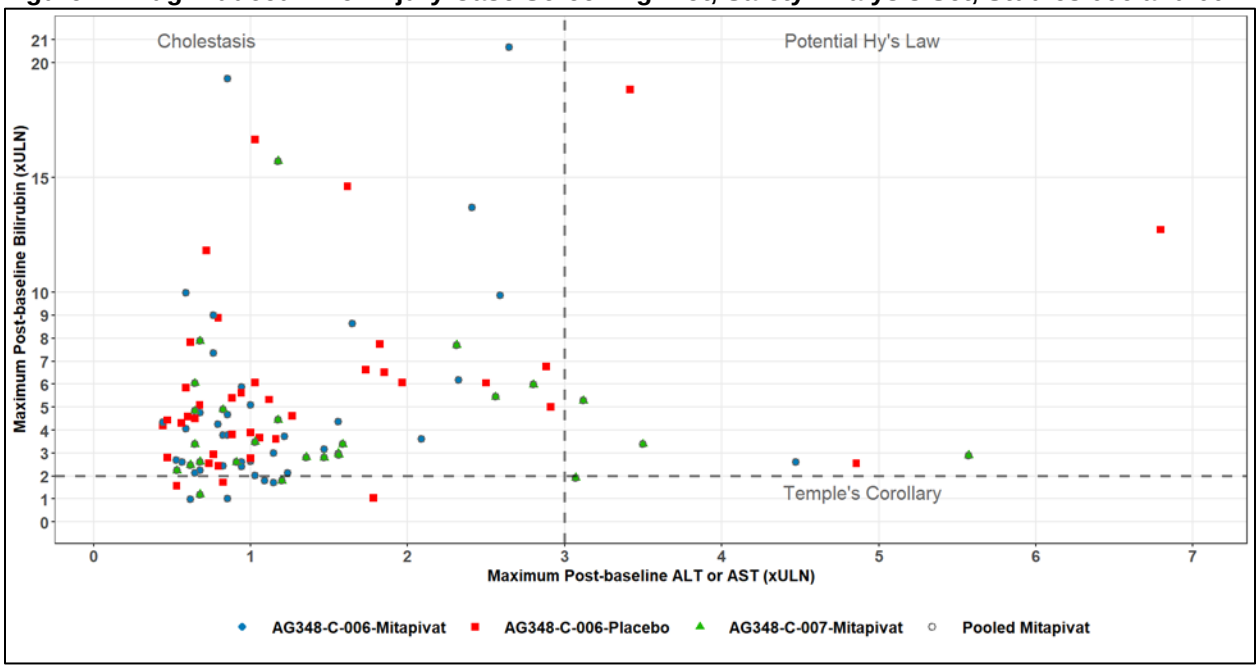
Effect on Transaminases	006 Mitapivat N=40 n (%)	006 Placebo N=39 n (%)	006 Mitapivat vs. Placebo (%) (95% CI)	007 Mitapivat N=27 n (%)	Pooled Mitapivat N=67 n (%)
All subjects	1 (2.5)	6 (15.4)	-12.9 (-25.2, -0.6)	10 (37.0)	11 (16.4)
ALT increased	1 (2.5)	6 (15.4)	-12.9 (-25.2, -0.6)	10 (37.0)	11 (16.4)
AST increased	1 (2.5)	3 (7.7)	-5.2 (-14.9, 4.5)	5 (18.5)	6 (9.0)
Maximum severity					
Death	0	0	0 (0, 0)	0	0
Grade 4	0	0	0 (0, 0)	0	0
Grade 3	0	1 (2.6)	-2.6 (-7.5, 2.4)	1 (3.7)	1 (1.5)
Grade 2	0	1 (2.6)	-2.6 (-7.5, 2.4)	2 (7.4)	2 (3.0)
Grade 1	1 (2.5)	4 (10.3)	-7.8 (-18.4, 2.9)	7 (25.9)	8 (11.9)
Serious	0	0	0 (0, 0)	0	0
Deaths	0	0	0 (0, 0)	0	0
Resulting in discontinuation	0	0	0 (0, 0)	0	0

Source: adae.xpt; software, R.
Treatment-emergent adverse events defined as adverse event onset was on or after the initiation of any study therapy through 28 days post last dose of any study therapy.
Duration is 26 weeks for Study 006 and 42 weeks for Study 007.
Risk difference column shows difference (with 95% confidence interval) between total treatment and comparator.
Relatedness is determined by investigator.
Grade 1: >ULN-3.0× ULN, Grade 2: >3.0-5.0× ULN, Grade 3: >5.0-20.0× ULN, Grade 4: >20.0× ULN.
Abbreviations: AE, adverse event; AESI, adverse events of special interest; CI, confidence interval; N, number of subjects in treatment arm; n, number of subjects with adverse event.Abbreviations: AE, adverse event; AESI, adverse events of special interest; CI, confidence interval; N, number of patients in treatment arm; n, number of patients with adverse event.

Evaluation for Drug-Induced Liver Injury

A total of four patients (mitapivat: one patient [ID: (b) (6), placebo: three patients) in Study 006 and three patients (IDs: (b) (6) and (b) (6)) in Study 007 had concurrent ALT or AST >3× ULN and total bilirubin >2× ULN, which met the criteria for potential drug-induced liver injury events (Figure 7). The narratives for the four patients who received mitapivat in Studies 006 and 007 and had concurrent ALT or AST >3× ULN and total bilirubin >2× ULN increases are presented in Section 17.3. In all four cases, although there were increases in the ALT and/or AST values compared to baseline values, their baseline bilirubin values were elevated and remained elevated during the study.

Figure 7. Drug-Induced Liver Injury Case Screening Plot, Safety Analysis Set, Studies 006 and 007



Source: ad b.xpt; software, R.
Each data point represents at least one visit (from a patient) with both ALT/AST and total bilirubin values in the postbaseline period.
A potential Hy's Law case was defined as having a maximum postbaseline total bilirubin equal to or exceeding 2× ULN within 30 days after maximum postbaseline ALT or AST equal to or exceeding 3× ULN, without findings of cholestasis (defined as ALP <2× ULN).
Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; TB, total bilirubin; DILI, drug-induced liver injury; ULN, upper limit of normal; ALP, alkaline phosphatase

Among patients who were treated with mitapivat in the mitapivat clinical studies (including in Studies 003 and 011), 11 met the Hy's law criteria (i.e., elevated aminotransferase of >3× ULN, alkaline phosphatase <2× ULN, and associated with an increase in bilirubin >2× ULN). However, all patients had Grade ≥2 bilirubin (>1.5 to 3.0× ULN) increase at baseline, and bilirubin on study decreased or remained at baseline values in nine (5.8%) subjects. These events were not assessed as potential drug-induced liver injury due to elevated baseline liver tests and no observed increase in bilirubin. The two patients (1.2%) who had increased bilirubin during the study at the time the criteria for a Hy's law event were met, had confounding factors (ongoing hemolysis: one patient [0.6%], acute cholelithiasis: one patient [0.6%]). In a total of nine patients (5.8%), ALT or AST elevations were attributed to acute and/or extrinsic alternative etiologies, and two patients (1.2%) had ALT/AST increase due to ongoing hemolysis secondary to PKD anemia. No SAEs of hepatic injury were reported in the completed or ongoing clinical studies with mitapivat. In the summary of clinical safety, it is stated that transaminase increase generally occurred during the first 16 weeks on study treatment and resolved within 16 days while continuing on study treatment. Other concomitant factors that could cause increases in transaminases included liver iron overload, treatment with iron chelators, concomitant acetaminophen use, recent history of infection, and recent history of alcohol use in addition to underlying hemolysis in patients with PKD.

In summary, all subjects with hepatobiliary events and or laboratory abnormalities had a plausible alternate etiology or had confounding factors such as ongoing hemolysis.

7.6.7.2. Insomnia

According to the Applicant, mitapivat has antagonist/inverse agonist activity against the histamine H3 receptor (H3R), known to control wakefulness. However, mitapivat does not penetrate the blood–brain barrier (brain-to-plasma AUC ratio of <0.1); therefore, it is unlikely that mitapivat exposure is sufficient to modulate the H3 receptors in the brain. However, events of insomnia in Study 003 were more frequent in patients who received >50 mg BID.

A request for information was sent to the Applicant to provide a possible mechanism of action for insomnia with mitapivat administration. The Applicant responded that mechanisms that have been assessed include antagonism/inverse agonism of H3R and changes in hormone levels due to aromatase inhibition. The Applicant consulted a neurology sleep expert, who postulated that despite its low penetration into the CNS, mitapivat might cause insomnia due to the wide distribution of H3R in the brain, including the area postrema, ventral lateral medulla, and other circumventricular organs. These areas of the brain have fenestrated capillaries that allow the brain to sample the contents of the blood. Interaction with the H3R in these areas could explain the occurrence of CNS effects despite low CNS penetration. Therefore, although mitapivat has low brain penetration, it may act on H3R in the circumventricular organs where the blood-brain barrier is more highly permeable, contributing to the insomnia observed in some subjects.

Changes in hormone levels due to aromatase inhibition is also a potential mechanism by which mitapivat may cause insomnia. As noted in Section 7.6.7.4, mitapivat treatment in male patients caused increased testosterone levels and decreased estrone and estradiol levels. Hormone changes in women during menopause (decreases in estrone/estradiol) have been associated with sleep disturbances; testosterone has been shown to increase the risk of sleep disturbances.

In Study 006, the incidence of insomnia was similar between the two arms (mitapivat: 17.5%, placebo: 17.9%). All events in the mitapivat arm were Grade 1 (mild difficulty falling asleep, staying asleep or waking up early), none was considered serious, and no dose modifications were conducted due to the TEAE. The TEAEs were not recovered or resolved in a total of six patients (mitapivat: three patients, placebo: three patients).

In Study 007, the incidence of insomnia was similar to that in Study 006 (22.2%). All events were Grade 1 or 2 (moderate difficulty falling asleep, staying asleep or waking up early), none were considered serious, and no dose modifications were conducted due to the TEAE. In all but one patient, the TEAE recovered or resolved with no change in the dose of the study treatment. In Studies 006 and 007, the incidence of insomnia was not associated with increasing doses of mitapivat.

Table 42. Insomnia TEAEs, Safety Analysis Set, Studies 006 and 007

Preferred Term	006 Mitapivat N=40 n (%)	006 Placebo N=39 n (%)	007 Mitapivat N=27 n (%)	Pooled Mitapivat N=67 n (%)
All	7 (17.5)	7 (17.9)	6 (22.2)	13 (19.4)
Middle insomnia	3 (7.5)	3 (7.7)	2 (7.4)	5 (7.5)
Insomnia	2 (5.0)	0	1 (3.7)	3 (4.5)
Initial insomnia	1 (2.5)	4 (10.3)	2 (7.4)	3 (4.5)
Terminal insomnia	1 (2.5)	0	1 (3.7)	2 (3.0)

Source: adae.xpt.
Incidences are based on the number of subjects, not the number of events. Although a patient may have had two or more clinical AEs, the patient is counted only once in a category. The same patient may appear in different categories.
Abbreviation: N, total number of subjects; n, number of subjects in each category; TEAE, treatment-emergent adverse event

The Applicant provided profile plots for patients who experienced insomnia in Studies 006 and 007 showing changes in hemoglobin and onset of insomnia. There was no general tendency or association between patients who experienced insomnia and hemoglobin changes.

In the dose-ranging Study 003, the incidence of insomnia was greater in the higher-dose regimen; among the 25 patients who received mitapivat 300 mg BID, 16 patients (64.0%) experienced insomnia; among the 27 patients who received mitapivat 50 mg BID, 5 patients (18.5%) experienced insomnia during the core period.

Although insomnia is a known side effect of aromatase inhibitors, the incidence of insomnia was similar in the mitapivat arm and the placebo arm at the doses of mitapivat proposed for marketing.

7.6.7.3. Acute Hemolysis and Need for Dose Taper

Study 003

Two patients (3.8%) in Study 003 experienced acute hemolysis after sudden withdrawal (without taper) of mitapivat during the 24-week core period due to concerns that hemoglobin would exceed the ULN. Both patients received an initial dose of 300 mg BID. One patient experienced serious Grade 3 acute hemolysis (transfusion or medical intervention indicated) and Grade 3 hemolytic anemia (hemoglobin <8.0 g/dL; transfusion indicated), which occurred shortly after onset of hemolysis; the other patient experienced Grade 2 hemolysis (evidence of hemolysis and ≥2 gm decrease in hemoglobin). Both patients experienced a rapid increase in hemoglobin during the first 3 weeks of treatment with mitapivat. Shortly after discontinuing mitapivat, acute hemolysis and anemia were observed. By contrast, patients who missed only a few doses of mitapivat later in their treatment course, or for whom the dose was tapered, did not experience events indicative of acute hemolysis.

Study 006

Based on previous clinical experience in Study 003, the Applicant implemented a dose taper of study treatment in Study 006 to address the risk of a rapid decrease in hemoglobin if there was a need to abruptly discontinue study drug. However, no patient with a hemoglobin response withdrew from the study; therefore, no analysis of acute hemolysis due to sudden study treatment discontinuation was performed. The Applicant, however, analyzed data from patients who tapered the dose at the end of treatment. In Study 006, a total of six patients who received treatment with mitapivat underwent a dose taper (five patients underwent a dose taper after Week 24 and 1 patient at Week 12). Three of the five patients who underwent the dose taper at Week 24 showed evidence of efficacy but did not meet the criteria for a responder during the study (either by an increase in hemoglobin or a decrease in other key markers of hemolysis). Follow-up data 28 days after completion of the dose taper indicated that the markers of hemolysis returned to baseline but did not indicate an acute hemolytic episode; mild hemolysis was seen in some cases based on the hemolytic markers. One patient who had dose taper at Week 12 was considered a hemoglobin responder. The mitapivat dose was decreased from 20 mg to 5 mg BID at Week 12. This patient showed signs of mild hemolysis in the period immediately after the dose decrease.

Study 007

In Study 007, no patients who were responding to treatment with mitapivat underwent a dose taper and no evidence of hemolysis was observed in nonresponding patients who underwent a dose taper to discontinue treatment. One patient (ID: (b) (6)) experienced nonserious Grade 2 hemolysis (evidence of hemolysis and ≥2 gm decrease in hemoglobin), which resolved within 2 days. This patient experienced hemolysis during treatment with mitapivat. The hemolysis did not occur following abrupt withdrawal of the study treatment, and more likely represents the underlying disease. This patient did not have increased hemoglobin in response to treatment with mitapivat, did not have any dose modifications of the study treatment, and did not experience a concomitant TEAE.

Additional Analyses Related to the Dose Taper

An information request was sent to the Applicant to provide the reasons for the dose taper for each patient (including the actual doses and period of each of the reduced doses the patient had been on) and subsequent dose changes (after the dose taper, if applicable) for each patient.

The Applicant responded that in Study 006, the eight subjects (mitapivat: six subjects, placebo: two subjects) who entered the Dose Taper Period completed the treatment period per protocol and dose tapers were initiated in accordance with the study protocol. In Study 007, of the nine subjects who entered the Dose Taper Period, four subjects completed the treatment period per protocol and five discontinued treatment due to the subject's withdrawal; all subjects initiated dose tapers in accordance with the study protocol.

Based on the data provided by the Applicant, the majority of patients (five of six patients in Study 006 and seven of nine patients in Study 007) who received treatment with mitapivat and underwent dose tapering had a dose taper of every other day for 7 days as part of the last step of the dose taper, which is more consistent with the "gradual dose taper regimen" in Study 007 and not consistent with the recommendation in Study 006 (and the proposed prescribing information to continue daily dosing for the last step of the dose taper) as summarized in Table 126. A request for information was sent to the Applicant to provide clarification.

The Applicant responded on August 18, 2021 that the seven subjects on Study 007 who underwent a dose taper with an every other day (QOD) regimen as the last step followed the taper regimen outlined in the protocol version that existed at that time. The recommended once-daily dose taper regimen, as shown in the table of the proposed prescribing information (Table 152), was not implemented in the Study 007 protocol before study completion. The single recommended dose taper, as shown in the table of the proposed prescribing information, was implemented in Study 006 as part of a protocol amendment (Protocol v. 4.0). Before this amendment was effective, subjects who underwent a dose taper followed the taper regimen outlined in the previous versions of the protocol.

- Three (Subjects (b) (6) and (b) (6)) of the five subjects in Study 006 who underwent a dose taper with a QOD regimen as the last step followed the taper regimen as outlined in the protocol version (i.e., v. 3.0) effective at the time of starting their taper.
- The other two subjects (Subjects (b) (6) and (b) (6)) received mitapivat in Study 006 under protocol v. 4.0. However, per physician decision, these subjects underwent a QOD regimen as the last step of their dose taper.

In both studies, all subjects who entered the Dose Taper Period either completed the treatment period per protocol or discontinued treatment due to the subject's withdrawal. Therefore, no dosing of mitapivat occurred after the end of the dose taper in either study.

On January 10, 2022, the Applicant provided additional information on the dose taper based on updated information for patients treated with mitapivat who underwent dose taper in Studies 006, 007, and the ongoing Study 011. Twenty-six patients underwent dose tapering (16had a dose taper regimen of every other day and 10 underwent the once daily dose as part of the last step of the dose taper at the update). The additional 11 patients (from Table 35) were those who continued in the Extension Study 011 and received treatment with mitapivat and underwent dose taper.

The Applicant also provided a summary of TEAEs with onset during the dose taper period for patients treated with mitapivat who underwent the once daily dose taper compared to patients treated with mitapivat who had a dose taper regimen of every other day as part of the last step of the dose taper regimen. Despite the small number of patients in each group, the overall incidence of TEAEs was higher in the every other day dose taper regimen compared to the once daily regimen (every other day taper regimen: 37.5%, once daily taper regimen: 20.0%). The majority of TEAEs reported during the dose taper period, however, do not appear likely to be caused by the dose taper.

Table 43. Summary of TEAEs During the Taper Period by Preferred Term in Patients Treated With Mitapivat Who Underwent Dose Taper

	Mitapivat	
Preferred Term	Once Daily Taper N=10 n (%)	Every Other Day Taper N=16 n (%)
All patients with events	2 (20.0)	6 (37.5)
Fatigue	0	2 (12.5)
ALT increased	0	1 (6.3)
Arthralgia	0	1 (6.3)
AST increased	0	1 (6.3)
Dyspnea	0	1 (6.3)
Influenza like illness	0	1 (6.3)
Oropharyngeal pain	0	1 (6.3)
Peripheral swelling	0	1 (6.3)
Syncope	0	1 (6.3)
Upper respiratory infection	0	1 (6.3)
Nasopharyngitis	1 (10.0)	0
Panic attack	1 (10.0)	0

Source: Applicant response to an IR.
Abbreviations: N, total number of subjects; n, number of subjects in each category; TEAE, treatment emergent adverse event

In Study 006 and 007, all patients who discontinued treatment underwent a dose taper except for one patient in Study 007 who was lost to follow-up. Therefore, it is not possible to conclusively show that the taper reduces the risk of hemolysis compared to stopping mitapivat abruptly. In addition, the two patients in Study 003 who experienced acute hemolysis after sudden withdrawal (without taper) of mitapivat were receiving a dose of 300 mg BID (which is six times the maximum recommended dose to be approved). Nonetheless, it is plausible that having an increase in red blood cell mass with mitapivat would provide a larger pool of red blood cells that would suddenly become susceptible to hemolysis when mitapivat is discontinued, predisposing

to considerable hemolysis with abrupt discontinuation. Therefore, the Applicant's proposed once daily dose taper regimen appears to be acceptable. The Applicant has proposed inclusion of a Warning and Precaution to communicate the risk for acute hemolysis with mitapivat. The clinical team agrees that providers should be aware of this possible risk.

7.6.7.4. Effects of Aromatase Inhibition

Aromatase is an enzyme of the cytochrome P450 (CYP) family and the product of the *CYP19* gene and is the primary enzyme for the synthesis of estrogen in postmenopausal women. Aromatase inhibitors decrease estrogen production by blocking aromatase and decrease peripheral conversion of testosterone to estradiol and of androstenedione to estrone.

Based on in vitro data that indicated mitapivat to be a weak aromatase inhibitor, the Applicant monitored sex hormone laboratory abnormalities, DXA scans to monitor bone density, TEAEs of endocrinological interest, and menstrual cycles (by diaries) during the study.

Sex Hormones

In Studies 006 and 007, in male patients treated with mitapivat 50 mg BID, testosterone levels generally increased in the first 4 to 8 weeks of treatment and remained fairly constant at the increased level throughout treatment; by contrast, estrone and estradiol levels decreased gradually over the course of treatment. The testosterone, estrone, and estradiol levels mostly remained within the normal ranges.

Male patients who were treated with placebo did not show consistent changes in testosterone, estrone, and estradiol levels. Table 44 lists the incidence of testosterone increase (to above normal levels); and of estrone and estradiol decreases (to below normal levels) in Studies 006 and 007.

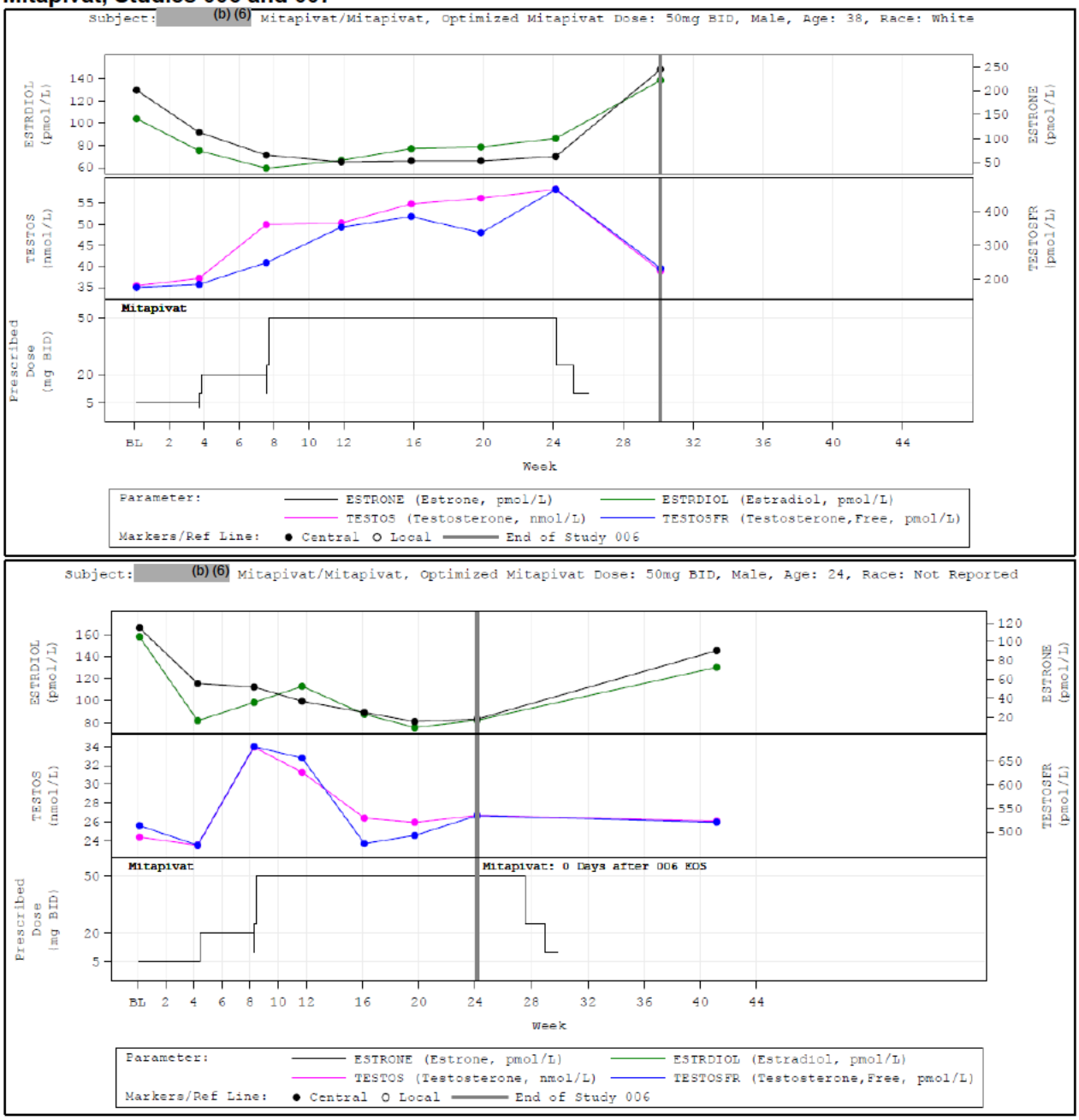
Table 44. Reproductive Hormone Analyses in Males, Safety Analysis Set, Studies 006 and 007*

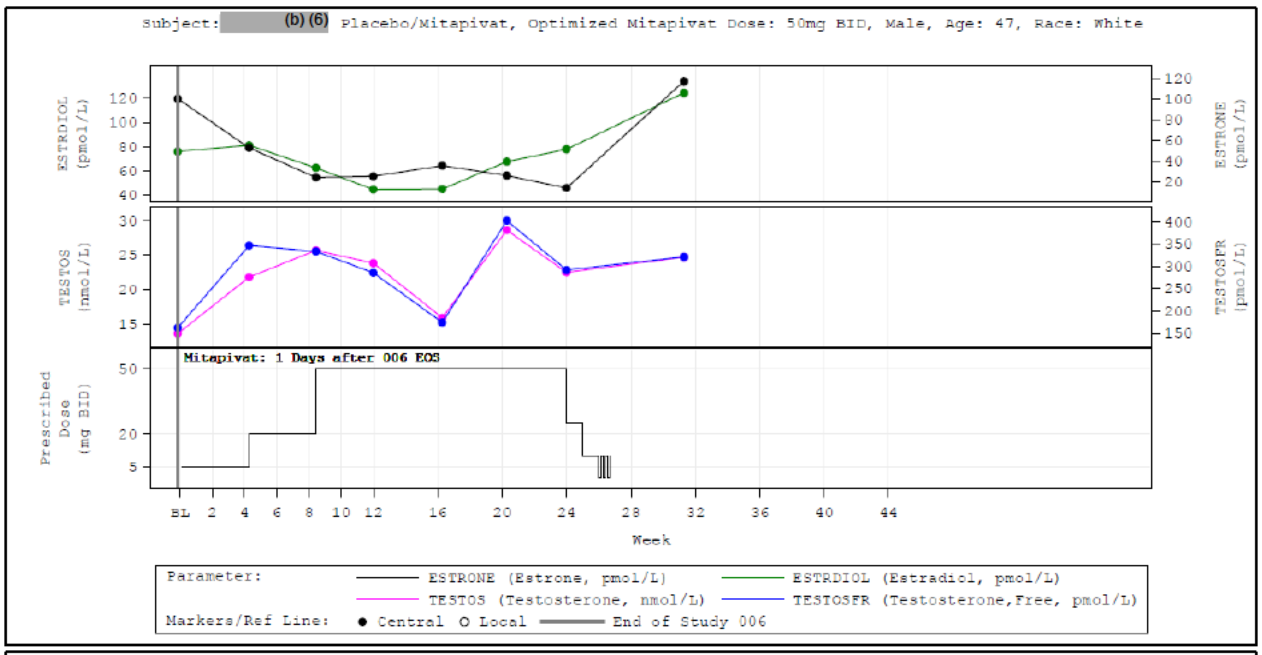
Laboratory Parameter	006 Mitapivat N=16 n (%)	006 Placebo N=15 n (%)	007 Mitapivat N=7 n (%)	Pooled Mitapivat N=23 n (%)
Estrone decreased	9 (56.3)	0	1 (14.3)	10 (43.5)
Estradiol decreased	2 (12.5)	1 (6.7)	0	2 (8.7)
Blood testosterone increased	1 (6.3)	1 (6.7)	0	1 (4.3)

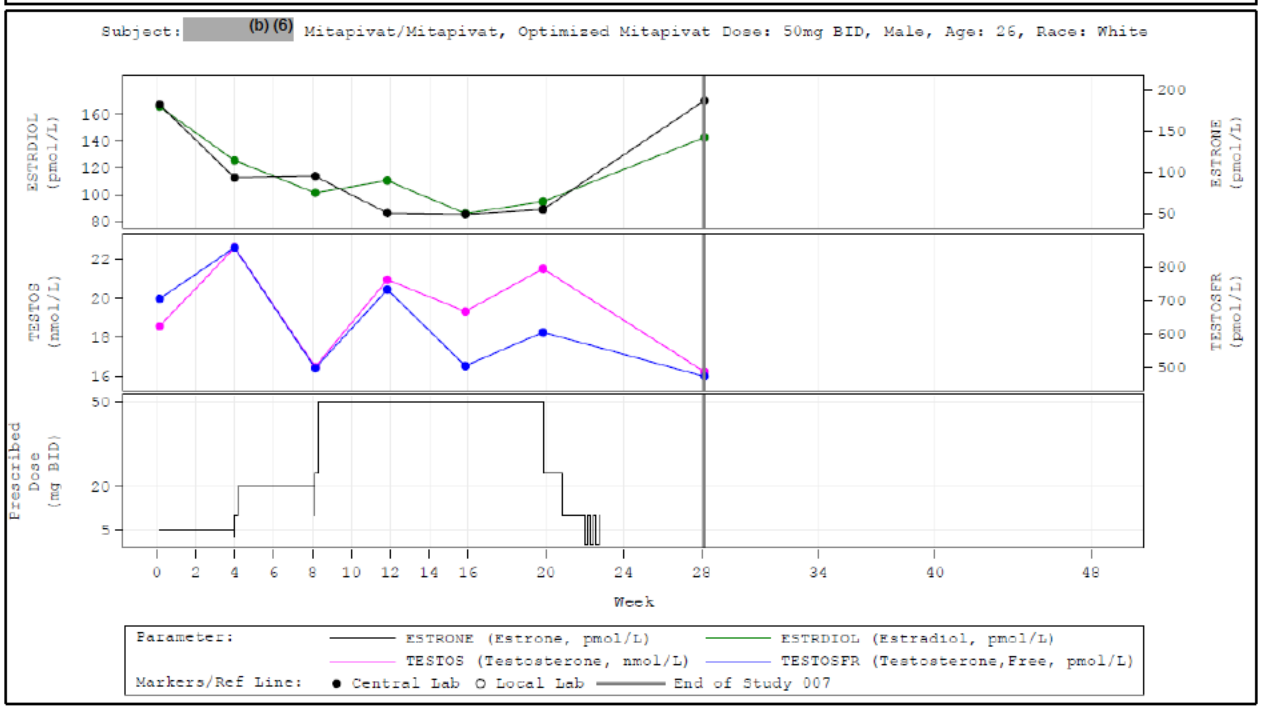
Source: ad b.xpt.
* Decreases in estrone and estradiol represent below the LLN and increases in testosterone represent above the ULN where baseline was within normal limits.
Abbreviations: N, total number of subjects; n, number of subjects in each category;

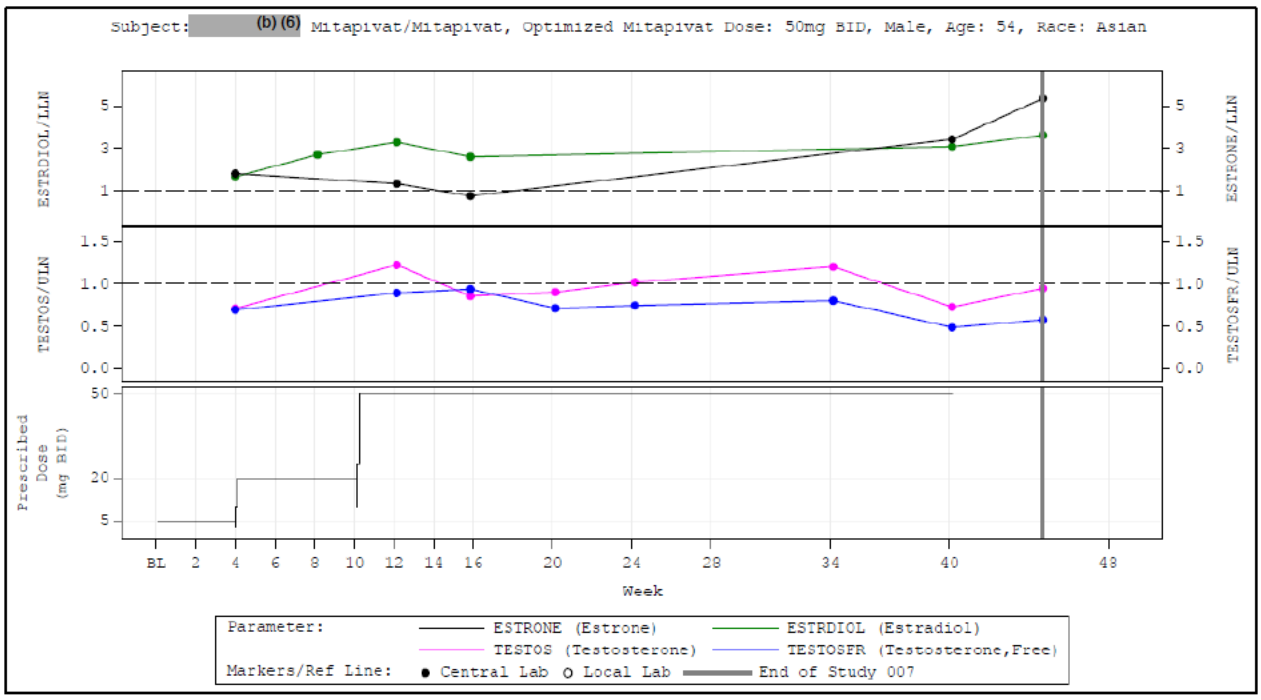
Per the Applicant, hormonal changes observed in male subjects during treatment with mitapivat were reversible upon study drug discontinuation. An information request was sent to the Applicant to provide the information supporting reversibility. The Applicant provided plots of sex hormone parameters of male patients in Studies 006 and 007 who discontinued mitapivat therapy (and have data through at least 28 days after the last dose) (Figure 8). The data show that the hormonal changes were generally reversible upon study treatment discontinuation.

Figure 8. Plots of Hormonal Parameters of Male Patients Who Discontinued Treatment With Mitapivat, Studies 006 and 007




Subject: (b) (6) Placebo/Mitapivat, Optimized Mitapivat Dose: 50mg BID, Male, Age: 47, Race: White
ESTRDIOL (pmol/L)
ESTRONE (pmol/L)
TESTOS (nmol/L)
TESTOSFR (pmol/L)
Prescribed Dose (mg BID)
Mitapivat: 1 Days after 006 EOS
BL 2 4 6 8 10 12 16 20 24 28 32 36 40 44
Week
Parameter:
ESTRONE (Estrone, pmol/L)
ESTRDIOL (Estradiol, pmol/L)
TESTOS (Testosterone, nmol/L)
TESTOSFR (Testosterone,Free, pmol/L)
Markers/Ref Line:
Central
Local
End of Study 006


Subject: (b) (6) Mitapivat/Mitapivat, Optimized Mitapivat Dose: 50mg BID, Male, Age: 26, Race: White
ESTRDIOL (pmol/L)
ESTRONE (pmol/L)
TESTOS (nmol/L)
TESTOSFR (pmol/L)
Prescribed Dose (mg BID)
0 2 4 6 8 10 12 14 16 20 24 28 34 40 48
Week
Parameter:
ESTRONE (Estrone, pmol/L)
ESTRDIOL (Estradiol, pmol/L)
TESTOS (Testosterone, nmol/L)
TESTOSFR (Testosterone,Free, pmol/L)
Markers/Ref Line:
Central Lab
Local Lab
End of Study 007



Source: Applicant's response to an Information Request.
Abbreviations: BID, twice daily; LLN, lower limit of normal; ULN, upper limit of normal

The Applicant was unable to analyze sex hormones in female patients due to physiologic variations in hormone levels expected throughout the normal menstrual cycle and the various types of hormonal contraceptives used by patients. In addition, due to the small number of postmenopausal women in the study (n=8 total), the Applicant was unable to fully interpretate the aromatase inhibitor effect of mitapivat in this subgroup.

Adverse events observed in male patients were generally consistent with those in female patients (Table 140 and Table 141). There were no differences in trends in TEAEs between female and male patients who could be affected by hormonal changes. TEAEs such as anxiety and depression that could be associated with hormonal changes were rare.

The Applicant proposed to include a discussion of variations in reproductive hormones in Section 6 of the labeling. The clinical review team agreed with this proposal. Labeling will include a tabular presentation of the abnormalities in reproductive hormones observed in Study 006. The changes in reproductive hormones returned to normal or close to baseline within 28 days after discontinuing mitapivat in males.

Dual-Energy X-Ray Absorptiometry Scans

In Studies 006 and 007, no clinically meaningful changes in BMD were observed from baseline to Week 24 (Study 006) or Part 2 Week 24 (Study 007), respectively. Most patients remained in the same baseline T-score category during the study. Consistent results were obtained in Studies 003 and 011. Table 45 summarizes the DXA score changes from baseline to Week 24 or Part 2 Week 24 in Studies 006 and 007.

Table 45. DXA Score Changes From Baseline to Week 24, Study 006, or to Week 24 of Part 2, Safety Analysis Set, Study 007

DXA Score	006 Mitapivat N=40 n (%)	006 Placebo N=39 n (%)	007 Mitapivat N=27 n (%)
Femoral total, n	33	31	20
BMD (g/cm^2)			
Median	-0.008	-0.007	0.007
Range	-0.050, 0.60	-0.036, 0.040	-0.054, 0.032
T-Score			
Median	-0.060	-0.060	0.050
Range	-0.40, 0.47	-0.30, 0.28	-0.36, 0.26
Adjusted spine, n	32	31	20
BMD (g/cm^2)			
Median	0.003	0.002	-0.007
Range	-0.068, 0.079	-0.088, 0.057	-0.029, 0.049
T-Score			
Median	0.025	0.020	-0.065
Range	-0.57, 0.66	-0.73, 0.52	-0.24, 0.44

Source: addxa.xpt.
Abbreviation: BMD, bone mineral density, DXA, dual-energy x-ray absorptiometry; N, total number of subjects; n, number of subjects in each category

Patients with PKD have early-onset osteoporosis due to underlying disease. The addition of a drug that may also cause osteoporosis or osteopenia warrants additional monitoring and information for providers. The evaluation of bone density over 24 weeks may not be sufficient to detect a significant change in BMD. Because mitapivat will likely be a chronic medication, we recommend that patients undergo a bone density scan at baseline then at least every 2 years. It is possible that a change in bone density was not seen because of the 24-week assessment period but at 48 weeks there may be a change.

Musculoskeletal Disorders

Aromatase inhibitors can induce or enhance musculoskeletal problems, including arthralgia and myalgia (Thorne 2007), which may be debilitating. In Study 006, the proportion of patients who experienced TEAEs in the Musculoskeletal and Connective Tissue Disorders SOC was higher in the mitapivat arm compared with the placebo arm (mitapivat: 35.0%, placebo: 25.6%). All TEAEs (except one serious Grade 3 TEAE of musculoskeletal pain that was reported in a patient in the mitapivat) were Grade 1 or 2 in severity; and none required treatment modification of the study treatment. The most frequently reported TEAEs (≥10%) in the mitapivat arm and at a higher incidence than with placebo were back pain and arthralgia.

In Study 007, a total of seven patients (25.9%) experienced TEAEs in the Musculoskeletal and Connective Tissue Disorders SOC. One patient experienced Grade 3 back pain and Grade 3 joint swelling that required dose reduction of mitapivat. All other TEAEs were considered nonserious and Grade 1 or 2 in severity.

Table 46. AEs in the Musculoskeletal and Connective Tissue Disorders SOC, Safety Analysis Set, Studies 006 and 007

Preferred Term	006 Mitapivat N=40 n (%)	006 Placebo N=39 n (%)	007 Mitapivat N=27 n (%)	Pooled Mitapivat N=67 n (%)
All	14 (35.0)	10 (25.6)	7 (25.9)	21 (31.3)
Back pain[1]	6 (15.0)	3 (7.7)	4 (14.8)	10 (14.9)
Arthralgia[2]	4 (10.0)	2 (5.1)	2 (7.4)	6 (9.0)
Osteopenia	1 (2.5)	0	1 (3.7)	2 (3.0)
Pain in extremity	2 (5.0)	3 (7.7)	0	2 (3.0)
Musculoskeletal pain[3]	2 (5.0)	3 (7.7)	0	2 (3.0)
Tendonitis	1 (2.5)	0	0	1 (1.5)
Neck pain	0	2 (5.1)	1 (3.7)	1 (1.5)
Osteoporosis	0	1 (2.6)	1 (3.7)	1 (1.5)
Muscular weakness	0	0	1 (3.7)	1 (1.5)
Groin pain	0	1 (2.6)	0	0

Source: adae.xpt.
[1] Back pain includes back pain and flank pain.
[2] Arthralgia includes arthralgia and joint swelling.
[3] Musculoskeletal pain includes musculoskeletal pain, muscle spasm, musculoskeletal chest pain and myalgia.
Incidences are based on the number of subjects, not the number of events. Although a patient may have had two or more clinical AEs, the patient is counted only once in a category. The same patient may appear in different categories.
Abbreviations: AE, adverse event; N, total number of subjects; n, number of subjects in each category; SOC, system organ class

Musculoskeletal disorders to include arthralgia and back pain are included in common adverse reactions in labeling for mitapivat.

Fractures

In Study 006, one patient (ID: (b) (6)) in the mitapivat arm who had a history of osteoporosis experienced a serious adverse event (SAE) of Grade 3 rib fracture (displaced or open wound with bone exposure; disabling; operative intervention indicated); and there were no fractures in the placebo arm.

In Study 007, one patient in Study 007 (ID: (b) (6)) who continued in Study 011 (Cohort 3) with a history of osteoporosis had a nonserious TEAE of Grade 2 thoracic vertebral fracture (symptomatic but nondisplaced; immobilization indicated).

In addition, two patients who received treatment with mitapivat in Study 006 and continued in Study 011 (Cohort 2) experienced fractures: one patient (ID: (b) (6)) had a serious Grade 3 tibia fracture and serious Grade 3 femur fracture on Study Day 137, and one patient ((b) (6) had a nonserious Grade 1 upper limb fracture (asymptomatic; clinical or diagnostic observations only; intervention not indicated) on Study Day 566.

There were no reported events of falls in the mitapivat clinical database. The patient narratives for the above cases are presented in Section 17.3.

Complications of PK deficiency include weakened bones and increased risk of bone fractures. According to Moreau et al. 2012, a low bone mass density and fractures are frequent in patients with hemolytic hemoglobinopathies (Moreau et al. 2012). However, the etiology is unknown. It is hypothesized that excessive hematopoiesis provokes bone marrow hyperplasia and reduces the amount of bone generated, causing bone distortion/fragility and enhancing bone resorption. It has been suggested that hemolysis exerts effects distinct from bleeding in the marrow and bone and may contribute to osteoporosis via a mechanism independent of erythropoietic stress. However,

it is not certain that the cases of fractures reported in patients treated with mitapivat in clinical studies are due to the disease or because the study treatment contributed to the fractures. Aromatase inhibitors are associated with accelerated bone loss and an increased risk of osteoporotic fractures. It cannot be excluded that the study treatment also contributed to events of fractures or worsened existing osteoporosis or osteopenia. Risk of fractures should be included in the Warnings and Precautions section of the labeling.

Adverse Events of Endocrinological Interest

TEAEs of endocrinological interest were identified by the Applicant based on the criteria outlined in the mitapivat program.

In Study 006, the overall incidence of TEAEs of endocrinological interest was higher in the mitapivat arm compared with the placebo arm (mitapivat: 25.0%, placebo: 15.4%). Adverse events that occurred in more than one patient in the mitapivat arm and at a higher incidence than with placebo were arthralgia, hot flush, and breast discomfort. All events were nonserious and Grade 1 or 2 in severity; none caused changes in dose modification.

In Study 007, a total of five patients (18.5%) had TEAEs of endocrinological interest. None was serious, greater than Grade 2 in severity, or required dose modification of the study treatment.

All these adverse events were reported in women expect for an adverse event of arthralgia and erectile dysfunction.

Table 47. Adverse Events of Endocrinologic Interest, Safety Analysis Set, Studies 006 and 007

Preferred Term	006 Mitapivat N=40 n (%)	006 Placebo N=39 n (%)	007 Mitapivat N=27 n (%)	Pooled Mitapivat N=67 n (%)
All	10 (25.0)	6 (15.4)	5 (18.5)	14 (20.9)
Arthralgia	4 (10.0)	2 (5.1)	1 (3.7)	5 (7.5)
Hot flush	3 (7.5)	0	1 (3.7)	4 (6.0)
Breast discomfort	2 (5.0)	0	0	2 (3.0)
Dysmenorrhea	1 (2.5)	3 (7.7)	1 (3.7)	2 (3.0)
Erectile dysfunction	1 (2.5)	0	0	1 (1.5)
Premenstrual pain	1 (2.5)	0	0	1 (1.5)
Vulvovaginal discomfort	1 (2.5)	0	0	1 (1.5)
Night sweats	0	1 (2.6)	0	0
Acne	0	0	1 (3.7)	1 (1.5)
Menstrual irregular	0	0	1 (3.7)	1 (1.5)

Source: adae.xpt.
Incidences are based on the number of subjects, not the number of events. Although a patient may have had two or more clinical AEs, the patient is counted only once in a category. The same patient may appear in different categories.
Abbreviations: N, total number of subjects; n, number of subjects in each category

Menstrual Cycle Diary

Menstrual diaries were collected from menstruating women. In Study 006, no significant differences were observed between the mitapivat and placebo arms in menstrual cycle diary data. In Study 007, no trends were observed in menstrual cycle diary data, which were generally consistent with those in the mitapivat arm of Study 006. No increase in the incidence of TEAEs in female subjects was observed. See Table 140 and Table 141.

7.6.7.5. Gastrointestinal Events

In Study 006, the overall incidence of gastrointestinal (GI) events was higher in the placebo arm (55.6%) compared to the mitapivat arm (37.5%). All events (except one patient in the placebo arm who had a Grade 3 obstructive pancreatitis and required study treatment interruption) were Grade 1 or 2 in severity and did not require dose modification of the study treatment.

In Study 007, one patient had a Grade 3 diarrhea (increase of ≥7 stools per day over baseline; incontinence; hospitalization indicated; limiting self-care ADL). All other events were Grade 1 or 2 in severity. There were no events that led to treatment modification of the study treatment. Table 48 summarizes the GI events that occurred in more than one patient in the mitapivat arms in Studies 006 and 007.

All reported GI AE preferred terms occurred at a similar or higher incidence than placebo in Study 006, except for constipation and dry mouth (Table 48).

Table 48. Gastrointestinal Events That Occurred in More Than One Patient in the Mitapivat Arm in Any Study, Safety Analysis Set, Studies 006 and 007

Preferred Term	006 Mitapivat N=40 n (%)	006 Placebo N=39 n (%)	007 Mitapivat N=27 n (%)	Pooled Mitapivat N=67 n (%)
All	15 (37.5)	20 (51.3)	15 (55.6)	30 (44.8)
Nausea	7 (17.5)	9 (23.1)	5 (18.5)	12 (17.9)
Abdominal pain[1]	5 (12.5)	7 (17.9)	4 (14.8)	9 (13.4)
Diarrhea	4 (10.0)	7 (17.9)	3 (11.1)	7 (10.4)
Gastritis[2]	2 (5.0)	5 (18.5)	4 (14.8)	6 (9.0)
Vomiting[3]	1 (2.5)	1 (2.6)	5 (18.5)	6 (9.0)
Constipation	2 (5.0)	0	1 (3.7)	3 (4.5)
Dry mouth	2 (5.0)	0	1 (3.7)	3 (4.5)

Source: adae.xpt.
Incidences are based on the number of subjects, not the number of events. Although a patient may have had two or more clinical adverse events, the patient is counted only once in a category. The same patient may appear in different categories.
[1] Abdominal pain includes abdominal pain upper/lower and discomfort/distension.
[2] Gastritis includes dyspepsia, gastric ulcer, gastroesophageal reflux disease, abdominal pain upper and gastrointestinal mucosal disorder.
[3] Vomiting includes hematemesis.
Abbreviations: N, total number of subjects; n, number of subjects in each category

7.6.7.6. Hypertriglyceridemia

In Studies 006 and 007, TEAEs of hypertriglyceridemia were underreported. Based on the laboratory abnormalities that occurred or worsened postbaseline (Table 50), the overall incidence of “triglycerides high” at any grade was higher in the placebo arm compared with the mitapivat arm (mitapivat: 40%, placebo: 54%). However, mitapivat is a weak aromatase inhibitor and aromatase inhibitors have effects on lipids (Wang et al. 2020). The median duration of mitapivat exposure in the placebo-controlled Study 006 was 24.1 weeks (range: 23.6, 27.4 weeks). The study duration may not have been sufficient to allow adequate evaluation of effects on lipids, considering that mitapivat treatment may be life-long.

In Study 006, all events except the TEAE that occurred in the patient in the placebo arm resolved. No patient required treatment modification of the study treatment.

In Study 007, all elevations were reported as transient; all events were resolved and none required dose changes of the study treatment.

Hypertriglyceridemia events were transient in nature in most patients. Many of the subjects who experienced Grade 3 and 4 events had confounding factors such as being overweight or obese, and some subjects had slight or moderate weight gain during the study. These subjects did not experience hypertension or other cardiovascular events in association with hypertriglyceridemia events. No safety trends were observed with cholesterol, low-density lipoprotein, high-density lipoprotein, or hypertriglyceridemia.

Hypertriglyceridemia is included in the Adverse Reactions Section 6.1 of the labeling for mitapivat. Hypertriglyceridemia and elevated cholesterol levels are reported with aromatase inhibitors. Although mitapivat is a weaker aromatase inhibitor, the drug may be given life-long. Monitoring of serum lipids will be an important consideration for providers and described in labeling.

7.6.7.7. Hypersensitivity

Per the Applicant, TEAEs of hypersensitivity (e.g., rash pustular, rhinitis allergic, swelling face in Study 003, eczema, rash macular, rash, dermatitis acneiform, and periorbital oedema) were observed in patients treated with mitapivat in the clinical studies. No case of anaphylaxis was reported in patients treated with mitapivat.

In Study 006, the incidence of TEAEs indicative of potential hypersensitivity was higher in the placebo arm (28.2%) compared with the mitapivat arm (12.5%). No TEAEs indicative of potential hypersensitivity were reported in more than one patient in the mitapivat arm. In Study 007, the incidence was 11.1%. Rash was the most common TEAE. All events reported in Studies 006 and 007 were Grade 1 or 2 in severity and none led to dose changes of the study treatment.

Table 49. Potential Hypersensitivity Reactions That Occurred in One or More Patient in the Mitapivat Arm in Any Study, Safety Analysis Set, Studies 006 and 007

Preferred Term	006 Mitapivat N=40 n (%)	006 Placebo N=39 n (%)	007 Mitapivat N=27 n (%)	Pooled Mitapivat N=67 n (%)
All	5 (12.5)	11 (28.2)	3 (11.1)	8 (11.9)
Skin and subcutaneous tissue disorders	5 (12.5)	8 (20.5)	3 (11.1)	8 (11.9)
Rash	0	3 (7.7)	3 (11.1)	3 (4.5)
Eczema	1 (2.5)	1 (2.6)	0	1 (1.5)
Erythema	1 (2.5)	0	0	1 (1.5)
Pruritis	1 (2.5)	0	0	1 (1.5)
Rash macular	1 (2.5)	0	0	1 (1.5)
Skin ulcer	1 (2.5)	0	0	1 (1.5)
Respiratory, thoracic, and mediastinal disorders	1 (2.5)	4 (10.3)	1 (3.7)	2 (3.0)
Eye disorders	1 (2.5)	0	0	1 (1.5)

Source: adae.xpt.
Incidences are based on the number of subjects, not the number of events. Although a patient may have had two or more clinical adverse events, the patient is counted only once in a category. The same patient may appear in different categories.
Abbreviations: N, total number of subjects; n, number of subjects in each category

7.6.8. Laboratory Findings, Studies 006 and 007

Study 006

Table 50 summarizes the incidences of newly occurring or worsening laboratory abnormalities postbaseline in Study 006. The shifts in hematology parameters were generally similar or greater in the placebo arm compared to the mitapivat arm. Grade 3 lymphopenia and neutropenia were each reported in one patient in the mitapivat arm. Both events resolved and no action was taken with the study treatment. The patient with the Grade 3 lymphopenia had low lymphocyte counts at screening, through Week 8, and then intermittently through Week 24. The patient with Grade 3 neutropenia had low neutrophil counts at Day 1 and intermittently through Week 10.

With regard to chemistry, based on the laboratory parameters reported for ALT high or AST high, the incidences of ALT high of any grade (mitapivat: 5.0%, placebo: 25.6%) and AST high of any grade (mitapivat: 10.0%, placebo: 17.9%) were higher in the placebo arm compared to the mitapivat arm.

Chemistry laboratory parameters that occurred in ≥5% in the mitapivat arm (with ≥5% higher incidence in the mitapivat arm compared to the placebo arm) of any grade included cholesterol high (mitapivat: 10.0%, placebo: 2.6%), potassium high (mitapivat: 7.5%, placebo: 0%), and urate high (mitapivat arm: 15.0%, placebo: 7.7%). All incidences of urate high were Grade 3 or 4 in severity. However, no TEAEs were reported due to a high urate level in Study 006.

Three patients (7.5%) experienced potassium high (range, 5.4 to 5.7 mmol/L) in the mitapivat arm. The narratives for these patients were not provided. TEAEs reported in these patients included vomiting, diarrhea, dizziness, syncope, and muscle spasm. No patient had potassium high in the placebo arm.

Table 50. Newly Occurring or Worsening Laboratory Abnormalities Postbaseline, Safety Analysis Set, Study 006

	Mitapivat N=40		Placebo N=39	
Laboratory Parameter	Any Grade n/N1 (%)	Grade 3-4 n/N1 (%)	Any Grade n/N1 (%)	Grade 3-4 n/N1 (%)
Hematology tests				
Hemoglobin high	0/40	0/40	0/39	0/39
Hemoglobin low	5/40 (12.5)	4/40 (10.0)	8/39 (20.5)	5/39 (12.8)
Leukocytes high	0/40	0/40	0/39	0/39
Leukocytes low	3/40 (7.5)	1/40 (2.5)	5/39 (12.8)	0/39
Lymphocytes high	14/40 (35.0)	0/40	15/39 (38.5)	0/39
Lymphocytes low	7/40 (17.5)	1/40 (2.5)	6/39 (15.4)	0/39
Neutrophils low	8/40 (20.0)	1/40 (2.5)	8/39 (20.5)	1/39 (2.6)
Platelets low	2/40 (5.0)	0/40	5/39 (12.8)	1/39 (2.6)
Chemistry tests				
ALT high	2/40 (5.0)	0/40	10/39 (25.6)	0/39
Albumin low	0/40	0/40	0/39	0/39
ALP high	1/40 (2.5)	0/40	1/39 (2.6)	0/39
AST high	4/40 (10.0)	0/40	7/39 (17.9)	1/39 (2.6)
Bilirubin high	1/40 (2.5)	0/40	7/39 (17.9)	5/39 (12.8)
Calcium high	0/40	0/40	0/39	0/39
Calcium low	0/40	0/40	2/39 (5.1)	0/39
Cholesterol high	4/40 (10.0)	0/40	1/39 (2.6)	0/39
Creatinine high	2/40 (5.0)	0/40	1/39 (2.6)	0/39

	Mitapivat N=40		Placebo N=39	
Laboratory Parameter	**Any Grade n/N1 (%)**	**Grade 3-4 n/N1 (%)**	**Any Grade n/N1 (%)**	**Grade 3-4 n/N1 (%)**
Glucose high	8/40 (20.0)	1/40 (2.5)	6/39 (15.4)	1/39 (2.6)
Glucose low	1/40 (2.5)	0/40	2/39 (5.1)	0/39
Haptoglobin low	2/40 (5.0)	0/40	1/39 (2.6)	0/39
Magnesium high	0/40	0/40	1/39 (2.6)	1/39 (2.6)
Magnesium low	0/40	0/40	0/39	0/39
Phosphate low	1/40 (2.5)	0/40	0/39	0/39
Potassium high	3/40 (7.5)	0/40	0/39	0/39
Potassium low	1/40 (2.5)	0/40	0/39	0/39
Sodium high	0/40	0/40	0/39	0/39
Sodium low	2/40 (5.0)	0/40	2/39 (5.1)	0/39
Triglycerides high	16/40 (40.0)	2/40 (5.0)	21/39 (53.8)	2/39 (5.1)
Urate high	6/40 (15.0)	6/40 (15.0)	3/39 (7.7)	3/39 (7.7)
Coagulation tests				
aPTT high	5/40 (12.5)	0/40	3/39 (7.7)	0/39
PT intl normalized ratio	5/40 (12.5)	0/40	11/39 (28.2)	0/39

Source: ad b.xpt, adapted from Study 006, Clinical Study Report.
Abbreviations: intl, international; N, total number of subjects; N1, denominator used to calculate percentages; n, number of subjects in each category

Study 007

In Study 007, the shifts in hematology parameters were generally consistent with those of the mitapivat arm in Study 006, except for hemoglobin low, which was observed more frequently in Study 007, consistent with the patient population in Study 007, which had more severe disease.

The shifts in chemistry parameters were also generally consistent with the results in the mitapivat arm in Study 006, except for ALT high and AST high, which occurred more frequently in Study 007, possibly due to the regular transfusions received by this patient population.

In Study 007, the incidence of high urate was 22.2% (six patients): a total of four patients had experienced increase from Grade 0 at baseline to Grade 3 during the study. However, no urate-related TEAEs were reported, and no treatment was administered to the four patients. In addition, two patients had an increase in urate from Grade 3 at baseline to Grade 4 during the study; of the two patients, one experienced a Grade 1 TEAE of hyperuricemia that was treated with allopurinol (confounding factors included a medical history of nephropathy and aspirin). The other patient experienced a nonserious TEAE of Grade 1 nephrolithiasis that was considered not related to study treatment, and no treatment was given. Another patient with a medical history of renal calculi experienced a Grade 2 SAE of renal colic that was considered not related to the study treatment; this SAE was treated with fluids, antibiotics, and pain medications. The urate levels were within the normal ranges for these two patients.

In Study 007, two patients (7.4%) developed potassium high (range: 5.3 to 6.6 mmol/L). TEAEs reported in these patients included diarrhea, nausea, and syncope.

Interpretation of the results of Study 007 is limited by its single-arm (there was no comparator arm) design.

Table 51. Newly Occurring or Worsening Laboratory Abnormalities Postbaseline, Safety Analysis Set, Study 007

	Mitapivat N=27	
Laboratory Parameter	**Any Grade n/N1 (%)**	**Grade 3-4 n/N1 (%)**
Hematology tests		
Hemoglobin high	0/27	0/27
Hemoglobin low	19/27 (70.4)	11/27 (40.7)
Leukocytes high	0/27	0/27
Leukocytes low	4/27 (14.8)	1/27 (3.7)
Lymphocytes high	10/27 (37.0)	0/27
Lymphocytes low	5/27 (18.5)	0/27
Neutrophils low	6/27 (22.2)	3/27 (11.1)
Platelets low	2/27 (7.4)	0/27
Chemistry tests		
ALT high	10/27 (37.0)	0/27
Albumin low	0/27	0/27
ALP high	3/27 (11.1)	0/27
AST high	12/27 (44.4)	1/27 (3.7)
Bilirubin high	2/27 (7.4)	2/27 (7.4)
Calcium high	1/27 (3.7)	0/27
Calcium low	1/27 (3.7)	0/27
Cholesterol high	3/27 (11.1)	0/27
Creatinine high	3/27 (11.1)	0/27
Glucose high	5/27 (18.5)	0/27
Glucose low	3/27 (11.1)	0/27
Haptoglobin low	5/27 (18.5)	0/27
Magnesium high	3/27 (11.1)	0/27
Magnesium low	0/27	0/27
Phosphate low	0/27	0/27
Potassium high	2/27 (7.4)	1/27 (3.7)
Potassium low	1/27 (3.7)	0/27
Sodium high	0/27	0/27
Sodium low	4/27 (14.8)	0/27
Triglycerides high	13/27 (48.1)	3/27 (11.1)
Urate high	6/27 (22.2)	6/27 (22.2)
Coagulation tests		
aPTT high	5/27 (18.5)	0/27
PT intl. normalized ratio	9/27 (33.3)	0/27

Source: ad b.xpt, adapted from Study 007, Clinical Study Report.
Abbreviations: aPTT, partial thromboplastin time; N, total number of subjects; N1, The denominator used to calculate percentages; n, number of subjects in each category; PT, prothrombin time

For laboratory results related to liver function test, changes in sex hormones and hypertriglyceridemia, refer to Section 7.6.7.

7.6.9. Study 011

For the safety results of Study 011, refer to Section III.17.4.

7.7. Key Review Issues Relevant to Evaluation of Risk

7.7.1. Long-Term Risk of Aromatase Inhibition With Chronic Administration

Issue and Background

Mitapivat is a weak aromatase inhibitor in vitro. Aromatase inhibitors decrease estrogen production and decrease conversion of testosterone to estradiol and of androstenedione to estrone. Adverse events with the use of aromatase inhibitors include risks of osteoporotic fractures, musculoskeletal pain, alterations in lipid metabolism, and insomnia. In addition, the long-term safety of aromatase inhibition in the proposed population is not well characterized.

Assessment

Abnormalities in Reproductive Hormones

The Applicant monitored laboratory abnormalities in reproductive hormones, DXA scans to evaluate bone density, TEAEs of endocrinological interest, and menstrual cycles (by diaries) during the study.

In male patients who were treated with mitapivat 50 mg BID, testosterone levels generally increased and then remained constant at the increased level throughout treatment; by contrast, estrone and estradiol levels decreased over the course of treatment. From available data in a small number of patients (through at least 28 days after the last dose), the hormonal changes observed in male subjects during treatment with mitapivat were generally reversible upon study drug discontinuation.

In Studies 006 and 007, no clinically meaningful changes in bone density were observed from baseline to Week 24 or Part 2 Week 24, respectively. The median changes from baseline were similar between the mitapivat and placebo arms in Study 006. Most patients remained within the same baseline T-score category during the study. However, the interval assessment time may be too short to detect changes in bone density.

The preliminary safety results from the extension study (011) and the extension period of the single arm study (003) were consistent with regard to the TEAEs caused by inhibition of aromatase. See Sections 17.4 and 17.5.

For more information regarding abnormalities in reproductive hormones observed in the mitapivat clinical trials, see Section 7.6.7.4.

Fractures

In the mitapivat studies, a total of five patients treated with mitapivat experienced fractures (Study 006: one patient, Study 007: one patient, Study 011: three patients, including one patient reported in the safety update). No placebo-treated patients in Study 006 reported a fracture. See Section 7.6.7.4 for more information.

Insomnia

The incidence of insomnia in the mitapivat dose-ranging Study 003 was greater in the higher-dose group >50 mg BID (64.0%) compared to the lower-dose group ≤50 mg BID (18.5%).

The incidences of insomnia in patients who received mitapivat in Studies 006 (17.5%) and 007 (22.2%) were consistent with the ≤50 mg BID group (18.5%) in Study 003. In Study 006, the incidence of insomnia in the placebo (17.9%) was similar. See Section 7.6.7.2. for details.

Conclusion

The long-term risks of chronic administration of a drug that causes aromatase inhibition in the proposed population are not well characterized. The paucity of long-term safety data does not preclude approval. Postmarketing requirements will further evaluate safety follow-up data and summaries including AEs from long-term aromatase inhibition from the extension Study 011 and the Extension Period of Study 003.

7.7.2. Understanding of the Dose Taper

Issue and Background

Most of the patients in Studies 006 (87.5%) and 007 (100%) received a mitapivat dose of 50 mg BID. Dose taper regimen(s) were recommended in Studies 006 and 007 for patients who discontinued or interrupted study treatment to avoid withdrawal hemolysis (Section 15.3). However, in Studies 006 and 007, the final version of the recommended dose taper regimen was not implemented in most of the patients who underwent dose taper because patients followed the taper regimen outlined in the previous protocol version (i.e., v. 3.0), which was effective at the time of starting their taper or followed the dose taper regimen in protocol v. 3.0 per physician decision in Study 006. In Study 007, the final version of the dose taper regimen was not implemented (Section 7.5).

Assessment

In Studies 006 and 007, all but one of the patients who received mitapivat and discontinued treatment underwent a dose taper. The median dose taper period in patients who had a dose taper was 3 weeks in both studies. Most of these patients (Study 006: five of six patients, Study 007: seven of nine patients) underwent a dose taper regimen of every other day for 7 days as part of the last step. For more information, see Section 7.5.

On January 10, 2022, the Applicant provided updated dose taper information for patients who received treatment with mitapivat and underwent dose taper in Studies 006, 007, and the ongoing Study 011. A total of 16 patients had a dose taper regimen of every other day and 10 patients underwent the once daily dose as part of the last step of the dose taper regimen at the update. Based on comparison of the incidence of TEAEs with onset during the dose taper period, the overall incidence of TEAEs was higher in the every other day dose taper regimen compared to the once daily regimen (every other day taper regimen: 37.5%, once daily taper regimen: 20.0%) with small numbers of patients in the two groups which makes any conclusions on estimates unstable.

Conclusion

The review team concluded that based on the updated analysis and the results of the dose taper and TEAEs, the once daily dose taper regimen appears acceptable and should be included in the label to avoid or ameliorate withdrawal hemolysis.

8. Therapeutic Individualization

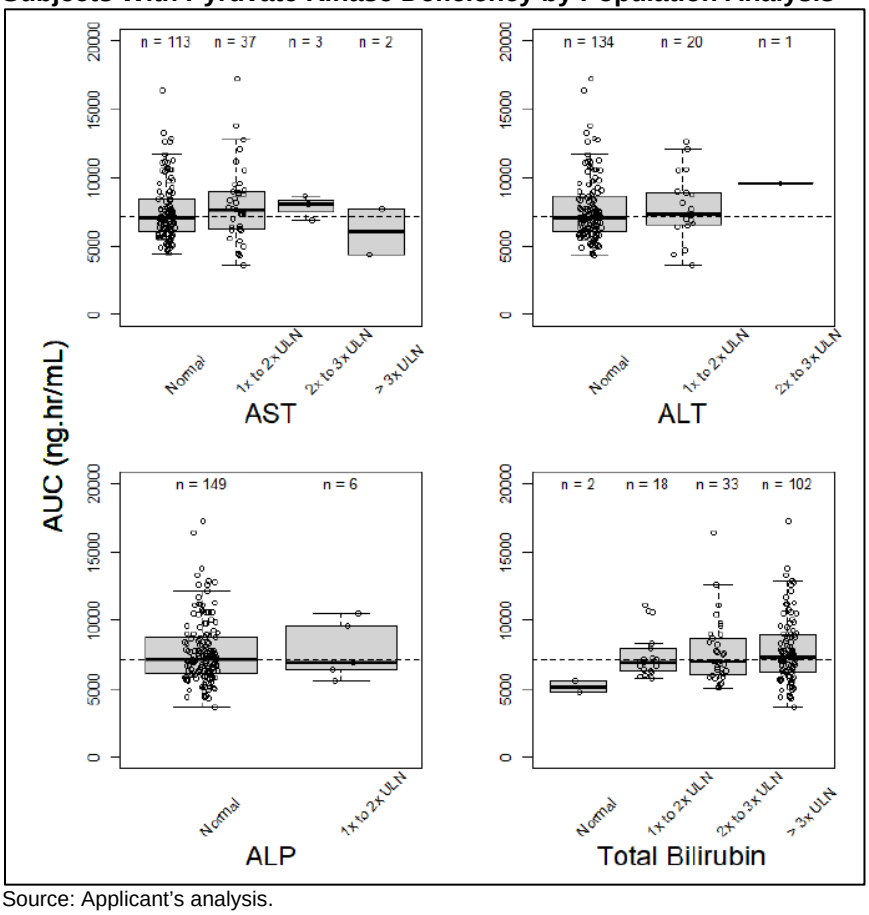
8.1. Intrinsic Factors

Hepatic Impairment

A dedicated hepatic impairment (HI) study was not conducted. The Applicant submitted a population pharmacokinetics analysis that categorized patients according to AST, ALT, alkaline phosphatase, and total bilirubin levels.

When compared to patients with normal liver enzyme levels, there was no significant difference in mitapivat exposure in the group of patients with 1× to 2× ULN elevated levels of AST, ALT, or alkaline phosphatase (Figure 9). The numbers of patients enrolled with 2× to 3× ULN or above 3× ULN elevated levels of AST or ALT were insufficient to allow comparison with patients with normal liver enzyme levels. Patients with elevated total bilirubin levels (1× to 2× ULN, 2× to 3× ULN, above 3× ULN) showed higher mitapivat exposure in comparison to patients with a normal total bilirubin level (Figure 9). Note that an elevated bilirubin level is commonly observed in patients with PKD; indeed, it is a characteristic of the disease.

Figure 9. Effect of Liver Enzymes and Total Bilirubin on Steady-State Mitapivat Exposure in Subjects With Pyruvate Kinase Deficiency by Population Analysis



Source: Applicant's analysis.
Abbreviations: ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate aminotransferase; AUC, area under the concentration-time curve; ULN, upper limit of normal; n, number of subjects in each category

The reviewer reanalyzed the Applicant's data based on the National Cancer Institute criteria for HI classification. However, a major drawback of using the National Cancer Institute criteria for HI classification in the case of mitapivat is the inability to determine whether the elevated bilirubin level is the result of HI or of the disease. The reviewer concluded that the submitted data to understand the impact of HI on mitapivat pharmacokinetics are inadequate.

Based on the results of the human absorption, distribution, metabolism, and excretion (ADME) Study AG-348-009, mitapivat has an oral bioavailability of 72.7% and undergoes extensive hepatic metabolism with 2.6% of the dose excreted unchanged in urine and <1% excreted unchanged in feces. Therefore, HI is expected to increase the systemic exposure of mitapivat. Patients with PKD can have concomitant HI during the course of their disease. Therefore, understanding the influence of HI based on the Child-Pugh classification on the pharmacokinetics of mitapivat is essential.

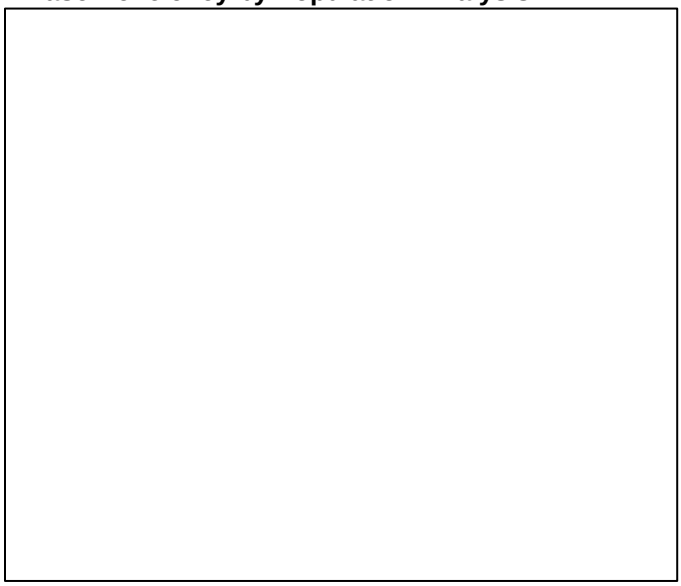
A postmarketing requirement is planned to mandate an HI study to characterize the pharmacokinetics of mitapivat in subjects with moderate (Child-Pugh B) and severe (Child-Pugh C) HI relative to those with normal hepatic function. Currently, the proposed labeling will recommend avoiding the use of mitapivat in patients with moderate or severe HI. During the Late-Cycle Meeting, the Applicant proposed to conduct a reduced design for HI study (normal versus moderate HI), given the difficulty of enrolling subjects with severe hepatic impairment.

On completion of this study, the Applicant will assess the necessity of evaluating subjects with mild (Child-Pugh A) and/or severe (Child-Pugh C) HI. The review team agrees with the plan to conduct a reduced design HI study.

Renal Impairment

A dedicated renal impairment (RI) study was not conducted. The effect of RI on the pharmacokinetics of mitapivat was assessed as part of the population pharmacokinetics analyses. The steady-state AUC was similar in patients with normal renal function (estimated glomerular filtration rate [eGFR] ≥90 mL/min/1.73 m^2) and mild RI (60 mL/min/1.73 m^2 ≤ eGFR <90 mL/min/1.73 m^2) (Figure 10). Four patients enrolled in the clinical trials had moderate RI (30 mL/min/1.73 m^2 ≤ eGFR <60 mL/min/1.73 m^2). Because of the small sample size, no meaningful conclusion can be derived on the exposure change in patients with moderate RI.

Figure 10. Effect of Renal Function on Steady-State Mitapivat Exposure in Patients With Pyruvate Kinase Deficiency by Population Analysis



APPEARS THIS WAY ON ORIGINAL

Source: Applicant's analysis.
Abbreviations: AUC, area under concentration-time curve; n, number of subjects in each category

Based on the results from the human ADME Study AG-348-009, mitapivat has an oral bioavailability of 72.7% but only 2.6% of the administered dose is excreted unchanged in urine, suggesting that renal elimination is not the major elimination pathway for mitapivat. The review team agrees that a dose adjustment is not needed for patients with mild or moderate RI. Mitapivat has not been studied in patients with severe RI. Because of the potential for uremic toxins to affect hepatic metabolism, the dose recommendation for severe RI should be reassessed when the results of the dedicated HI study are available.

PKLR Genotype

PKD is an autosomal recessive disorder caused by variants in the PKLR gene. The clinical variability observed in patients may be explained by the wide genetic heterogeneity of PKD and poor genotype-phenotype correlation. The most common variants are missense substitutions affecting residues critical for the structure and/or function of PK; less common are frameshift

and splicing variants and premature stop codons, which were categorized by the Applicant as nonmissense variants.

The results from Study 003 demonstrated that carriers of nonmissense/nonmissense variants do not respond to mitapivat therapy. In addition, a limited hemoglobin response was observed in carriers of p.R479H variants. Based on the results of Study 003, patients who were homozygous for the p.R479H variant or have two nonmissense variants, without the presence of another missense variant, in the PKLR gene were not included in Studies 006 and 007. For additional details on therapeutic individualization, refer to Section III.14.5.

Other Intrinsic Factors

The covariate effect analysis in population pharmacokinetics and pharmacodynamics models indicated that weight, age, race, and sex did not have a significant effect on the E-R relationship for mitapivat (see Section III.14.3). Hence, dose adjustment is not needed for these intrinsic factors.

Clinical studies of mitapivat did not include a sufficient number of patients aged ≥65 years to determine whether elderly patients respond differently from younger patients. Other reported clinical experience has not identified differences in the response to mitapivat between elderly and younger patients.

8.2. Drug Interactions

Metabolic Pathway

Mitapivat is predominantly metabolized by CYP3A4/5 in vitro. Drugs that are inhibitors or inducers of CYP3A have the potential to affect mitapivat exposures. The Applicant conducted clinical drug interaction studies to evaluate the impact of a strong CYP3A inhibitor and a strong CYP3A inducer on the pharmacokinetics of mitapivat. The effects of moderate CYP3A inhibitors and inducers were evaluated by physiologically based pharmacokinetic (PB-pharmacokinetic) modeling and simulations.

Effects of Other Drugs on Mitapivat

Effects of CYP3A Inhibitors on Mitapivat

Itraconazole, a strong CYP3A inhibitor, was dosed (200 mg once daily) to steady-state and its effect was evaluated on a single dose of 20 mg mitapivat. Itraconazole increased the AUC_{∞} and C_{max} of mitapivat by 4.9- and 1.7-fold, respectively. The effects of two strong CYP3A inhibitors, itraconazole and ketoconazole, and one moderate inhibitor, fluconazole, were also evaluated using PB-pharmacokinetic modeling and simulations.

PB-pharmacokinetic simulations showed that itraconazole increased the mitapivat AUC_{0-12} and C_{max} by 3.6- and 2.2-fold, respectively, following a mitapivat dosage of 50 mg twice daily. Ketoconazole, a strong CYP3A inhibitor, increased the mitapivat AUC_{0-12} and C_{max} by approximately 3.9- and 2.4-fold, respectively, following mitapivat doses of 5, 20, or 50 mg twice daily. Fluconazole, a moderate CYP3A inhibitor, increased the mitapivat AUC_{0-12} and C_{max} by approximately 2.6- and 1.6-fold, respectively, following mitapivat doses of 5, 20, or 50 mg twice daily.

The review team agrees with the Applicant that the use of mitapivat with strong CYP3A inhibitors should be avoided due to the large increase in mitapivat exposure. For use with moderate CYP3A inhibitors, the team recommends monitoring of Hb levels and for the increased risks of adverse reactions including capping of the mitapivat dose at 20 mg BID. Based on the PB-pharmacokinetic simulations, mitapivat exposure at 20 mg BID concomitantly with a moderate CYP3A inhibitor is similar to the exposure achieved following administration of mitapivat 50 mg BID alone (see Section III.14.4 for details).

Effects of CYP3A Inducers on Mitapivat

Rifampin, a strong CYP3A inducer, was dosed (600 mg once daily) to steady-state and its effect was evaluated on a single dose of 50 mg mitapivat. Rifampin decreased mitapivat AUC_{∞} and C_{max} by 91% and 77%, respectively. The effects of a strong CYP3A inducer, rifampin, and a moderate inducer, efavirenz, were evaluated by PB-pharmacokinetic modeling and simulations.

Based on the PB-pharmacokinetic simulations, rifampin decreased the mitapivat AUC_{0-12} and C_{max} by approximately 95% and 85%, respectively, following mitapivat doses of 5, 20, or 50 mg twice daily. Efavirenz, a moderate CYP3A inducer, decreased the mitapivat AUC_{0-12} and C_{max} by approximately 60% and 30%, respectively, following a mitapivat dose of 5 or 20 mg twice daily. Efavirenz decreased the mitapivat AUC_{0-12} and C_{max} by 55% and 24%, respectively, following a mitapivat dose of 50 mg twice daily.

The review team agrees with the Applicant that the use of mitapivat with strong CYP3A inducers should be avoided due to the large decrease in mitapivat exposure. For use with moderate CYP3A inhibitors, the team recommends monitoring of Hb levels for reduced activity of mitapivat and further recommends titration beyond 50 mg twice daily dose if necessary but not to exceed 100 mg twice daily. For patients on stable moderate CYP3A inducers, the review team recommends initiating mitapivat at 10 mg twice daily. These recommendations are supported by PB-pharmacokinetic simulations, which show that mitapivat exposures at 100 mg and 10 mg twice daily concomitantly with a CYP3A inducer is similar to 50 mg and 5 mg twice daily administered alone, respectively (See Section III.14.4 for details).

Effect of Mitapivat on Other Drugs

In vitro studies indicated that mitapivat is an inducer of CYP3A, CYP2B6, CYP2C8, CYP2C9, CYP2C19, and uridine 5'-diphospho-glucuronosyltransferase (UGT)1A1, as well as an inhibitor of the P-gp transporter. Refer to Section III.14.1 for details of the in vitro studies of mitapivat. Clinical drug-drug interaction (DDI) studies were not conducted for mitapivat as a perpetrator. Induction by mitapivat of CYP3A, CYP2B6, CYP2C8, CYP2C9, CYP2C19, and inhibition of P-gp transporter was evaluated by the Applicant by PB-pharmacokinetics modeling and simulations.

Effect of Mitapivat on CYP3A Substrates

Autoinduction was observed with multiple administrations of mitapivat in clinical studies. The mean plasma C_{max} and AUC_{0-12} increased less-than-dose-proportionally and the accumulation ratios were less than 1.0 with repeated doses of 120 mg to 700 mg twice daily in the Phase 1 Study 002. The accumulation ratios were ~1.0 with 5 mg to 50 mg of mitapivat twice daily in the Phase 3 Study 006. Based on PB-pharmacokinetic simulations, the AUC_{inf} and C_{max} of

midazolam, a CYP3A substrate, decreased by 21% and 19%, respectively, following coadministration with mitapivat 5 mg twice daily. The midazolam AUC_{inf} and C_{max} decreased by 43% and 39%, respectively, following coadministration with mitapivat at 20 mg twice daily, and 57% and 52%, respectively, at 50 mg twice daily. The review team concludes that the submitted PB-pharmacokinetics model is validated to predict exposure of a sensitive CYP3A substrate when coadministered with mitapivat (see Section III.14.4 for details).

The Applicant proposed the following labeling language for concomitant use of mitapivat with sensitive CYP3A substrates:

(b) (4) *PYRUKYND* (b) (4) *decrease concentrations of drugs that are sensitive CYP3A4 substrates. Monitor for loss of therapeutic effect.*

The review team agrees with the overall proposal with regard to the concomitant use of sensitive CYP3A4 substrates. The review team recommends specifying sensitive CYP3A substrates with narrow therapeutic index (NTI), because minimal concentration changes of NTI drugs may lead to meaningful loss of therapeutic effect. In addition, according to the Guidance for Industry: Clinical Drug Interaction Studies with Combined Oral Contraceptives, we recommend adding the following language to the mitapivat labeling:

Advise patients using hormonal contraceptives to use an alternative nonhormonal contraceptive method or add a barrier method of contraception during treatment with PYRUKYND.

Effect of Mitapivat on CYP2B6, CYP2C, and UGT1A1 Substrates

In vitro studies showed that the treatment of cultured human hepatocytes with mitapivat up to 100µM caused concentration-dependent increases in CYP3A4/5, CYP2B6, CYP2C8, CYP2C9, CYP2C19, and UGT1A1 activities with EC_{50} of these enzymes ranging from 0.2 to 7.6 µM, as well as more than two-fold increases in the mRNA levels of these enzymes. The mean C_{max} value at steady-state with 50 mg twice daily doses is approximately 1 µM. Based on the in vitro drug interaction guidance (In Vitro Drug Interaction Studies-Cytochrome P450 Enzyme-and Transporter-Mediated Drug Interactions Guidance for Industry), these findings cannot rule out the in-vivo induction potential of mitapivat. In addition, the review team finds the submitted PB-pharmacokinetic modeling and simulation to be inadequate to evaluate the effect of mitapivat on the pharmacokinetics of CYP2C and CYP2B6 probe substrates because of the limited evidence supporting in vitro-in vivo extrapolation of the induction potential of CYP2C isoforms (see Section III.14.4).

Because of the concern for potential induction effect based on in vitro studies and lack of clinical drug interaction data, the review team proposes to add the following labeling language regarding concomitant use of mitapivat with substrates of these enzymes:

PYRUKYND has the potential to induce CYP2B6, CYP2C8, CYP2C9, CYP2C19 and UGT1A1 enzymes, and may decrease the systemic concentrations of drugs that are substrates of these enzymes. Monitor patients for loss of therapeutic effect of sensitive substrates with narrow therapeutic index when co-administered with PYRUKYND.

Effect of Mitapivat on P-gp Substrates

In vitro studies showed that mitapivat inhibited human P-gp–mediated transport of digoxin (10µM) with an IC_{50} of 12.8µM. Based on the static model (I_{gut} [intestinal concentration] ÷ IC_{50} >10), there is a potential for DDIs with P-gp substrates. The PB-pharmacokinetics model submitted by the Applicant also suggested that mitapivat may increase exposure to probe P-gp substrates. However, the PB-pharmacokinetics model prediction was based on a parameter sensitivity analysis and could not provide definitive DDI estimates. Coadministration of mitapivat with drugs that are sensitive substrates of P-gp may result in a clinically relevant increase in their plasma concentrations, especially those with NTI, for which a minimal concentration change may lead to serious adverse reactions. Therefore, the review team proposes adding the following language to the label:

PYRUKYND has the potential to inhibit the P-gp transporter. Coadministration of PYRUKYND may increase systemic concentrations of drugs that are P-gp substrates. Monitor patients for adverse reactions to P-gp substrates with a narrow therapeutic index when co-administered with PYRUKYND.

8.3. Plans for Pediatric Drug Development

Mitapivat received orphan drug designation for the treatment of PKD on March 24, 2015; therefore, mitapivat is exempt from the requirements Pediatric Research Equity Act for the indication.

(b) (4)

8.4. Pregnancy and Lactation

Nonclinical reproduction studies covering the entire cycle of reproduction in rats, and the period of organogenesis in rabbits, were conducted to inform potential risks to humans during pregnancy and lactation, as well as the potential to affect fertility. No adverse treatment-related effects on fertility, embryo-fetal development, and pre- and postnatal development were observed at clinically relevant exposure levels. Based on the nonclinical data, no warning or contraindication for mitapivat in pregnancy is warranted in the label. The following section describes the outcomes and conclusions of these studies. More details of the reproductive toxicity studies are provided in Section III.13.1.1.6.4. and the final recommended labeling is shown in Section III.21.

1 Page of Draft Labeling has been Withheld in Full as b4 (CCI/TS) immediately following this page

Table 53. Reproductive Toxicity Safety Margins

Study	NOAEL (mg/kg/BID)	Nonclinical Exposure (ng.hr/mL)	Safety Margins* (Multiples)
Fertility, Rat	(M) 150	(M) 160,000	(M) 45×
	(F) 100	(F) 174,000	(F) 48×
EFD, Rat	25	46,800	13×
EFD, Rabbit	30	5360	1.5×
PPND, Rat	(F0) 10	12,500	(F0) 3.5×#
	(F1) 25	46,800	(F1) 13×#

Source: Nonclinical Reviewer.
* Safety margins were based on the simulated population pharmacokinetic data generated by the clinical pharmacology population reviewer simulated exposure every 12 hours (dosing interval) AUC0-12 at steady-state with a geometric mean of 3591.4 h*ng/mL (%CV=28%) for the MRHD of 50 mg BID.
# Exposure based on AUC from pregnant rats in the EFD study.
Abbreviations: AUC, area under the concentration-time curve; BID, twice daily; CV, coefficient of variation; F, female; M, male; NOAEL, no observed adverse effect level; EFD, embryo-fetal development; PPND, pre- and postnatal development

Clinical studies have not been conducted with mitapivat to evaluate the effect on fertility or the unborn fetus.

A total of two pregnancies was reported in the mitapivat clinical studies (i.e., Study 003). The first case occurred in a female patient, and the second case occurred in the female partner of a male patient who received treatment with mitapivat. The outcomes of both pregnancies were reported as successful live births with normal Apgar scores (at 1 and 5 minutes). A serious postpartum hemorrhage that required blood transfusion was reported in the female partner of the male patient who received mitapivat. Follow-up information for the female patient who received mitapivat (information received 9 and 21 months after the birth) indicated that the baby was developing normally and doing well.

9. Product Quality

The Office of Pharmaceutical Quality Review team has assessed mitapivat with respect to chemistry, manufacturing, and controls and has determined that it meets all applicable standards to support the identity, strength, quality, and purity that it purports to have. As such, the Office of Pharmaceutical Quality recommends approval of this NDA from a quality perspective. Refer to the review by Theodore Carver and the quality review team for additional details.

9.1. Device or Combination Product Considerations

Not applicable to this product.

10. Human Subjects Protections/Clinical Site and Other Good Clinical Practice Inspections/Financial Disclosure

Compliance With Good Clinical Practices

Both Studies 006 and 007 were reviewed and approved by the Independent Ethics Committees or Institutional Review Boards and conducted in accordance with good clinical practices and the

Declaration of Helsinki. Written informed consent was obtained from each subject prior to performance of study-specific procedures.

Financial Disclosure

The Applicant provided FDA financial certification form 3454 signed by Jonathan Biller, the Chief Financial Officer and Head of Legal & Corporate Affairs for Agios Pharmaceuticals, dated May 19, 2021. The Applicant certified to the following statement:

"As the Sponsor of the submitted studies, I certify that I have not entered into any financial arrangement with the listed clinical investigators (enter names of clinical investigators below or attach list of names to this form) whereby the value of compensation to the investigator could be affected by the outcome of the study as defined in 21 CFR 54.2(a). I also certify that each listed clinical investigator required to disclose to the Sponsor whether the investigator had a proprietary interest in this product or a significant equity in the Sponsor as defined in 21 CFR 54.2(b) did not disclose any such interests. I further certify that no listed investigator was the recipient of significant payments of other sorts as defined in 21 CFR 54.2(f)."

The submission contained a list of clinical investigators that participated in Studies 006 (203 principal/subinvestigators) and 007 (86 principal/subinvestigators).

In Studies 006 and 007, a total of two principal investigators (b) (6) disclosed payments in an aggregate amount of $27,593.84 U.S. dollars and (b) (6) disclosed payments in an aggregate amount of more than $25,000 U.S. dollars) and one clinical investigator (b) (6) respectively, had financial disclosure interest/arrangements.

The Applicant provided FDA 3455 forms for (b) (6) and (b) (6) and information on their financial interests and arrangements, and a description of the steps taken to minimize the potential for bias resulting from the disclosed financial interests and arrangements.

The Applicant reported that Study 006 was initiated on (b) (6) and is closed. (b) (6) disclosed payments from Agios of $27,593.84 for research funding and honoraria/consultation payments. Per the Applicant, payments were related to consultations and to research funding for exploratory research that was unrelated to the primary and secondary objectives of Study 006. Of the 80 patients enrolled in Study 006, (b) (6) patients (b) (6)%) were enrolled at site (b) (6) where (b) (6) was the principal investigator. Of the (b) (6) patients, there were (b) (6) patients who achieved the primary endpoint. The Office of Scientific Investigations (OSI) inspection included site (b) (6). The OSI inspection results at this site concluded that (b) (4). No under-reporting of adverse events was found. See Section 20.

Per the Applicant, (b) (6) received payments from (b) (6) of $20,472.17 for consultations and research funding. In addition, (b) (6) site, (b) (6) received $1,855,516.76 for research funding of the (b) (6) The Applicant states that payments to (b) (6) were related to consultations and research funding. Additional payments to (b) (6) were also related to research funding for exploratory research that was unrelated to the primary and secondary objectives of Study 006. Of the 80 patients enrolled in Study 006 (b) (6) patients (b) (6)%) were enrolled at site

(b) (6), where (b) (6) was the principal investigator. There were no patients who achieved the primary endpoint at site (b) (6)

In Study 007, (b) (6)%) of the 27 enrolled patients was enrolled at site (b) (6) where (b) (6) was the principal investigator. The patient enrolled at this site did not achieve the primary endpoint.

Per the Applicant, investigator compensation was not affected by the outcome of the clinical study as defined in 21 CFR § 54.2. Compensation was not higher for a favorable outcome than for an unfavorable outcome, whether in the form of cash payments or equity interest, nor was it tied to sales of the product (i.e., royalty interest).

Therefore, it is unlikely that these financial conflicts of interest affected the overall results of the studies. In both studies, none of the investigators were full- or part-time employees of the Applicant. Also see Section 23.

Office of Scientific Investigations

The OSI participated in discussions regarding the need for clinical site inspections. For details, see Section 20.

Protocol Deviations

Study 006

In Study 006, all randomized patients (100%) had protocol deviations. The incidence of protocol deviations in each category was generally similar between the two arms (Table 54). All patients had protocol deviations that were considered minor. The most common minor deviations were visit/assessment not performed (100%), visit/assessment out of window (80%), deviations due to principal investigator oversight (71.3%), study treatment deviations (48.8%), and deviations related to study drug accountability (37.5%).

A total of 21 patients (26.3%) also had major protocol deviations; 6 patients (7.5%) did not reconsent with the amended informed consent before conducting study procedures, the informed consent process was not documented in the medical file in 6 patients (7.5%), five patients (6.3%) had unblinding deviations (all at or after the Week 24 visit), three patients (3.8%) had study treatment deviations, one patient (1.3%) had other deviation (informed consent form not countersigned), and one patient (1.3%) had an SAE-related deviation (transaminase increase not reported within 24 hours). Per the Applicant, during preparation of the clinical study report, a further two patients with unblinding deviations were identified that met the criteria for major but were miscategorized in the database as minor. One patient had unintentional unblinding at Week 24; the other was regarding the sharing of restricted Hb data, which did not affect treatment unblinding. Per the Applicant, these two deviations did not have a negative impact on data integrity.

A request for information was sent to the Applicant to determine whether the protocol for Study 006 allowed investigators to access restricted data (i.e., RBC parameters and hemolysis parameters) and to provide the measures implemented in the study that ensured that data integrity was maintained. The Agency also asked how cases of unblinding were handled.

The Applicant responded that standard operating procedures for blinding maintenance and an unblinding plan were prospectively developed and detailed the measures implemented to ensure that data integrity was maintained throughout the study. The processes in the standard operating procedures were followed. Unblinding of subjects, investigators, and site personnel occurred as planned at the Week 24 visit after all assessments in Study 006 were completed for the subjects who expressed intent to enter the open-label extension Study 011. There were no instances of emergency unblinding or unintentional unblinding of subjects, investigators, or site personnel throughout the study.

Table 54. Protocol Deviations, Full Analysis Set, Study 006

Deviations	Mitapivat (N=40)	Placebo (N=40)	Total (N=80)
All patients with deviations	40 (100%)	40 (100%)	80 (100%)
Patients with minor deviations	40 (100%)	40 (100%)	80 (100%)
ICH/GCP deviations	34 (85.0%)	32 (80.0%)	66 (82.5%)
PI oversight	30 (75.0%)	27 (67.5%)	57 (71.3%)
Study drug accountability	12 (30.0%)	18 (45.0%)	30 (37.5%)
Other	0	1 (2.5%)	1 (1.3%)
Protocol deviations	40 (100%)	40 (100%)	80 (100%)
Visit or assessment not performed	40 (100%)	40 (100%)	80 (100%)
Visit or assessment out of window	33 (82.5%)	31 (77.5%)	64 (80.0%)
Study treatment deviation	18 (45.0%)	21 (52.5%)	39 (48.8%)
Use of prohibited concomitant treatment	3 (7.5%)	1 (2.5%)	4 (5.0%)
Unblinding deviation	3 (7.5%)	1 (2.5%)	4 (5.0%)
Other	2 (5.0%)	3 (7.5%)	5 (6.3%)
Patients with major deviations	10 (25.0%)	11 (27.5%)	21 (26.3%)
ICH/GCP deviations	6 (15.0%)	7 (17.5%)	13 (16.3%)
Informed consent	3 (7.5%)	3 (7.5%)	6 (7.5%)
Source document	3 (7.5%)	3 (7.5%)	6 (7.5%)
Other	0	1 (2.5%)	1 (1.3%)
Protocol deviations	5 (12.5%)	4 (10.0%)	9 (11.3%)
Study treatment deviation	2 (5.0%)	1 (2.5%)	3 (3.8%)
SAE-related deviation	1 (2.5%)	0	1 (1.3%)
Unblinding deviation	2 (5.0%)	3 (7.5%)	5 (6.3%)

Source: ADDV.xpt.
Based on number of subjects. A patient can appear in more than one category.
Abbreviations: GCP, good clinical practice; ICH, international conference on harmonization; N, total number of subjects; PI, prescribing information; SAE, serious adverse event

A sensitivity analysis of the primary endpoint was conducted excluding the patients with major protocol deviations (Table 55). The results were consistent with the primary analysis of the primary endpoint. Therefore, it is unlikely that these protocol deviations affected the overall results of the study.

Table 55. Analysis of Hemoglobin Response in Patients Without Major Protocol Deviation, Mantel-Haenszel Stratum Weighted Method, Study 006

Hemoglobin Response	Mitapivat N=30	Placebo N=29
Hb responders, n(%)	13 (43.3)	0
Adjusted difference in response rate (mitapivat vs. placebo), %		42.7
95% CI		(24.7, 60.7)
Two-sided p-value		<0.0001

Source: ad beff.xpt and addv.xpt.
Hb responders were defined as subjects with a ≥15 g/L (1.5 g/dL) increase in Hb concentration from baseline at two 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.
Abbreviations: CI, confidence interval; Hb, hemoglobin; N, total number of subjects; n, number of subjects in each category

Study 007

Protocol deviations also occurred in all patients (100%) in Study 007. All patients had minor protocol deviations, most of which were due to visit or assessment not performed (96.3%), visit or assessment out of window (85.2%), deviation in study treatment (81.5%), study drug accountability (66.7%), and deviations due to principal investigator oversight (63.0%).

Major protocol deviations also occurred in 15 patients (55.6%). The most common major protocol deviations (>15%) were related to informed consent (25.9%), and study treatment deviation (18.5%).

Table 56. Protocol Deviations, Full Analysis Set, Study 007

Deviation	Mitapivat (N=27)
All patients with deviations	27 (100%)
Patients with minor deviations	27 (100%)
ICH/GCP deviations	20 (74.1%)
PI oversight	17 (63.0%)
Study drug accountability	18 (66.7%)
Other	5 (18.5%)
Protocol deviations	27 (100%)
Visit or assessment not performed	26 (96.3%)
Visit or assessment out of window	23 (85.2%)
Study treatment deviation	22 (81.5%)
Use of prohibited concomitant treatment	1 (3.7%)
Other*	1 (3.7%)
Patients with major deviations	15 (55.6%)
ICH/GCP deviations	9 (33.3%)
Informed consent	7 (25.9%)
PI oversight	2 (7.4%)
Source document	1 (3.7%)
Protocol deviations	10 (37.0%)
Study treatment deviation	5 (18.5%)
Visit or assessment not performed	4 (14.8%)
SAE-related deviation	3 (11.1%)
Other**	3 (11.1%)

Source: ADDV.xpt.
* eDiary was returned late.
** Subject transfused, but was not administered the MNU, when ITT was reached.
Based on number of subjects. A patient can appear in more than one category.
Abbreviations: GCP, good clinical practice; ICH, international conference on harmonization; N, total number of subjects; PI, prescribing information; SAE, serious adverse event

A sensitivity analysis of the primary endpoint excluding the patients with major protocol deviations was also conducted for Study 007 (Table 57). The results show that major protocol deviations did not affect the result of the primary endpoint.

Table 57. Analysis of Transfusion Reduction Response in Patients Without Major Protocol Deviations, Study 007

Transfusion Reduction Response	Total N=12
Transfusion reduction responders, n (%)	5 (41.7)
95% CI	(15.2, 72.3)

Source: adtf.xpt and addv.xpt.
Transfusion reduction response is defined as a ≥33% reduction in total number of red blood cell (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 the Clopper-Pearson method.
Efficacy is demonstrated if the lower bound of the two-sided 95% CI for the transfusion reduction response rate is >10%.
Abbreviations: CI, confidence interval; N, total number of subjects; n, number of subjects in each category

11. Advisory Committee Summary

The application did not raise safety or effectiveness issues requiring external expert advice. Therefore, an Advisory Committee meeting was not held.

III. Appendices

12. Summary of Regulatory History

Table 58. Summary of Regulatory History

Date	Activity	Key Outcome(s)
December 16, 2013	Pre-IND Meeting to discuss their novel product, AG-348, for the treatment of patients with pyruvate kinase deficiency (PKD).	FDA advised the Applicant that quality of life instruments are not validated in the PKD population and encouraged the development of a PKD-specific instrument.
March 5, 2014	IND 119825 for mitapivat sulfate was submitted, which included clinical protocol #AG-348-C-001 entitled, "*A Phase 1, Randomized, Double-Blind, Placebo-Controlled, Single Ascending Dose, Safety, Pharmacokinetic, and Pharmacodynamic Study of Orally Administered AG-348 in Healthy Volunteers*."	The IND was deemed Safe to Proceed on March 28, 2014.
May 2, 2014	Granted as a Type C, Guidance meeting. The Applicant requested feedback on the clinical study design for the proposed Phase 2 study in adult subjects, guidance on the proposed approach to initiate clinical studies in the pediatric population, and guidance on the proposed toxicology development plan to support clinical development.	FDA advised the Applicant as follows: All patients should have at least 6 months of treatment to allow for adequate collection of efficacy and safety data to possibly support an initial accelerated approval. Regular approval will require at least 12 months of follow-up data. Agency encouraged the development of patient-reported outcomes in rare diseases that incorporate measures specific to the disease (PKD) and focus on key symptoms of the disease. For the pivotal study, we encouraged the Applicant to collect data to enable development of a specific instrument for PKD, which may be implemented in later trials.

Date	Activity	Key Outcome(s)
March 5, 2015	GLP surveillance inspection	The FDA-requested inspection was conducted for two studies: (b) (4) 553093 and (b) (4) 888066. A four-item Form FDA 483 was issued for the following: 1) (b) (4) [redacted] The ORA District decision and the OSIS final classification are both voluntary action indicated (VAI).
March 27, 2017	Requested FastTrack Designation	On April 27, 2017, FDA approved the fast track designation request for the treatment of patients with PKD.
April 3, 2017	Type B, End-of-Phase 2 meeting request to discuss the results from Phase 2 Study AG-348-C-002, the recommended pivotal dose, the proposed pivotal trial designs, and the overall nonclinical and clinical development plans for AG-348.	The Agency recommended analysis of safety and efficacy in the intent-to-treat population and not only responders.
October 12, 2017	Requested waiver of the IRB requirements under 21 CFR Part 56 for the use of AG-348 in all foreign investigational studies conducted under this IND.	FDA Granted the IRB waiver on November 17, 2017, stating that the IEC provide the same mechanism for ensuring the protection of the rights and welfare of human subjects as described in your submission.
December 11, 2017	Requested a special carcinogenicity protocol assessment for clinical protocol *"A 2-Year Oral Gavage Carcinogenicity Study of AG-348 in Sprague-Dawley Rats."*	The Agency issued an SPA agreement on January 24, 2018, with executive CAC recommendations. Recommendations included, but were not limited, to dose recommendations.
July 7, 2018	Type B, End-of-Phase 2 CMC, meeting to discuss the acceptability of the proposed starting materials and starting material control strategy.	The Agency recommended that the Applicant provide comparative dissolution data for plain-face/printed tablets for AG-348 as part of a complete NDA submission package and FDA agrees that imprinting is not expected to affect tablet quality.

Date	Activity	Key Outcome(s)
November 21, 2018	Requested a special carcinogenicity protocol assessment for clinical protocol *"A 26-Week Carcinogenicity Study of AG-348 by Oral Gavage in CByB6F1/Tg rasH2 Hemizygous Mice."*	The Agency issued an SPA agreement on December 20, 2018, with executive CAC recommendations. Recommendations included but were not limited to: Dosing intervals for both males and females.
March 18, 2019	Type C Guidance meeting to discuss the proposed clinical pharmacology development plan.	Preliminary comments were sent to the Applicant on May 24, 2019. The Applicant withdrew this meeting request, stating that further discussion was not required.
April 5, 2019	Type C, Guidance WRO meeting was requested to discuss the acceptability to request a claim of categorical exclusion under 21 CFR 25.31(b) for mitapivat sulfate at the time of submission of a future NDA.	The Agency provided the Applicant with final written responses on June 18, 2019.
[redacted]		(b) (4)
September 12, 2019	Proprietary name	FDA agreed on March 4, 2020, that the name Pyrukynd was conditionally acceptable and that a request for proprietary name review for Pyrukynd should be submitted once the NDA is submitted.
February 5, 2020	Type B, pre-NDA to discuss the adequacy of the proposed content and format of this NDA.	The Agency advised the Applicant to provide information that the endpoints and patient populations chosen for Studies 006 and 007 will demonstrate the benefit of mitapivat therapy in PKD and that topline data are needed to assess the endpoints and patient populations at the pre-NDA meeting.
May 29, 2020	Type C, Guidance, WRO to discuss a proposed PRO and PerfOs measurement strategy for a planned Phase 3 study.	The Agency provided the Applicant with final written responses on August 14, 2020.
June 17, 2021	Formally submitted an NDA for PKD.	

Source: Regulatory Project Manager.
Abbreviations: CAC, Carcinogenicity Assessment Committee; GLP, good laboratory practices; IEC, independent ethics committee; IND, investigational new drug application; NDA, new drug application; PerfO, performance outcome; PKD, pyruvate kinase deficiency; PRO, patient reported outcome; SPA, Special Protocol Assessment; WRO, written response only

13. Pharmacology Toxicology: Additional Information and Assessment

13.1. Summary Review of Studies Submitted Under the Investigational New Drug Application

13.1.1. Pharmacology

13.1.1.1. Primary Pharmacology

In Vitro and Ex Vivo Activity Consistent With the Proposed Mechanism of Action of Mitapivat (AG-348)

PK activity was estimated from the rate of nicotinamide adenine dinucleotide plus hydrogen oxidation in recombinantly expressed and purified wild-type (WT) and mutant red cell isoform of PK (PK-R) receptors incubated with mitapivat at a range of concentrations. Mitapivat potently activated the WT and several mutant PK-R isoforms with a half-maximal activation concentration (AC_{50}) ranging from 13nM for WT and 9nM to 60nM for the mutant PKRs. Mitapivat also potently activated M2-type pyruvate kinase (PKM2) and L-type pyruvate kinase (PKL) with three-fold lower potency (38nM) compared to WT PK-R (13nM). PK-R is expressed selectively in erythrocytes and erythrocyte precursors, whereas PKL is expressed in the liver, kidney, and in parts of the small intestine and PKM2 is expressed in the lung, adipocytes, epithelial cells, and proliferating cells, including embryonic cells and adult stem cells.

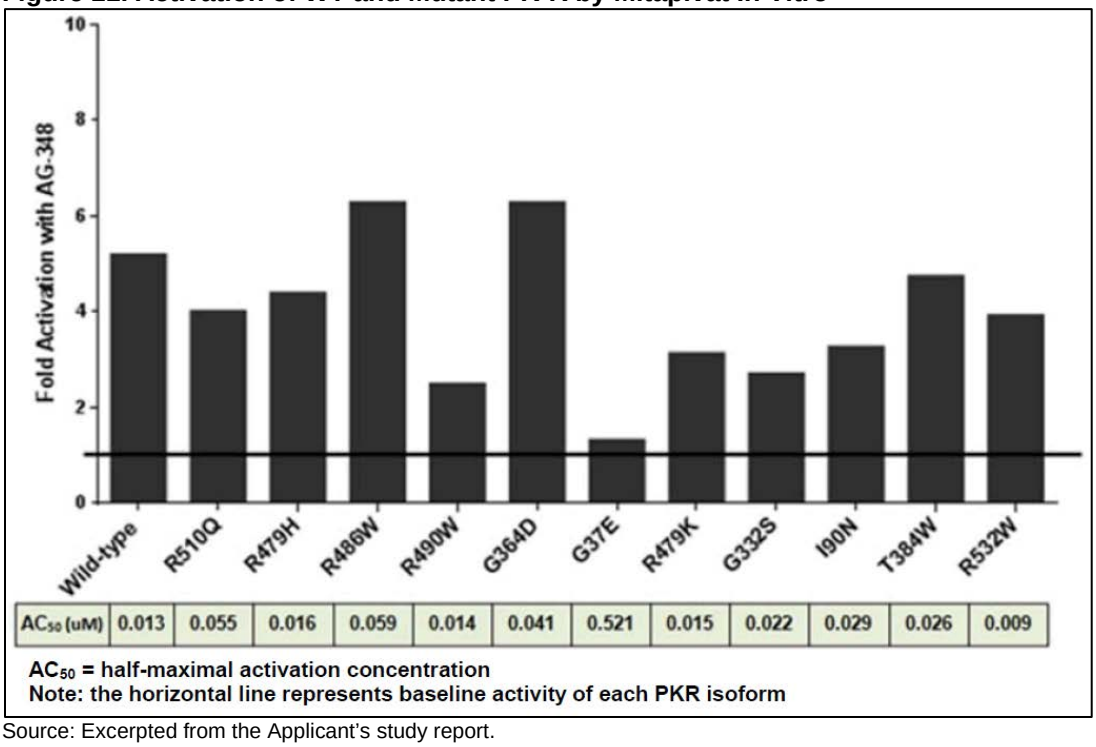
Table 59. Activation of WT and Mutant PK-R Isoforms by Mitapivat

PKR Isoform	FBP		AG-348	
	AC_{50} (µM)	Percent Activation (%)	AC_{50} (µM)	Percent Activation (%)
PKR WT	0.006	467.8	0.013	519.2
PKR R510Q	0.008	478.2	0.055	400.5
PKR R479H	0.034	550.7	0.016	438.7
PKR R486W	0.012	462.1	0.059	628
PKR R490W	0.01	520.4	0.014	248.5
PKR G364D	0.012	372.4	0.041	627.6
PKR G37E	No fit	128.4	0.521	131.1
PKR R479K	0.032	509	0.015	312.8
PKR G332S	0.015	499.6	0.022	269.8
PKR I90N	0.068	412.7	0.029	325.8
PKR T384W	0.023	448.7	0.026	474.2
PKR R532W	No fit	93.8	0.009	392.3
PKM2	0.012	450.8	0.038	587.4
PKL	0.014	381	0.037	292.6

Abbreviations: AC_{50} = half-maximal activation concentration; FBP = fructose-1,6,-bisphosphate

Source: Excerpted from the Applicant's study report.

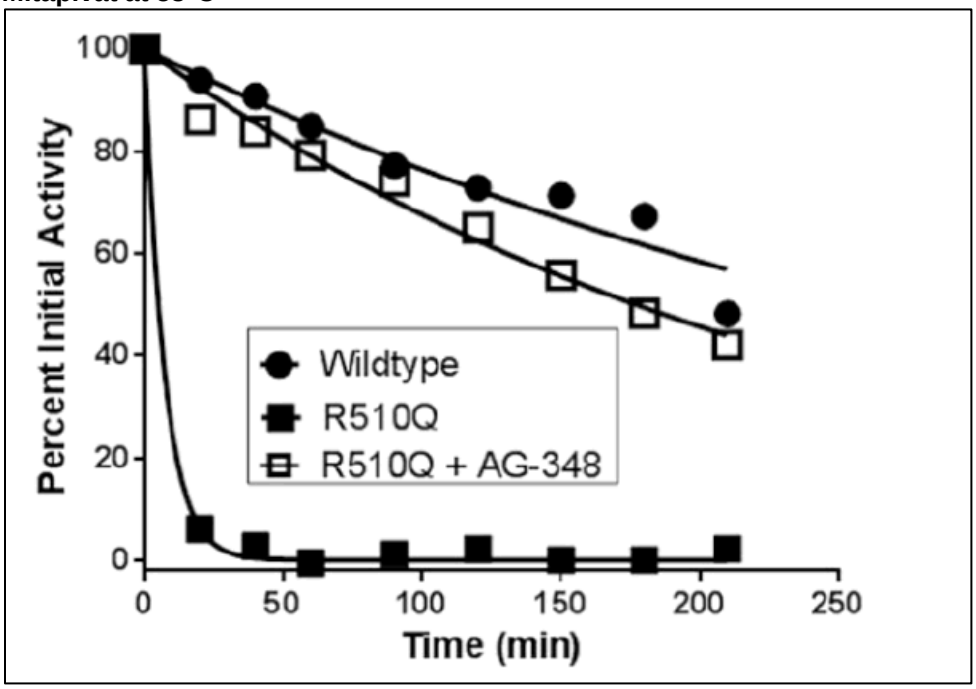
Figure 11. Activation of WT and Mutant PK-R by Mitapivat In Vitro



Source: Excerpted from the Applicant's study report.

The ability of mitapivat and its metabolite AGI-8702 to protect against the thermal denaturation of five PK-R mutant isoforms was evaluated. AG-438 (or AGI-8702), WT PK-R, and the five mutant PK-R enzymes were incubated at room temperature for 30 minutes, subsequently at 53°C for the specified amount of time when activity was measured. Of the five tested isoforms, three (R510Q, G364D, and R532W) had a significant deficiency in protein stability at elevated temperature (i.e., greater thermolability than the WT enzyme). The AC_{50} values for mitapivat to stabilize the R510Q, G364D, and R532W isoforms were 0.54, 1.18, and 1.93µM, respectively. The stabilizing effect of mitapivat was best demonstrated with the R510Q mutant (Figure 12). Preincubation with mitapivat at 53°C attenuated the loss of R510Q activity and improved R510Q stability with calculated half-lives of 257.5 minutes for WT PK-R, 5.2 minutes for R510Q without mitapivat preincubation, and 176.9 minutes for R510Q with mitapivat preincubation.

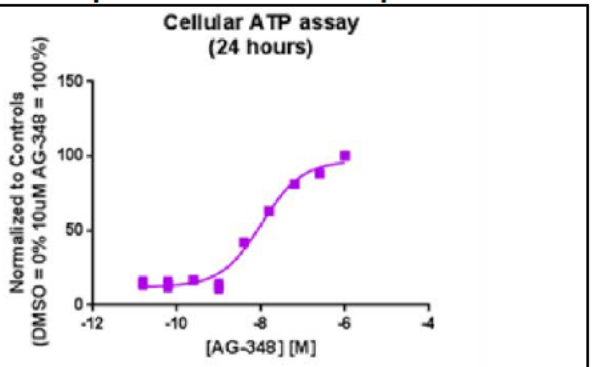
Figure 12. Stability of Wild-Type PK-R and R510Q Isoform With and Without Preincubation With Mitapivat at 53°C



Source: Excerpted from the Applicant's study report.
Abbreviations: PK-R, red blood cell isoform of pyruvate kinase

Mitapivat caused a potent and dose-dependent increase in cellular adenosine triphosphate (ATP; AC50 10.9±7nM) in healthy human red blood cells (RBCs). Compared to mitapivat, it's major metabolite, AG-8702, was a weak activator (~200-fold weaker) of PK-R with an AC_{50} of 2.3µM for ATP production in human RBCs and 337µM for PK-R activation in human whole blood. The maximal PK-R activation with AG-8702 was ~50% that of mitapivat.

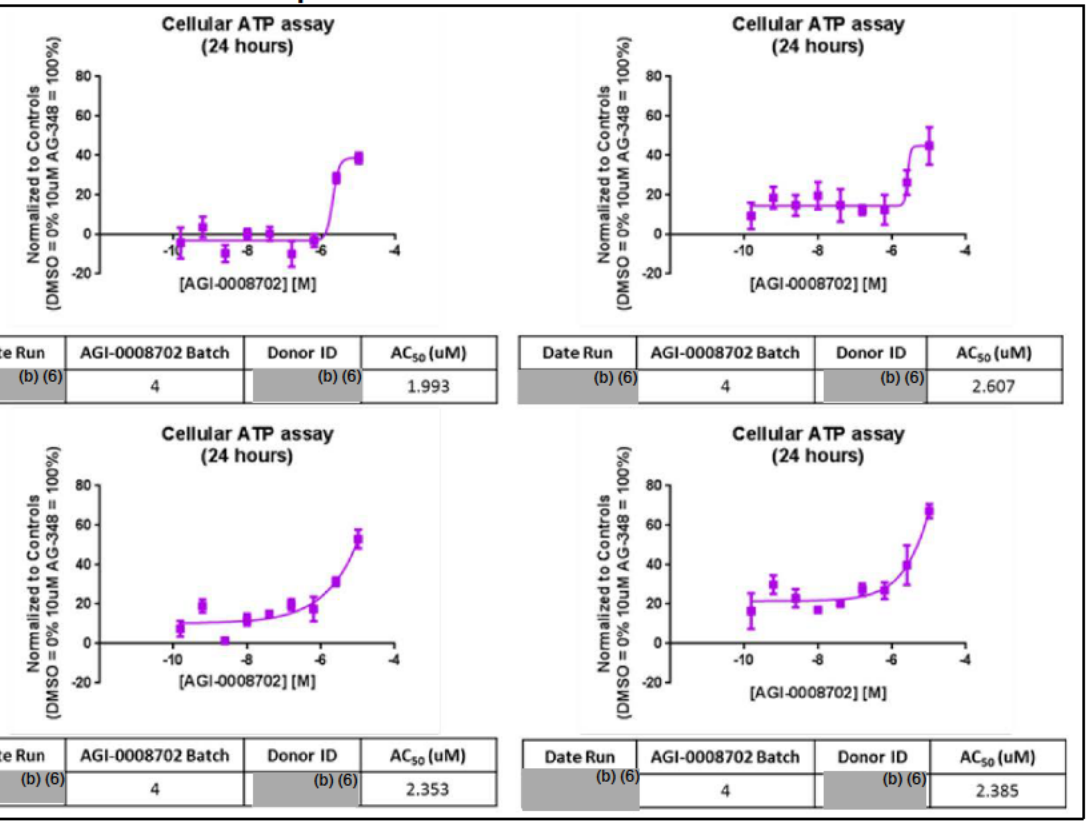
Figure 13. Representative Dose-Response Curve of Mitapivat-Induced ATP In Human RBCs



Date Run	AG-348 Batch	Donor ID	AC_{50} (nM)
(b) (6)	8	(b) (6)	10.2

Source: Excerpted from the Applicant's study report.
Abbreviations: AC, activity concentration; ATP, adenosine triphosphate; RBC, red blood cell

Figure 14. AG-8702 Dose-Response Curves for ATP Levels in RBCs In Vitro



Date Run	AGI-0008702 Batch	Donor ID	AC_{50} (uM)
(b) (6)	4	(b) (6)	1.993

Date Run	AGI-0008702 Batch	Donor ID	AC_{50} (uM)
(b) (6)	4	(b) (6)	2.607

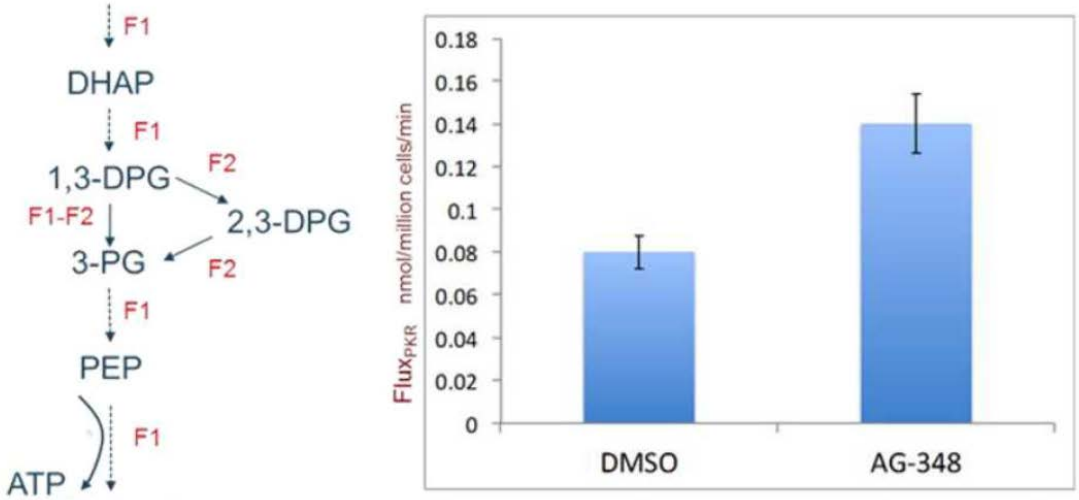
Date Run	AGI-0008702 Batch	Donor ID	AC_{50} (uM)
(b) (6)	4	(b) (6)	2.353

Date Run	AGI-0008702 Batch	Donor ID	AC_{50} (uM)
(b) (6)	4	(b) (6)	2.385

Source: Excerpted from the Applicant's study report.
Abbreviations: AC, activity concentration; ATP, adenosine triphosphate; RBC, red blood cell

The effect of mitapivat on PK-R flux, e.g., the rate of carbon flow through the PK-R enzyme reaction, was assessed in whole blood from mice treated with mitapivat using a stable isotope strategy (^{14}C-labeled glucose). Mitapivat increased flux through PK-R by 80% in C57BL/6 mouse whole blood. This shows that mitapivat bound and activated the PK-R enzyme, and enhanced the glycolytic pathway in RBCs. These data support the ability of mitapivat to activate the WT PK-R enzyme in vivo and alter RBC metabolism.

Figure 15. Effect of Mitapivat (AG-348) on PK-R Catalytic Activity



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

Note: F1 and F2 represent the modeled fluxes through individual steps.

Source: Extracted from the nonclinical review in IND 119825.
Abbreviations: ATP, adenosine triphosphate; DHAP, dihydroxyacetone phosphate; 2,3-DPG, 2,3-bisphosphoglyceric acid; IND, investigational new drug; PG, phosphoglycerate ; PEP, phosphoenolpyruvic acid; PK-R, red blood cell isoform of pyruvate kinase

In Vivo Studies Supporting the Proposed Indication

Compared to vehicle or untreated healthy mice, mice administered mitapivat for 7 days at the clinical exposures exhibited a dose-dependent decrease in plasma 2,3-bisphosphoglyceric acid (2,3-DPG) and dose-dependent increases in plasma ATP and ATP/2,3-DPG ratio.

Table 60. Effect of Mitapivat (AG-348) on Plasma ATP, 2,3-DPG, and ATP/2.3-DPG Ratio

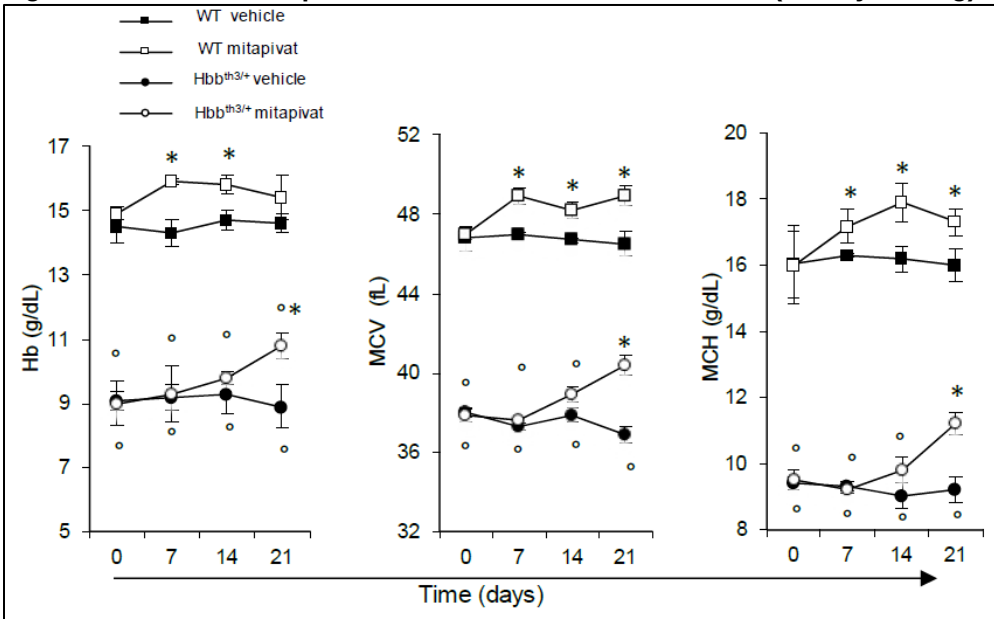
Groups	AG-348	ATP	2,3-DPG	ATP/2,3-DPG
	Plasma	Blood	Blood	Blood
$AUC_{0\text{-}12hr}$ (hr•ng/mL)				
PO-vehicle	NA	3827625	23201250	0.165
PO-1 mg/kg	41.3	4424250	22788750	0.194
PO-10 mg/kg	120	4720125	19991250	0.236
PO-50 mg/kg	685	5249250	19327500	0.272
PO-150 mg/kg	4076	5500875	18851250	0.292
Untreated	NA	3786750	22968750	0.165

Source: Excerpted from the Applicant's study report.
Abbreviations: ATP, adenosine triphosphate; AUC, area under the concentration-time curve; 2,3-DPG, 2,3-bisphosphoglyceric acid; NA, not applicable; PO, by mouth

Mitapivat administration improved RBC morphology, and increased total hemoglobin, mean corpuscular volume, and mean corpuscular hemoglobin and significantly reduced reticulocyte counts in $Hbb^{th3/+}$ mice administered mitapivat compared to vehicle for 21 days; similar findings

were obtained after dosing for 56 days. Hbb^{th3}/+ mice exhibit defective β-globin chains and are considered a model of β-thalassemia. This mouse model does not reflect a deficiency in PK activity, but does reflect impaired RBC structure, function, and longevity and thus provides a means to evaluate parameters of anemia at the RBC level. In the mitapivat-administered Hbb$^{th3/+}$ mice, significant improvements in RBC indices, hemoglobin and RBC morphology were associated with a significant reduction in α-globin membrane aggregates, increased soluble Hb in RBC, lower RBC oxidative stress, reduced plasma total bilirubin, and increased plasma ATP levels. In these mice, mitapivat improved iron regulation, as evidenced by increased expression of hepcidin; decreased expression of iron transporters; and decreased iron overload in duodenal enterocytes, hepatocytes and Kupffer cells.

Figure 16. Effect of Mitapivat on RBC Indices in Hbb$^{th3/+}$ Mice (21-Day Dosing)

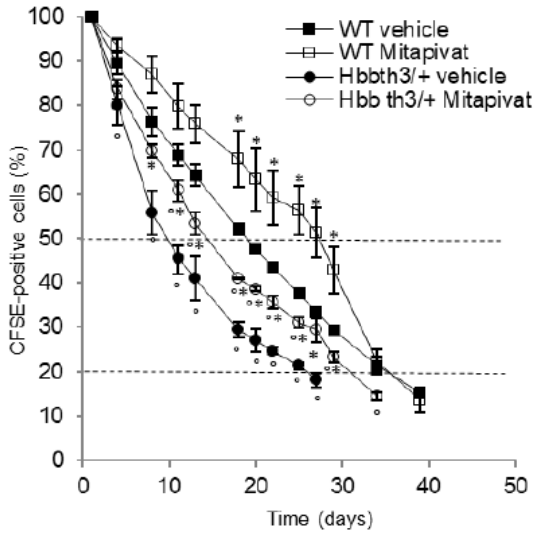


Abbreviations: ANOVA = analysis of variance; Hb = hemoglobin; MCH = mean corpuscular hemoglobin; MCV = mean corpuscular volume; SD = standard deviation; WT = wild-type.
Note: Data are mean±SD (n=6/group; experiment performed in single replicate).
°P<0.05 compared with WT vehicle and *P<0.05 compared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

Source: Excerpted from the Applicant's study report.

Figure 17. Effect of Mitapivat on Survival of RBCs From Hbb$^{th3/+}$ Mice

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



Abbreviations: ANOVA = analysis of variance; CFSE = carboxyfluorescein succinimidyl ester; RBC = red blood cell; SD = standard deviation; T_{20} = day at which 20% of labeled RBCs remained ; T_{50} = day at which 50% of labeled RBCs remained; WT = wild-type.
Note: Data are mean±SD (n=4 for wild-type vehicle group; n=3 for WT vehicle group and Hbb$^{th3/+}$ and WT mitapivat groups; experiment performed in single replicate).
°P<0.05 compared with WT vehicle and *P<0.05 compared with vehicle-treated animals of the same genotype by 1-way ANOVA with Dunnett's test for longitudinal comparison.

Source: Excerpted from the Applicant's study report.

13.1.1.2. Secondary Pharmacology

Off-target effects identified for mitapivat (AG-348) were an antagonistic or inverse agonistic effect on histamine H3 receptors with a half-maximal inhibitory concentration (IC_{50}) of 0.102 and 0.012µM, respectively, and inhibition of aromatase activity in human placental microsomes with an IC_{50} of 2.05µM.

Table 61. Inhibition of Histamine H3 Receptor by Mitapivat

Target	AG-348	AGI-8702
Histamine receptor	**% binding inhibition at 10 or 30 µM**	**% binding inhibition at 10 µM**
H3	72 (10 µM)	<50
H2	64 (10 µM)	<50
H1	55 (30 µM)	<50
Histamine receptor	**IC_{50} (µM)**	**IC_{50} (µM)**
H3	1.25	ND
H2	6.4	ND
H1	ND	ND
Histamine receptor	**Functional antagonism/inverse agonism IC_{50} (nM)**	**Functional antagonism/inverse agonism IC_{50} (nM)**
H3	102/12	ND
H2	No activity/No activity	ND
H1	ND	ND

Source: Excerpted from the Applicant's report.
Abbreviations: IC, inhibitory concentration; ND, not done

The inhibition of aromatase in human placental microsomes is relevant in humans because the maximum plasma concentration (C_{max}) in humans at the recommended dose of 50 mg twice daily (BID) is 2.14µM (965 ng/mL), which is comparable to the IC_{50} in human placental microsomes. Mitapivat inhibited rat aromatase activity (in rat ovarian microsomes) with a potency four-fold greater than that of human aromatase (human placental microsomes). Compared to the parent drug, its major metabolite AG-8702 did not significantly activate PK-R (200-fold weaker) and did not exhibit significant off-target binding of H3 histamine or inhibition of human aromatase.

Table 62. Inhibition of Rat and Human Aromatases by Mitapivat (AGI-0001480) and Other Known Aromatase Inhibitors

Compound ID	Enzyme Species	IC_{50} (nM)	IC_{50} (µM)
AGI-0001480	Human	**2050 nM**	**2.05**
Anastrozole	Human	**138 nM**	**0.138**
Exemestane	Human	**397 nM**	**0.397**
Fadrozole	Human	**12.1 nM**	**0.0121**
Letrozole	Human	**8.29 nM**	**0.00829**
AGI-0001480	Rat	**493 nM**	**0.493**
Anastrozole	Rat	**2.87 nM**	**0.00287**
Exemestane	Rat	**0.672 nM**	**0.000672**
Fadrozole	Rat	**21.6 nM**	**0.0216**
Letrozole	Rat	**0.392 nM**	**0.000392**

Source: Excerpted from the Applicant's study report.
Abbreviations: IC, inhibitory concentration

13.1.1.3. Safety Pharmacology

Table 63. Safety Pharmacology Studies and Findings

Study/Study No.	Findings
AG-348-N-023: Effect of mitapivat on cloned hERG potassium channels expressed in human embryonic kidney cells	Mitapivat inhibited hERG potassium channel current at an IC_{50} of 29.4µM and an IC_{20} of 8.6µM (Hill coefficient, 1.1), suggesting a low potential for inhibition of the hERG current at the clinical exposure (clinical Cmax: 935.2 ng/mL). As expected, terfenadine, the positive control, caused a significant 83% inhibition of hERG at 60nM.
Potential neurobehavioral effects in male Sprague-Dawley rats: AG-348-N-046-R1 (b) (4) 888061) Sprague-Dawley rats 6 males/group 0, 30, 150, 300 mg/kg Oral, single dose	There were no treatment-related adverse effects on behavioral, physiological, or neurological parameters assessed up to the highest dose tested (29× MRHD, based on Day 1 AUC in the chronic toxicity study).
Potential cardiovascular effects in male cynomolgus monkeys: AG-348-N-048-R1 Cynomolgus monkeys (telemetered conscious) 4 males/group Nasogastric gavage: 10, 25, and 75 mg/kg (5 mL/kg) single dose with a 3-7-day wash-out period between doses.	There were no treatment-related effects on heart rate, arterial blood pressure, pulse pressure, body temperature, and ECG waveforms (from which the ECG intervals PR, QRS, RR, QT, and heart rate-corrected QT [QTcB] were derived) up to the highest dose tested (2.5× MRHD, based on AUC extrapolated from plasma exposures on Day 1 in the 9-month oral toxicity study).

Study/Study No.	Findings
AG-348-N-047-R1 (b) (4) 888063): Mitapivat: Respiratory assessment following oral dosing to plethysmograph-restrained Sprague-Dawley rats 8 males/group Single oral gavage dose: 30, 150, and 300 mg/kg (10 mL/kg)	There were no drug-related effects on respiratory rate, tidal volume, and minute volume up to the highest dose tested (40× MRHD, based on AUC in the chronic toxicity study).
AG-348-N-013-R1(TK:AG-348-N-013-R2): Emetic activity of mitapivat in ferret Male ferrets, n=5 Nasogastric gavage: 20, 100, and 500 mg/kg (5 mL/kg)	Oral treatment induced marked dose-dependent emetic activity in ferrets at exposures ≥100 mg/kg (21-fold MRHD based on AUC), which is consistent with the observation in monkeys in the general toxicity study at exposures comparable to clinical exposure. NOAEL was 20 mg/kg (2-fold MRHD based on AUC).

Source: Nonclinical Reviewer.
Abbreviations: AUC, area under the concentration-time curve; ECG, electrocardiogram; IC, inhibitory concentration; MRHD, maximum recommended human dose; NOAEL, no observable adverse effect level

13.1.1.4. ADME/pharmacokinetics

Absorption

- Mitapivat was administered to rats, dogs, and monkeys at a single oral dose of 10 mg/kg or as a single intravenous injection at 1 mg/kg. Following oral administration, mitapivat was rapidly absorbed in all three species, with rapid clearance and a high volume of distribution. Half-life ranged from 3.7 and 3.9 hours in dogs and monkeys to 9.5 hours in rats. Oral bioavailability was highest in dogs (81.7%), followed by rats (50/4% and monkeys (24.4%).

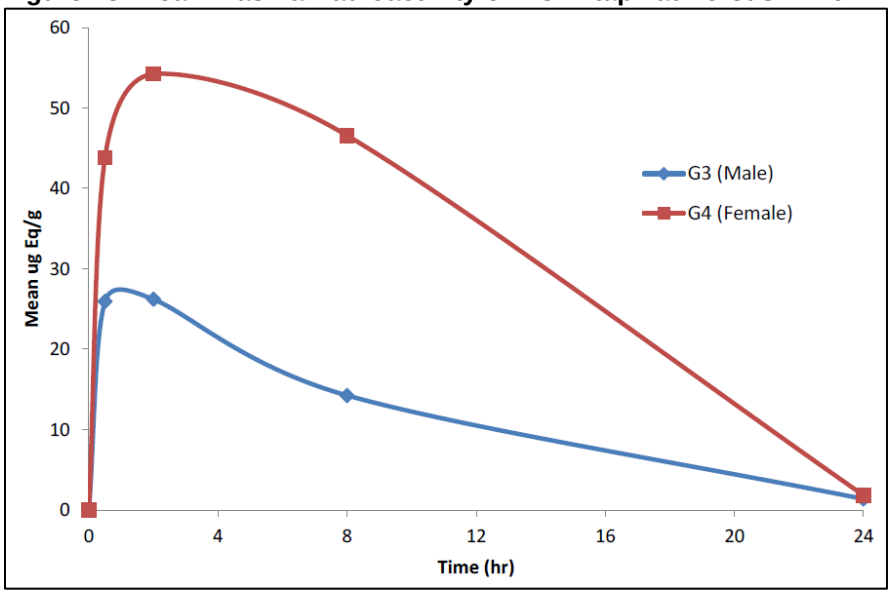
Table 64. Pharmacokinetics of Mitapivat Following a Single Oral (10 mg/kg) Administration

Species[1]	Sex	$t_{½}$ (hr)	C_{max} (ng/mL)	t_{max} (hr)	AUC_{0-24hr} (hr•ng/mL)	$AUC_{0-\infty}$ (hr•ng/mL)	F[2] (%)	Report Number
Sprague Dawley rats	M	9.5 ± 2.3	3,750 ± 1,970	0.19 ± 0.096	2,760 ± 1,340	2,800 ± 1,330	50.4 ± 23.9	AG348-N-032-R1
Beagle dogs	M	3.7 ± 0.37	2,610 ± 1,770	0.58 ± 0.38	5,870 ± 1,470	5,900 ± 1,480	81.7 ± 20.5	AG348-N-034-R1
Cynomolgus monkeys	M	3.9 ± 0.26	1,080 ± 83.3	2.0 ± 0.0	2,310 ± 148	2,320 ± 152	24.4 ± 1.59	AG348-N-035-R1

Abbreviations: $AUC_{0-\infty}$ = area under the concentration-time curve extrapolated to infinity; AUC_{0-24hr} = area under the concentration-time curve from 0 to 24 hours; C_{max} = maximum concentration; F = absolute bioavailability; M = male; $t_{½}$ = terminal elimination half-life; t_{max} = time to maximum concentration.
Note: Mitapivat was formulated as a suspension in 0.5% methylcellulose with 0.2% Tween 80 in water.
[1] Animals were fasted before dosing.
[2] F was calculated using the $AUC_{0-\infty}$ (oral) from this table and the $AUC_{0-\infty}$ (intravenous) from Table 3.
Source: Excerpted from the Applicant's study report.

- Following single oral administration of ^{14}C mitapivat, absorption was rapid, with C_{max} at around 2 hours. Absorption was higher in females compared to males and mitapivat was detectable up to 24 hours in both sexes.

Figure 18. Mean Plasma Radioactivity of ^{14}C Mitapivat Versus Time in Rats



Source: Excerpted from the Applicant's study report.

Distribution

- In rats, [^{14}C]mitapivat was absorbed and distributed to tissues quickly following oral administration (time to maximum concentration [T_{max}] 1 to 4 hours). [^{14}C]Mitapivat was rapidly eliminated, and by 168 hours radioactivity was below the limit of quantitation in most tissues.
- Highest distribution of [^{14}C]mitapivat was in the ocular tissues (eye, uveal tract), gastrointestinal tract, and tissues of the metabolic and excretory systems. The distribution of [^{14}C]mitapivat to melanin-containing tissues, such as pigmented skin and the uveal tract of the ocular system, indicates that mitapivat has the potential to bind melanin. No adverse effects were observed in the above systems in the chronic toxicity studies in rats and monkeys. These findings do not appear to be clinically relevant.
- The distribution of mitapivat in rat brain was low, with a mean area under the concentration-time curve (AUC) (brain): AUC (plasma) ratios of 0.05 and 0.09 after single and repeated doses, respectively.
- The ability of mitapivat to partition into RBCs from plasma was low in mouse, rat, dog, monkey, and human blood (RBC partitioning coefficient [$K_{RBC/PL}$] <1).

Table 65. Protein Binding of Mitapivat in Plasma of Human, Monkey, Dog, Rat, Mouse, and Ferret

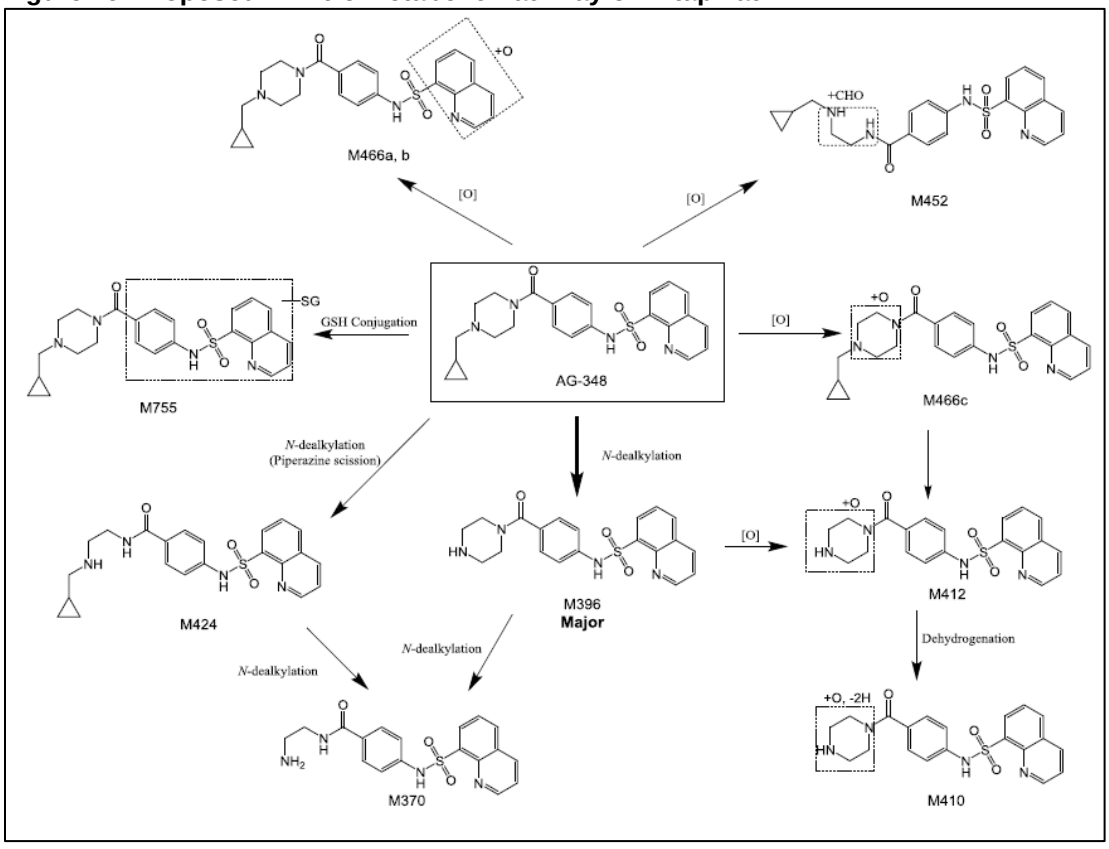
Compound	Test Concentration (µM)	Mean % Bound					
		Human	Monkey	Dog	Rat	Mouse	Ferret
AG-348	0.2	97.67	93.84	90.09	98.25	96.17	-
	1	97.70	93.64	88.93	97.98	95.55	82.88
	10	97.66	92.27	88.22	97.78	95.45	-
Mean		97.67	93.25	89.08	98.00	95.72	82.88

Source: Excerpted from the Applicant's study report.

Metabolism

- There were no disproportional or uniquely human metabolites for mitapivat.
- AG-8702, an N-dealkylated metabolite, was the most abundant metabolite formed from mitapivat in animals and humans but was not a major circulating metabolite in humans (<10% of mitapivat).
- In plasma from human and multiple animal species, including those used in the general toxicity studies, mitapivat exhibited high plasma protein binding (93 to 98% in humans, rats, and monkeys), with comparable and concentration-independent binding.
- By contrast, AG-8702, the major metabolite, exhibited moderate plasma protein binding (~50%) at concentrations up to 1µM in human plasma.
- Cytochrome p450 (CYP)3A4 and 3A5 were the primary isoforms metabolizing mitapivat to AG-8702. Trace amounts of AG-8702 were formed by CYP1A2 and 2D6.
- In vitro, mitapivat was cleared faster in rat, dog, and monkey microsomes compared to human microsomes, as evidenced by a shorter $t_{1/2}$ and higher intrinsic and hepatic clearance as well as a higher hepatic extraction ratio.

Figure 19. Proposed In Vitro Metabolic Pathway of Mitapivat



Source: Excerpted from the Applicant's study report.
Abbreviation: GSH, glutathione

Excretion

- The elimination route of mitapivat was evaluated in male and female Sprague-Dawley rats that were bile duct cannulated or noncannulated following a single oral administration of [^{14}C]mitapivat at 100 mg/kg (200 µCi/kg).
- Mean percentage radioactivity recovered from bile, urine, and feces was comparable in males, whereas in females there was a slight increase in radioactivity in bile compared to urine. Elimination of radioactivity was rapid; most was eliminated within 48 hours.
- Orally administered mitapivat was primarily eliminated by hepatic metabolism and the biliary route. Very minimal mitapivat (~1.5%) was eliminated unchanged in urine and bile in rats.

Table 66. Mean Cumulative Percentage Excretion of Radioactivity in BDC Rats Following a Single Oral Administration of ^{14}C Mitapivat

Group	Matrix	Percent of Dose (%) (N=3)		
		0-24 hr	0-48 hr	0-168 hr
G1 (Male)	Bile	33.1 ± 7.68	33.9 ± 7.45	34.4 ± 7.27
	Urine	28.5 ± 6.39	30.5 ± 7.51	31.8 ± 8.76
	Feces	25.3 ± 4.11	29.0 ± 5.74	30.1 ± 4.80
	Total*	**86.9 ± 6.79**	**93.4 ± 7.07**	**97.0 ± 4.73**
G2 (Female)	Bile	46.3 ± 4.38	48.1 ± 5.39	48.8 ± 5.49
	Urine	11.8 ± 1.57	13.1 ± 1.82	13.9 ± 2.53
	Feces	24.5 ± 1.83	28.7 ± 3.43	30.4 ± 1.56
	Total*	**82. 7 ± 2.02**	**89.9 ± 4.67**	**93.5 ± 4.25**

*Includes cage wash at 168 hours post-dose

Source: Excerpted from the Applicant's study report.
Abbreviations: BDC, bile duct cannulated; G1, group 1; G2, group 2; N, total number of subjects

Pharmacokinetic Drug Interactions

- In human cultured hepatocytes, mitapivat caused induction of CYP3A4/5 (EC_{50}: ~1µM), CYP2B6 (1.2 to 3.7µM), CYP2C8 (0.2 to 7.6µM), CYP2C9 (0.4 to 0.6µM), CYP2C19 (2 to 3.3µM), and UGT1A1 (1.4 to 11µM), which was consistent with observations in the rat and monkey chronic toxicity studies of hepatocellular hypertrophy and increased liver weight in both species, and increased thyroid weight in rats. Induction of CYP450, particularly CYP3A4/5 (the major metabolizing enzymes), suggests the potential for interaction of mitapivat with drugs that inhibit or induce these enzymes.
- Furthermore, a metabolism-based inhibition of CYP3A4/5 by mitapivat was observed.

- Mitapivat is an inhibitor of p-glycoprotein (P-gp) with IC_{50} values of 12.8 uM and potential for drug-drug interations based on the static model (I_{gut} [intestinal concentration] ÷ IC_{50} >10). Mitapivat does not inhibit BCRP and organic anion transporting polypeptide OATP1B3, but does inhibit bile salt export pump (BSEP), organic anion transporter (OAT3), organic cation transporter (OCT2), and multidrug and toxin extrusion-1 (MATE1) with IC_{50} values of, 22.0, 12.1, 7.76, and 7.17μM, respectively.

13.1.1.5. Toxicokinetic Data

Table 67. Toxicokinetic Data Studies and Findings

Study/Study No.	Major Findings
General Toxicology Studies	
AG-348-N-083-R1: 6-Month (Twice Daily) Oral (Gavage) Toxicity and Toxicokinetic Study of Mitapivat in Sprague-Dawley Rats Sample collection times: predose and 1, 2, 4, 8, and 12 hours postdose Mitapivat and AG-8702 Accumulation: None Dose proportionality: (F) Greater than dose proportional; (M) greater than dose-proportional at medium and high doses Gender differences: Mitapivat exposure 1.5- to 3.6-fold greater in females. AG-8702 exposure ~10-fold greater in males **NOAEL: (M) 60 mg/kg/day** **Safety Margin: 7×** **(F) 50 mg/kg/day** **Safety Margin: 18×**	**Table 68. Toxicokinetic Parameters of Mitapivat and AG-8702 in Rats** $AUC_{0\text{-}12}$ (ng•hr/mL); C_{max} (ng/mL); T_{max} (hr) Source: Excerpted from the Applicant's study report. Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum serum concentration; t_{max}, time for drug maximum concentration

Dosage	$AUC_{0\text{-}12}$ (ng•hr/mL)			C_{max} (ng/mL)			T_{max} (hr)		
	Day 0	Day 90	Day 180	Day 0	Day 90	Day 180	Day 0	Day 90	Day 180
AG-348									
Males									
40 mg/kg/day	5100	10,700	20,400	1730	2970	7310	1.0	2.0	1.0
60 mg/kg/day	7310	16,300	23,900	3160	4770	9770	1.0	1.0	1.0
150 mg/kg/day	35,600	44,200	70,600	9710	12,300	25,800	1.0	1.0	1.0
300 mg/kg/day	105,000	122,000	144,000	17,600	18,500	32,600	1.0	1.0	1.0
Females									
10 mg/kg/day	2420	4890	7880	1180	1710	2790	1.0	1.0	1.0
20 mg/kg/day	6630	14,500	23,400	2530	5520	6950	1.0	1.0	1.0
50 mg/kg/day	27,200	42,800	66,000	8460	8860	16,700	1.0	2.0	1.0
200 mg/kg/day	250,000	201,000	292,000	35,200	23,800	63,900	1.0	1.0	1.0
AGI-8702									
Males									
40 mg/kg/day	1230	2510	2970	431	857	835	1.0	1.0	1.0
60 mg/kg/day	1810	4210	5090	650	1170	1520	1.0	1.0	1.0
150 mg/kg/day	9910	8710	10,500	2420	2120	3070	1.0	1.0	1.0
300 mg/kg/day	27,500	23,900	25,100	5190	4430	5870	1.0	1.0	1.0
Females									
10 mg/kg/day	18.3	49.0	93.0	10.2	14.8	32.1	1.0	1.0	1.0
20 mg/kg/day	60.2	183	252	24.3	76.7	78.5	1.0	1.0	1.0
50 mg/kg/day	259	583	792	77.8	151	260	1.0	1.0	1.0
200 mg/kg/day	2110	2780	4360	321	440	1110	1.0	1.0	1.0

Study/Study No. | **Major Findings**

AG-348-N-082-R1: A 9-Month (Twice Daily) Oral (Nasogastric) Toxicity and Toxicokinetic Study of Mitapivat in Cynomolgus Monkeys
Sample collection times: predose and 1, 2, 4, 8, and 12 hours postdose
Mitapivat and AG-8702
Accumulation: none
Dose proportionality: proportional
Gender differences: None
AG-8702/Mitapivat, 1.4-2.4
NOAEL: (M) 50 mg/kg/day
Safety margin: 1×
(F) 100 mg/kg/day
Safety margin: 2×

Table 69. Mitapivat Toxicokinetic Parameters in Monkeys (M+F)

Dosage	$AUC_{0\text{-}12\ hr}$ (ng•hr/mL)		C_{max} (ng/mL)		T_{max} (hr)	
	Male	Female	Male	Female	Male	Female
Study Day 0						
50 mg/kg/day	5020	4150	1700	1530	1.7	1.3
100 mg/kg/day	12,800	11,300	4310	3300	2.3	1.5
200 mg/kg/day	31,800	27,900	6500	5910	2.7	2.3
Study Day 90						
50 mg/kg/day	3710	3150	1530	1040	1.2	1.3
100 mg/kg/day	7750	10,100	2250	4090	1.8	1.5
200 mg/kg/day	17,400	16,700	4870	4880	2.0	2.0
Study Day 180						
50 mg/kg/day	4220	3880	1640	1150	1.0	1.3
100 mg/kg/day	8980	9850	2620	3320	1.5	1.8
200 mg/kg/day	23,300	24,900	6950	6890	1.8	2.7
Study Day 270						
50 mg/kg/day	4370	4100	1570	1270	1.2	1.3
100 mg/kg/day	9100	8040	3380	2350	1.8	2.0
200 mg/kg/day	21,300	19,100	6720	4680	1.5	3.0

Source: Excerpted from the Applicant's study report.
Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum serum concentration; $t_{1/2}$, drug half-life; t_{max}, time for drug maximum concentration

Table 70. AG-8702 Toxicokinetic Parameters in Monkeys (M+F)

Daily Dose (mg/kg/day)	Day		C_{max} (ng/mL)	t_{max} (h)	$AUC_{0\text{-}12}$ (ng•h/mL)	$t_{1/2}$ (h)	$AUC_{0\text{-}12}$ Ratio (AGI-8702/ AG-348)
50	0	Mean	2230	1.5	8470	2.5	2.42
		SD	729	0.90	1750	0.83	1.78
		n	12	12	12	10	12
	90	Mean	2030	1.3	6950	3.5	2.32
		SD	971	0.45	2360	1.2	1.44
		n	12	12	12	11	12
	180	Mean	1850	1.4	7670	3.3	2.24
		SD	842	0.51	2570	1.3	1.40
		n	12	12	12	10	12
	270	Mean	1960	1.4	7930	2.9	2.18
		SD	504	0.51	2060	0.78	1.40
		n	12	12	12	12	12
100	0	Mean	4720	2.0	19600	2.4	2.21
		SD	1370	1.0	5120	0.98	1.89
		n	12	12	12	8	12
	90	Mean	4030	1.8	14600	2.6	1.74
		SD	1610	0.45	3990	0.64	0.464
		n	12	12	12	11	12
	180	Mean	3760	1.8	15400	2.6	1.71
		SD	1190	0.39	3660	0.42	0.475
		n	12	12	12	10	12
	270	Mean	3850	2.1	14400	2.6	1.74
		SD	1770	0.67	4700	0.47	0.391
		n	12	12	12	8	12
200	0	Mean	7200	2.8	38400	2.6	1.41
		SD	2390	1.0	12300	0.54	0.701
		n	12	12	12	7	12
	90	Mean	6500	2.2	26200	2.9	1.54
		SD	3010	0.58	9870	0.80	0.234
		n	12	12	12	10	12
	180	Mean	7910	2.3	34500	2.4	1.45
		SD	2110	0.78	11300	0.59	0.147
		n	12	12	12	10	12
	270	Mean	7560	2.3	32000	2.6	1.59
		SD	2380	1.1	7410	0.62	0.125
		n	12	12	12	8	12

Study/Study No.	Major Findings
	Source: Excerpted from the Applicant's study report. Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum serum concentration; n, number of subjects in each category; SD, standard deviation; $t_{1/2}$, drug half-life; t_{max}, time for drug maximum concentration
Reproductive Toxicology Studies	
AG-348-N-086: A Combined Fertility and Early Embryonic Development Study of Mitapivat Administered by Oral Gavage in Rats **NOAEL: (M) 300 mg/kg/day; safety margin: 45×** **(F) 200 mg/kg/day; safety margin 49×**	**Table 71. TK Parameters for Mitapivat in the Rat FEED Study, on Day 84 in Males and Day 29 in Females** Summary of Toxicokinetic Parameters

Gender	Study Day	BID Dose (mg/kg/day)	C_{max} (ng/mL)	T_{max} (h)	$AUC_{0\text{-}12h}$ (ng•h/mL)
Male	84	40	5680	1	11700
		60	8060	1	21800
		150	24300	1	83700
		300	28500	1	160000
Female	29	10	1760	1	3760
		20	5480	1	15800
		50	36600	1	69300
		200	35000	1	174000

Source: Excerpted from the Applicant's study report.
Abbreviations: AUC, area under the concentration-time curve; BID, twice daily; C_{max}, maximum serum concentration; SD, standard deviation; t_{max}, time for drug maximum concentration

AG-348-N-080-R1: An Oral (Gavage) Study of the Effects of Mitapivat on Embryo/Fetal Development in Rats
NOAEL: 50 mg/kg/day
Safety margin: 13×

Table 72. TK Parameters of Mitapivat and AG-8702 in Rats, EFD Study

AG-348 Dosage Level (mg/kg/day)	Gestation Day	C_{max} (ng/mL)	t_{max} (h)	$AUC_{0\text{-}12}$ (ng•hr/mL)	$t_{1/2}$ (h)
10	6	906	1	2020	1.84
	17	1410	1	4250	2.36
20	6	1990	1	4790	1.94
	17	3480	1	12500	2.14
50	6	8400	1	23900	1.77
	17	11400	1	46800	2.92
200	6	32400	1	253000	ND
	17	30700	1	227000	6.41

ND = not determined (r^2 <0.85).

Text Table 4. Toxicokinetic Parameters of AGI-8702

AG-348 Dosage Level (mg/kg/day)	Gestation Day	C_{max} (ng/mL)	t_{max} (h)	$AUC_{0\text{-}12}$ (ng•hr/mL)	$t_{1/2}$ (h)
10	6	9.67	1	22.6	ND
	17	18.1	1	61.4	2.10
20	6	21.1	1	52.8	2.24
	17	47.1	1	161	2.30
50	6	88.8	1	267	2.33
	17	122	1	519	2.92
200	6	321	1	2110	4.81
	17	433	1	2930	6.53

Source: Excerpted from the Applicant's study report.
Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum serum concentration; n, number of subjects in each category; ND, not determined; SD, standard deviation; $t_{1/2}$, drug half-life; t_{max}, time for drug maximum concentration

Study/Study No.	Major Findings
AG-348-N-081-R1: An Oral (Gavage) Study of the Effects of Mitapivat on Embryo/Fetal Development in Rabbits **NOAEL: 30 mg/kg/BID** **Safety margin: 1.5×**	**Table 73.TK Parameters of Mitapivat in Rabbits, EFD Study**

BID Dose (mg/kg/dose)	Dosage Level (mg/kg/day)	Gestation Day		C_{max} (ng/mL)	t_{max} (hr)	AUC_{0-12} (ng•hr/mL)
12.5	25	7	Mean	610	1.0	1370
			SD	336	0.0	643
			N	3	3	3
		20	Mean	942	1.0	2390
			SD	121	0.0	755
			N	3	3	3
30	60	7	Mean	2320	1.0	3930
			SD	796	0.0	1050
			N	3	3	3
		20	Mean	2430	1.0	5360
			SD	768	0.0	1470
			N	3	3	3
62.5[a]	125[a]	7	Mean	4270	1.0	9190
			SD	1130	0.0	1140
			N	3	3	3
		20	Mean	5390	1.0	11200
			SD	1880	0.0	1060
			N	3	3	3

[a] = Female no. 72039 was nongravid and was included in the calculations.
Source: Excerpted from the Applicant's study report.
Abbreviations: AUC, area under the concentration-time curve; BID, twice daily; C_{max}, maximum serum concentration; N, number of subjects; SD, standard deviation; t_{max}, time for drug maximum concentration

Table 74. TK Parameters for AG-8702 in Rabbits, EFD Study

BID Dose (mg/kg/dose)	Dosage Level (mg/kg/day)	Gestation Day		C_{max} (ng/mL)	t_{max} (hr)	AUC_{0-12} (ng•hr/mL)
12.5	25	7	Mean	92.0	1.0	246
			SD	30.7	0.0	91.6
			N	3	3	3
		20	Mean	196	1.0	596
			SD	34.3	0.0	89.7
			N	3	3	3
30	60	7	Mean	273	1.0	598
			SD	61.7	0.0	105
			N	3	3	3
		20	Mean	848	1.0	2730
			SD	244	0.0	702
			N	3	3	3
62.5[a]	125[a]	7	Mean	532	1.0	1480
			SD	179	0.0	387
			N	3	3	3
		20	Mean	1830	1.0	6310
			SD	559	0.0	1270
			N	3	3	3

[a] = Female no. 72039 was nongravid and was included in the calculations.
Source: Excerpted from the Applicant's study report.
Abbreviations: AUC, area under the concentration-time curve; BID, twice daily; C_{max}, maximum serum concentration; N, number of subjects; SD, standard deviation; t_{max}, time for drug maximum concentration

Study/Study No.	Major Findings
Carcinogenicity studies	
AG-348-N-088: A 2-Year Oral Gavage Carcinogenicity Study of Mitapivat in Sprague-Dawley Rats Sample collection times: predose and 1, 2, 4, 8, and 12 hours postdose Mitapivat and AG-8702 Accumulation: minimal Dose proportionality: Greater than dose proportional except Day 182 in high-dose males Gender differences: Mitapivat exposure 2.4-fold greater in females. AG-8702 exposure ~10-fold greater in males **NOAEL: (M) 150 mg/kg/BID Safety margin: 47×** **(F) 100 mg/kg/BID Safety margin: >100×**	**Table 75. Toxicokinetic Parameters of Mitapivat After Chronic Oral Administration of Mitapivat in Rats** Source: Excerpted from the Applicant's study report. Abbreviations: AUC, area under the concentration-time curve; BID, twice daily; C_{max}, maximum serum concentration; F, female; M, male; N, number of subjects; ND, not determined; SD, standard deviation; $t_{1/2}$, drug half-life; t_{max}, time for drug maximum concentration **Table 76. Toxicokinetic Parameters of AG-8702, a Metabolite of Mitapivat, After Chronic Oral Administration of Mitapivat in Rats** Source: Excerpted from the Applicant's study report. Abbreviations: AUC, area under the concentration-time curve; BID, twice daily; C_{max}, maximum serum concentration; F, female; M, male; N, number of subjects; ND, not determined; SD, standard deviation; $t_{1/2}$, drug half-life; t_{max}, time for drug maximum concentration

Sex	Day	AG-348 BID Dose (mg/kg)	C_{max} (ng/mL)	t_{max} (h)	$AUC_{0\text{-}12h}$ (ng•h/mL)	$t_{1/2}$ (h)	C_{max}/Dose (ng/mL)/(mg/kg)	$AUC_{0\text{-}12h}$/Dose (ng•h/mL)/(mg/kg)
F	1	10	1360	1	4340	2.34	136	434
		25	4990	1	20300	2.12	200	811
		100	26000	8	222000	ND	260	2220
	28	10	4690	1	13200	2.17	469	1320
		25	15000	1	54700	2.53	599	2190
		100	63300	1	390000	ND	633	3900
	182	10	10900	1	28600	2.15	1090	2860
		25	27300	1	102000	2.44	1090	4080
		100	74400	1	409000	4.33	744	4090
M	1	15	565	1	1940	2.55	37.6	129
		50	3040	2	11900	1.91	60.9	238
		150	11000	1	85000	ND	73.2	567
	28	15	1350	1	3940	2.71	89.9	263
		50	11900	1	42700	ND	238	854
		150	31600	1	136000	ND	211	905
	182	15	6020	1	13000	1.98	401	866
		50	25300	1	83100	1.64	506	1660
		150	50000	1	168000	2.55	333	1120

Sex	Day	AG-348 BID Dose (mg/kg)	C_{max} (ng/mL)	t_{max} (h)	$AUC_{0\text{-}12h}$ (ng•h/mL)	$t_{1/2}$ (h)	C_{max}/Dose (ng/mL)/(mg/kg)	$AUC_{0\text{-}12h}$/Dose (ng•h/mL)/(mg/kg)
F	1	10	13.5	1	54.1	3.29	1.35	5.41
		25	52.5	1	230	2.56	2.10	9.19
		100	251	2	2140	3.27	2.51	21.4
	28	10	50.8	1	139	2.18	5.08	13.9
		25	164	1	632	2.62	6.57	25.3
		100	746	1	4330	3.30	7.46	43.3
	182	10	73.6	1	271	2.71	7.36	27.1
		25	322	1	1010	3.16	12.9	40.5
		100	1010	1	5950	4.78	10.1	59.5
M	1	15	108	1	487	2.90	7.22	32.4
		50	630	1	2900	2.02	12.6	57.9
		150	3970	1	24600	ND	26.5	164
	28	15	385	1	1340	2.75	25.7	89.6
		50	3160	1	11700	ND	63.2	234
		150	9520	1	41500	ND	63.5	277
	182	15	903	1	2680	2.02	60.2	178
		50	3310	1	10900	1.99	66.2	218
		150	7690	1	29200	2.62	51.3	195

Study/Study No.	Major Findings
AG-348-N-095: A 26-Week Carcinogenicity Study of Mitapivat by Oral Gavage in CByB6F1/Tg rasH2 Hemizygous Mice Sample collection times: predose and 1, 2, 4, 8, and 12 hours postdose Mitapivat and AG-8702 Accumulation: none Dose proportionality: Greater than dose proportional Gender differences: none **NOAEL: (M) 250 mg/kg/BID; (F) 125 mg/kg/BID**	**Table 77. Toxicokinetics of Mitapivat in Male and Female Transgenic ras H2 Mice** Source: Excerpted from the Applicant's study report. Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum serum concentration; SE, standard error; T_{max}, time for drug maximum concentration **Table 78. Toxicokinetics of AG-8702 in Male and Female Transgenic ras H2 Mice** Source: Excerpted from the Applicant's study report. Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum serum concentration; SE, standard error; T_{max}, time for drug maximum concentration

Analyte	Sex	Day	Dose (mg/kg /dose)	T_{max} (hr)	C_{max} (ng/mL)	SE C_{max} (ng/mL)	$AUC_{(0-12)}$ (hr*ng/mL)	SE $AUC_{(0-12)}$ (hr*ng/mL)
AG-348	Male	1	30	1	186	9.70	393	24.5
			100	1	2720	713	6530	1230
			250	1	9490	390	32500	6880
		28	30	1	314	128	573	131
			100	1	642	124	2820	470
			250	1	6380	2260	16500	2640
		182	30	1	261	54.8	718	66.4
			100	1	4020	686	6570	926
			250	1	11200	3710	22800	6640
	Female	1	12.5	1	112	8.52	322	21.3
			37.5	1	427	52.5	1180	79.6
			125	1	5860	1690	13000	1850
		28	12.5	1	113	8.21	349	36.9
			37.5	1	378	72.2	1370	96.3
			125	1	1850	307	7280	476
		182	12.5	1	168	4.51	567	57.5
			37.5	1	545	38.3	1890	190
			125	1	4380	1950	9180	2170

Analyte	Sex	Day	Dose (mg/kg /dose)	T_{max} (hr)	C_{max} (ng/mL)	SE C_{max} (ng/mL)	$AUC_{(0-12)}$ (hr*ng/mL)	SE $AUC_{(0-12)}$ (hr*ng/mL)
AGI-8702	Male	1	30	1	55.5	1.99	129	11.6
			100	1	728	260	1740	320
			250	1	1580	45.1	5290	748
		28	30	1	124	33.7	220	34.6
			100	1	505	93.2	1480	147
			250	1	4020	786	11300	1180
		182	30	1	96.2	8.50	236	17.6
			100	1	1480	181	2830	325
			250	1	6000	983	12500	2680
	Female	1	12.5	2	180	170	315	255
			37.5	1	379	97.0	728	128
			125	1	901	221	3480	899
		28	12.5	2	50.2	40.4	126	60.8
			37.5	1	115	14.4	266	15.2
			125	1	497	50.3	1910	475
		182	12.5	1	19.3	2.30	77.9	7.38
			37.5	2	944	675	1690	1010
			125	4	1540	805	7170	2520

Source: Nonclinical Reviewer.
Safety margins were based on the AUC_{0-12} at steady-state with a geometric mean of 3591.4 h*ng/mL (%CV 28%) at the MRHD of 50 mg BID.
Abbreviations: AUC, area under the curve; BID, twice daily; C_{max}, maximum plasma concentration; CV, coefficient of variation; F, female; NOAEL, no observed adverse effect level; M, male

13.1.1.6. Toxicology

13.1.1.6.1. General Toxicology

AG-348-N-083-R1: A 6-Month (Twice Daily) Oral (Gavage) Toxicity and Toxicokinetic Study of AG-348 (Mitapivat) in Sprague-Dawley Rats

Key Study Findings

- Dose-dependent and statistically significant increase in liver weight was observed in males (up to 22%) at ≥30 mg/kg/BID (7× maximum recommended human dose [MRHD], based on AUC) and in females (16%) at 100 mg/kg/BID (82× MRHD, based on AUC) which correlated microscopically with dose-dependent hepatocellular hypertrophy in males (minimal) and females (minimal to mild). Liver findings were not associated with clinical chemistry changes or microscopic changes indicative of liver injury, were reversible, and thus appeared to be an adaptive nonadverse response to mitapivat, which is an inducer of CYP450 enzymes.
- Reproductive toxicity, likely secondary to aromatase inhibition, was evident in both males and females. Tubular degeneration in the testes, spermatid retention in the epididymis, abnormal sperm morphology, and reduced sperm density occurred at ≥75 mg/kg/BID (≥20× MRHD). In females, incomplete corpora lutea, uterine atrophy, and increased mucification of the vagina were observed at 200 mg/kg (81× MRHD). All findings were reversible or trended towards reversibility at dose cessation.
- No observed adverse effect level (NOAEL) is 30 mg/kg/BID (7× MRHD, based on AUC) and 25 mg/kg/BID (18× MRHD, based on AUC) in males and females, respectively.

Table 79. Methods of the 26-Week Oral Toxicity Study in Rats

Study Feature	Method Details
GLP compliance:	Yes
Dose and frequency of dosing:	(M): 0 (vehicle), 20, 30, 75, and 150 mg/kg/BID (40, 60, 150, and 300 mg/kg/day) for 6 months (F): 0 (vehicle), 5, 10, 25, and 100 mg/kg/BID (10, 20, 50, and 200 mg/kg/day) for 6 months
Route of administration:	Oral gavage
Formulation/vehicle:	0.5% methylcellulose, 400 cps/5% povidone K30 in deionized water
Species/strain:	Rat/Sprague-Dawley
Number/sex/group:	30/sex/group
Age:	8 weeks at initiation
Satellite groups/unique design:	TK 9/sex/group for mitapivat-administered groups 3/sex/group for vehicle control
Deviation from study protocol affecting interpretation of results:	None

Source: Nonclinical Reviewer.
Abbreviations: BID, twice daily; F, female; GLP, good laboratory practices; M, male; TK, toxicokinetics

Table 80. Observations and Results of the 26-Week Oral Toxicity Study in Rats

Parameter	Major Findings
Mortality	No mitapivat-related mortalities at any dose level in either sex.
Clinical signs	Unremarkable.

Parameter	Major Findings
Body weights	In males, mitapivat caused a consistent reduction in body weight gain over the study duration, resulting in statistically significant decreases in body weight of 8.1% and 9.2% at 75 and 150 mg/kg/BID, respectively, compared to vehicle control. Although body weight gain was similar to controls during the recovery period, mean body weight remained 9.0% and 10.2% lower (not statistically significant) at 75 and 150 mg/kg/BID, respectively, compared to control. In females, there was a statistically significant increase in body weight at the high dose (11.2%) compared to vehicle control, which remained higher (16.7%, not statistically significant) at the end of recovery period. The decrease in body weight in male rats was mild, not associated with clinical signs of ill health, and unlikely to be adverse. There was no mitapivat-related change in food consumption in male rats at any dose level. Consistent with increased body weight, food consumption was increased in females at 100 mg/kg/BID compared to vehicle control. At several points during the study period, the increase was statistically significant.
Ophthalmoscopy	Unremarkable.
Hematology	There were no mitapivat-related adverse effects on hematologic and coagulation parameters at any dose.
Coagulation	No mitapivat-related adverse effects on coagulation parameters in either sex.
Clinical chemistry	There were no mitapivat-related adverse effects on clinical chemistry parameters in either sex at any dose level.
Urinalysis	There were no mitapivat-related adverse urinalysis changes in either sex at any dose level.
Gross pathology	There was mitapivat-related paleness of adrenal glands in three male rats at 300 mg/kg/day. Paleness correlated microscopically with vacuolation of the zona glomerulosa.
Organ weights	There were mitapivat-related statistically significantly increases in absolute and relative (to body weight) liver weight in males at ≥60 mg/kg/day and in females at 100 mg/kg/BID (highest dose), which correlated microscopically with dose-dependent hepatocellular hypertrophy (minimal in males; minimal to mild in females). In females, increased ovary/oviduct, kidney, and thyroid/parathyroid weights (absolute and relative to body weight or brain weight) were observed at the HD, but there were no correlative microscopic changes.
Histopathology Adequate battery: Yes	Adrenal: Dose-dependent increase in the incidence and severity of minimal to mild vacuolation of zona glomerulosa was seen in all male dose groups and in females at ≥10 mg/kg/BID but was not associated with adrenal gland dysfunction. Therefore, the microscopic changes in adrenal gland are likely secondary to stress due to chronic drug administration and not likely adverse. Liver (males at ≥75 mg/kg/BID and in females at ≥10 mg/kg/day): Mitapivat caused a dose-dependent increase in the incidence of centrilobular hepatocyte hypertrophy in males (minimal) and females (minimal to mild). Hepatocellular hypertrophy was not associated with clinical chemistry (AST, ALT, ALP) or microscopic (necrosis) markers of liver injury and appears to be secondary to induction of liver metabolic enzymes and not likely adverse. Male reproductive tract (at ≥75 mg/kg/BID): In the epididymis there was increased incidence and severity of cellular debris and severely reduced luminal sperm, which correlated with increased spermatid retention, tubular degeneration, and increasing incidence of minimal Leydig cell hypertrophy. Leydig cell hypertrophy was characterized by increasing eosinophilic cytoplasm resulting in a swollen appearance of the cell and was not associated with other morphological changes characteristic of injury to testes and hence not likely adverse. Spermatid retention was characterized by the presence of elongating spermatids (step 19) after physiologic release (Stage VIII). Abnormal late stage (VIII to XIV) residual bodies along the luminal surface, early stage (I to VII) free within the lumen consistent with malformed tail

Parameter	Major Findings
	cytoplasm of the developing elongated spermatids, and occasional vacuolation of the seminiferous epithelium characterized tubular degeneration. Retained spermatids and tubular degeneration at ≥75 mg/kg/BID are likely adverse in this study due to their potential for adverse impact on fertility. Female reproductive tract (ovaries, uterus, and vagina at ≥25 mg/kg/BID): There was mitapivat-related increase in the incidence and/or severity of incomplete corpora lutea at ≥25 mg/kg/BID Incomplete corpora lutea was characterized by a wall of luteinized cells and an empty or fibrous center. Increased incidence of luteinized follicles indicating retained oocyte and lack of ovulation was observed at 100 mg/kg/BID. Incomplete corpora lutea and increased luteinized follicles have been previously observed with aromatase inhibitors and thus likely secondary to aromatase inhibition by mitapivat but not likely to affect fertility based on observations in the dedicated fertility study in rats. At 100 mg/kg/BID, among some rats with estrogen dominant morphology, there was mitapivat-related uterine atrophy, characterized by decreased overall diameter of the uterus, which was most prominent in the uterine stroma, and increased mucification of the vagina. Although reproductive senescence is a normal physiological phenomenon, and was observed in control rats, the two above changes indicate a mitapivat-induced shift in hormonal balance (likely due to aromatase inhibition) and are likely adverse to the animal. Recovery period histopathology findings: At the end of the recovery period, vacuolation of zona glomerulosa was absent at 5 mg/kg/BID but persisted, albeit with lesser severity, at ≥10 mg/kg/BID indicating recovery from the mitapivat-induced effects. There was no mitapivat-related tubular degeneration at the end of recovery, but two animals were observed with retained spermatids. This is likely due to the short recovery period. Hepatocellular hypertrophy fully recovered in males. In females, minimal hepatocellular hypertrophy was observed at ≥25 mg/kg/BID but with reduced incidence and severity compared to the study phase indicating reversibility.
Special evaluations	None

Source: Nonclinical Reviewer.
Abbreviations: ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate aminotransferase; BID, twice daily; HD, high dose

AG-348-N-082-R1: A 9-Month (Twice Daily) Oral (Nasogastric) Toxicity and Toxicokinetic Study of Mitapivat in Cynomolgus Monkey

Key Study Findings

- Mitapivat, at ≥25 mg/kg/BID (at clinical exposure level based on AUC), caused transient and sporadic emesis in monkeys with no associated adverse clinical symptoms or body weight loss.
- Mitapivat, at all doses, caused increased liver weight in males (up to 44%) and females (up to 39%) compared to vehicle controls, which correlated microscopically with diffuse hepatocellular hypertrophy, that resulted in minimal subcapsular inflammation and necrosis at ≥50 mg/kg/BID in males (2.5× MRHD, based on AUC) and at 100 mg/kg/BID in females (5× MRHD, based on AUC), likely due to increased pressure from hypertrophy. This finding was likely adverse but was reversible during the recovery period. The hypertrophic changes were reversible during recovery phase.

- NOAEL is 25 mg/kg/BID (1.2× MRHD, based on AUC) and 50 mg/kg/BID (2.2× MRHD, based on AUC) in males and females, respectively, based on the occurrence of subcapsular inflammation/necrosis at higher doses.

Table 81. Methods of the 9-Month Oral Toxicity Study in Monkeys

Study Feature	Method Details
GLP compliance	Yes
Dose and frequency of dosing:	0 (vehicle), 25, 50, and 100 mg/kg/BID (50, 100, and 200 mg/kg/day) orally for 9 months
Route of administration:	Oral gavage
Formulation/vehicle:	0.5% methylcellulose 400 cps/5% povidone K30 in deionized water
Species/strain:	Monkey/Cynomolgus
Number/sex/group:	6/sex/group
Age:	2.5-3.9 years; young adults at initiation
Satellite groups/unique design:	None
Deviation from study protocol affecting interpretation of results:	None

Source:Nonclinical Reviewer
Abbreviations: BID, twice daily; GLP, good laboratory practices

Table 82. Observations and Results of the 9-Month Oral Toxicity Study in Monkeys

Parameter	Major Findings
Mortality	No mitapivat-related mortality in either sex at any dose level.
Clinical signs	There was transient and sporadic emesis in males and females 1 to 2 hours postdosing at ≥25 mg/kg/BID Emesis was not associated with adverse clinical symptoms such as dehydration, diarrhea or thinness and did not negatively impact body weight. Therefore, emesis is not considered adverse in these animals.
Body weights	No mitapivat-related significant change in body weight at any dose level in either sex.
Ophthalmoscopy	Unremarkable.
ECG	No mitapivat-related adverse quantitative or qualitative ECG changes.
Hematology	There were no mitapivat-related adverse effects on hematology and coagulation parameters at any dose level in either sex.
Coagulation	No mitapivat-related adverse effects on coagulation parameters.
Clinical chemistry	There was no statistically significant change in clinical chemistry values in animals administered mitapivat up to the high dose when compared to vehicle control animals and when accounting for pretreatment values.
Urinalysis	There were no mitapivat-related statistically significant and adverse changes to urinalysis values up to the high dose in either sex.
Gross pathology	In the main study, mitapivat-related macroscopic finding included a white deposit in the liver in one female at 100 mg/kg/BID, indicative of minimal subcapsular hepatocellular inflammation/necrosis resulting from hepatocellular hypertrophy. A white deposit was observed in another female monkey at 100 mg/kg/BID but was not likely mitapivat-related as this lesion was capsular and not associated microscopically with hepatocellular hypertrophy. There were no mitapivat-related macroscopic observations at any dose level in the recovery group.

Parameter	Major Findings
Organ weights	In the main study group, there were mitapivat-related, dose-dependent increases in liver weight reaching statistical significance at the high dose in males (44%), but not in females (39%). The higher liver weight correlated with diffuse hepatocellular hypertrophy observed microscopically at all dose levels in both sexes. In the main study group, in females, there was a trend towards an increase in adrenal weight in females at >50 mg/kg/BID, not dose-related, that correlated microscopically with mild diffuse hypertrophy of the zona fasciculata and hence considered likely mitapivat-related. Liver and adrenal findings showed reversibility during recovery.
Histopathology Adequate battery: Yes	Mitapivat caused a dose-dependent increase in incidence and severity of diffuse hepatocellular hypertrophy that ranged from minimal to severe in males and minimal to moderate in females. In the liver, minimal subcapsular inflammation and hepatocellular necrosis was observed at mid and high doses in males and at the high dose in females and is likely due to increased subcapsular pressure from hepatocellular hypertrophy. The hepatocellular hypertrophy is likely due to induction of CYP450 enzymes in the liver and is an adaptive change and thus not generally considered adverse. Furthermore, serum levels of liver injury markers did not increase significantly in these dose groups. Nevertheless, due to the association of hypertrophy with subcapsular inflammation and hepatocyte necrosis, this microscopic change is considered adverse. No adverse hepatocellular histopathology was observed during the recovery period indicating that the microscopic changes were reversible. In females, there was mitapivat-related, dose-dependent increase in incidence and severity (minimal to mild) of diffuse zona fasciculata hypertrophy at ≥50 mg/kg/BID, characterized by enlarged cytoplasm and rarefaction of cells. This finding correlated with increased adrenal gland weight at these doses. However, there were no correlative clinical chemistry changes indicative of adverse adrenal function. Zona fasciculata hypertrophy was not observed in the recovery groups.
Special Evaluations	There were blue to grey-blue pigmented macrophages observed in the lungs and lymph nodes in all dose groups and in the Kupffer cells in the liver in the low and mid dose in males and low and high dose in females. Pigmented macrophages were also seen in control animals but appear to be exacerbated in treated animals. Special staining for mucin (Alcian blue and toluidine blue), fat (oil red-O), iron (Prussian blue), lipofuscin, and glycoproteins/ proteoglycans and glycogen (PAS with and without diastase) of lung, liver, and lymph nodes of one male and female each from control and high dose group revealed that the pigments were identical, endogenous, and exacerbated by mitapivat administration. PAS staining for glycogen/proteoglycans was mostly observed. During recovery, pigmented macrophages were present in only one male at 100 mg/kg/BID indicating recovery of histological changes at 25 and 50 mg/kg/BID and recovery in females in all dose groups.

Source: Nonclinical Reviewer.
Abbreviations: BID, twice daily;CYP450, cytochrome P450; ECG, electrocardiogram; PAS, periodic acid-Schiff

13.1.1.6.2. Genetic Toxicology

The Applicant completed a battery of in vitro and in vivo genotoxicity studies (Table 83) with mitapivat (AG-348).

Table 83. Summary of Genotoxicity Studies

Study Title/ Study No.	Key Study Findings
In vitro reverse mutation assay in bacterial cells/AG-348-N-002-R1 GLP compliance: Yes Study is valid: Yes	Mitapivat was tested in four *Salmonella typhimurium* strains (TA98, TA100, TA1535 and TA1537) and an *Escherichia coli* strain (WP2 uvrA) at 50 to 5000 µg/plate with and without S9 metabolic activation. *Negative:* No mutagenicity or toxicity was observed in any strain at all tested doses with or without metabolic activation. Precipitate was observed beginning at 1500 or at 5000 µg per plate.
Modified bacterial reverse mutation assay in 24-well plates (Ames Test)/ AG-348-N-043-R1 GLP compliance: Non-GLP Study is valid: Yes	Mitapivat was assessed via Ames test, using the modified plate incorporation method (incubation duration: 48-72 hours). The bacterial strains used were *S. typhimurium* TA98, TA100, TA1535, and TA97a and the *E. coli* tester strain WP2 uvrA at 0.075 to 250 µg/plate. *Negative:* No mutagenicity, precipitate, or toxicity was observed in any strain at all tested doses with or without metabolic activation.
Modified bacterial reverse mutation assay in 24-well plates/ AG349-N-044-R1 GLP compliance: NonGLP Study is valid: Yes	AG-8702, the major metabolite of mitapivat, was assessed by Ames test using the modified plate incorporation method (incubation duration: 48-72 hours). The bacterial strains used were *S. typhimurium* TA98, TA100, TA1535, and TA97a and the *E. coli* tester strain WP2 uvrA at 0.075 to 250 µg/plate. *Negative:* No mutagenicity, precipitate, or toxicity was observed in any strain at all tested doses with or without metabolic activation.
In vitro mammalian cell micronucleus assay in human peripheral blood lymphocytes (HPBLs)/ AG-348-N-003-R1 GLP compliance: Yes Study is valid: Yes	Human peripheral blood lymphocytes (HPBLs) were exposed to mitapivat for 4 and 24 hours at 35, 75, 150, 245, 350, and 500 µg/mL with and without metabolic activation. *Negative:* Mitapivat was not clastogenic or aneugenic in the in vitro micronucleus test using HPBLs with or without metabolic activation.
In vivo micronucleus assay in rats/ AG-348-N-004-R1 GLP compliance: Yes Study is valid: tested up to the limit dose of 2000 mg/kg body weight	Mitapivat was administered to SD rats (n=5/group) at 0, 500, 1000, and 2000 mg/kg body weight (half dose administered BID). *Negative:* Mitapivat did not induce cytotoxicity as determined by lack of mitapivat-related changes in PCE/CE ratio between drug-administered and vehicle control animals. Compared to vehicle control, mitapivat did not induce a statistically significant increase in frequency of mnPCEs.

Source: Nonclinical Reviewer.
Abbreviations: BID, twice daily; CE, chromatic erythrocytes; GLP, good laboratory practices; HPBL, human peripheral blood lymphocytes; mnPCE, micronucleated polychromatic erythrocytes; PCE, polychromatic erythrocytes; SD, Sprague-Dawley

13.1.1.6.3. Carcinogenicity

13.1.1.6.3.1. A 2-Year Oral Gavage Carcinogenicity Study of AG-348 (Mitapivat) in Sprague-Dawley Rat / AG-348-N-088

Mitapivat was administered to rats (n=60/sex/group) orally at doses of 0 (water), 0 (vehicle), 15, 50, and 150 mg/kg BID in males and 10, 25, and 100 mg/kg BID in females for 87 and 95 weeks, respectively. Doses recommended by Executive Carcinogenicity Assessment Committee were used in the study. The dose selection for the 2-year rat carcinogenicity study was based on the maximum tolerated dose and ≥25-fold the clinical exposure in male rats from a 6-month general toxicity study, and ≥25-fold the clinical exposure in female rats from the 6-month study. The low and mid doses were selected based on adequate spacing of AUC values. The low and mid doses represent exposure multiples of approximately 5- and 15-fold the projected clinical mitapivat AUC values, and are anticipated to provide similar exposure values in males and females.

No statistically significant dose-response relationship and pairwise comparisons in mortality was noted for both male and female rats.

The carcinogenicity study was adequate and there was no evidence of drug-related neoplasms in the 2-year study in either males or females at systemic exposures of 47× and >100× MRHD, respectively, based on AUC.

13.1.1.6.3.2. A 26-Week Carcinogenicity Study of AG-348 (Mitapivat) by Oral Gavage in CByB6F1/Tg ras H2 Hemizygous Mice/AG-348-N-095

Transgenic ras H2 mice (n=25/sex/group) were administered mitapivat orally at doses of 0 (water), 0 (vehicle), 30, 100 and 250 mg/kg BID in males and 0 (water), 0 (vehicle), 12.5, 37.5, and 125 mg/kg BID in females for 26 weeks. Dose selection was based on a dose range finding 28-day study in wild-type mice given 150, 375, and 750 mg/kg/BID mitapivat. Mortality was observed at 750 mg/kg/BID in males and females and at 375 mg/kg/BID in females. The high dose was one-third the lethal dose for males or females in the 28-day toxicity study. The mid and low doses for both males and females were based on adequate spacing of AUC values.

There was no mitapivat-related mortality up to the high dose in either sex. There were no mitapivat-related tumors in male or female transgenic ras H2 mice at doses up to 500 mg/kg/day in males and 250 mg/kg/day in females when given orally for 26 weeks.

13.1.1.6.4. Reproductive and Developmental Toxicity

13.1.1.6.4.1. A Combined Fertility and Early Embryonic Development Study of AG-348 (Mitapivat) Administered by Oral Gavage in Rat/AG-348-N-086

Key Findings

- There were no mitapivat-related adverse effects on male and female fertility up to the highest dose tested (45× and 49× MRHD, respectively), both when animals were cohabited immediately after dosing or following a recovery period (10 weeks in males, 4 weeks in females).

Table 84. Methods of the Fertility and Early Embryonic Development to Implantation Study in Rats

Parameter	Method Details
GLP compliance	Yes
Dose and frequency of dosing:	(M): 0 (vehicle), 20, 30, 75, and 150 mg/kg, BID (F): 0 (vehicle), 5, 10, 25, and 100 mg/kg, BID
Route of administration:	Oral gavage
Formulation/vehicle:	0.5% methylcellulose, 400 cps/5% povidone (PVP K30) in deionized water
Species/strain:	Rat/Sprague-Dawley
Number/sex/group:	30/sex/dose group (15/sex/cohort)
Satellite groups:	None
Study design:	Cohort A Male rats were treated BID for 84 days beginning 10 weeks before cohabitation with untreated females, through the cohabitation period (maximum 14 days), and until the day before scheduled euthanasia. Female rats were given the test article and/or the vehicle control BID (approximately 12 hours apart) for 14 days before cohabitation (maximum 14 days) with untreated male breeder rats and continuing through GD 7. Cohort B Male rats were given the test article and/or the vehicle BID for 84 days. The rats then received a 10-week dose-free (recovery) period before cohabitation (maximum 14 days) with untreated females. Female rats were given the test article and/or the vehicle control BID for 29 days. The females then received a 4-week dose-free (recovery) period before cohabitation (maximum 14 days) with untreated male breeder rats.
Deviation from study protocol affecting interpretation of results:	None.

Source: Nonclinical Reviewer.
Abbreviations: BID, twice daily; F, female; GD, gestational day; GLP, good laboratory practices; M, male; PCE, polychromatic erythrocytes

Table 85. Observations and Results of the Fertility and Early Embryonic Development to Implantation Study in Rats

Parameter	Major Findings
Mortality	No mitapivat-related mortality
Clinical signs	No mitapivat-related adverse clinical signs
Body weights	No mitapivat-related adverse body weight or food consumption changes

Parameter	Major Findings
Necropsy findings Cesarean section data	No mitapivat-related adverse effects on male or female fertility in either cohort. The following findings were not considered adverse because they did not impact fertility: Statistically significant decreases in the number of estrous cycling stages were observed at the HD in Cohort A (↓20%) during the pre-cohabitation treatment period (14 days) and in Cohort B (13%) over 36 days. In Cohort B, the overall decrease was driven primarily by the mitapivat-related effect during the last 8 days of dosing. Decrease in sperm motility (up to 17%) at ≥75 mg/kg/bid, decrease in sperm density (33%) and increase in abnormal sperm (sperm with no head) at the HD were observed in Cohort A but not in Cohort B. Similarly, reduced sperm and cell debris (minimal to mild) in the epididymis, and seminiferous tubule degeneration (minimal to moderate), spermatid retention, and atypical residual bodies in the testes were observed at ≥75 mg/kg/bid in Cohort A but not B, indicating reversibility of the findings. Hormone analysis (testosterone, estradiol, and prolactin) was conducted in Cohorts A and B but was not interpretable due to high variability between groups, between cohorts, and within individual animals.

Source: Nonclinical Reviewer.
Abbreviations: HD, high dose

13.1.1.6.4.2. Embryo-Fetal Development Toxicity

13.1.1.6.4.2.1. An Oral (Gavage) Study of the Effects of AG-348 (Mitapivat) on Embryo/Fetal Development in Rats/ AG-348-N-080-R1

Key Findings

- One female in the high-dose (HD) group (63× MRHD) was euthanized due to mitapivat-related adverse clinical signs, which included nasal discharge and red deposits around the nose and urogenital area.
- Net body weight gain (without gravid uterine weight) and food consumption were statistically significantly lower (-14%) at the high dose (100 mg/kg/BID) compared to the vehicle control and were considered adverse due to associated clinical signs and premature euthanasia of one female at this dose.
- Statistically significant increases in late resorptions (12%) and total resorptions (four-fold), resulting in decreases in the mean litter proportion of pup viability (-22%) and combined fetal weight (-8%) at the HD (63× MRHD) compared to the vehicle control.
- Mitapivat-related external, visceral, and skeletal malformations were observed at the HD.
- The NOAEL for maternal and embryo-fetal toxicity is 25 mg/kg/BID (13× MRHD,) due to adverse effects of mitapivat on maternal health and embryo viability at the HD (100 mg/kg/BID).

Table 86. Methods of the Oral Embryo-Fetal Developmental Study in Rats

Parameter	Method Details
GLP compliance	Yes
Dose and frequency of dosing:	0 (vehicle), 5, 10, 25, and 100 mg/kg/BID; GD 6 to GD 17
Route of administration:	Oral gavage
Formulation/vehicle:	0.5% methylcellulose (400 cps) and 5% povidone K30 in deionized water
Species/strain:	Female rat/Sprague-Dawley
Number/sex/group:	25/sex/group
Satellite groups:	Toxicokinetics: 8 females/group
Study design:	Standard; pregnant female Wistar Han rats were dosed from GD 6 to GD 17 and euthanized on GD 20.
Deviation from study protocol affecting interpretation of results:	Deviations reported did not affect interpretation of the study findings.

Source: Nonclinical Reviewer.
Abbreviations: BID, twice daily; GD, gestation day

Table 87. Observations and Results of the Oral Embryo-Fetal Development Study in Rats

Parameter	Major Findings
Mortality	One female in the high-dose group (100 mg/kg/bid) was euthanized due to worsening physical condition. This animal had red deposits around the nasal and urogenital areas, clear nasal discharge, clear material around the mouth, and salivation. A cause of death was not identified. These clinical signs were similar to those in the surviving females; thus, the mortality was considered drug-related.
Clinical signs	Surviving females in the high dose group had clear nasal discharge, clear material around mouth and increased salivation 2 hours following dosing on GD 12-17.
Body weights	Body weight loss was observed in the HD group after the first dose (-9 g versus +1 g in the control group); however, body weight gain was observed thereafter and was similar across all study groups for the remainder of the dosing period. Net body weight gain (-14%; without gravid uterine weight) and food consumption were statistically significantly lower at the HD compared to the vehicle control.
Necropsy findings Cesarean section data	At the HD, mitapivat-related macroscopic observations included enlarged or fused placenta and/or distended amniotic sac in 12 of 24 animals.

Parameter	Major Findings
Necropsy findings Offspring	At the HD, there were mitapivat-related increases in early resorptions (16%, not statistically significant, but outside the range of the historical control for the test facility) and late resorptions (12%) resulting in a four-fold increase in total resorptions and a decrease in mean litter proportion of pup viability (72.1% per litter) compared to concurrent controls. An ~8% decrease in combined fetal weight was noted in the HD group compared to the vehicle control. At the HD, there were five fetuses in five different litters with malformations. External malformations included fetal anasarca, anophthalmia (bilateral), microstomia, malpositioned pinnae, absent naris, mandibular agnathia, anal atresia, vertebral agenesis (filamentous tail, 4 mm in length), omphalocele, and domed head. Skeletal (skull anomaly consisting of medial fusion of the premaxilla or absent intraparietal bone and squamosal suture), and visceral (stenotic pulmonary trunk) malformations were also observed. The mean litter proportions of malformations at 100 mg/kg/BID were higher than those for the concurrent control, and for omphalocele, microstomia, malpositioned pinnae, and absent naris, exceeded the maximum mean values of the test facility's historical control values. Although marketed aromatase inhibitors carry warnings for embryo-fetal toxicity, in the case of mitapivat it is unclear whether the fetal adverse effects are related to aromatase inhibition, given that mitapivat is 5- to 247-fold weaker than marketed aromatase inhibitors and the effects are observed at exposures not clinically relevant and are not independent of maternal toxicity.

Source: Nonclinical Reviewer.
Abbreviations: BID, twice daily; GD, gestation day; HD, high dose

13.1.1.6.4.2.2. An Oral (Gavage) Study of the Effects of Mitapivat on Embryo/Fetal Development in Rabbits/ AG-348-N-081-R1

Key Findings

- At the high dose (62.5 mg/kg/BID, 3× MRHD, based on AUC) there were body weight losses in dams at several points during the dosing period. These led to a statistically significant absolute body weight loss (-15g vs. +150g in controls) during the dosing period (GD 7 to 20).
- Body weight loss was associated with a statistically significant reduction in feed consumption at the high dose.
- There were no mitapivat-related malformations or variations.
- NOAEL for mitapivat is 30 mg/kg/BID (exposure 1.5× MRHD, based on AUC) due to mitapivat-related adverse body weight and feed consumption effects in maternal animals and adverse body weight loss in fetus at the high dose (62.5 mg/kg/BID).

Table 88. Methods of the Oral Embryo-Fetal Developmental Study in Rabbits

Parameter	Method Details
GLP compliance	Yes
Dose and frequency of dosing:	0 (vehicle), 12.5, 30, and 62.5 mg/kg; BID; GD 7 to GD 20
Route of administration:	Oral gavage
Formulation/vehicle:	0.5% methylcellulose (400 cps) and 5% povidone K30 in deionized water
Species/strain:	Time mated female rabbits/New Zealand White Hra:(NZW)SPF
Number/sex/group:	22/sex/group
Satellite groups:	Toxicokinetics: 3 time-mated females/Control group
Study design:	Pregnant female rabbits were dosed from GD 7 to GD 20 and euthanized on GD 29.
Deviation from study protocol affecting interpretation of results:	Deviations reported to not have affected interpretation of the study findings.

Source: Nonclinical Reviewer.
Abbreviations: BID, twice daily; GD, gestation day

Table 89. Observations and Results of the Oral Embryo-Fetal Development Study in Rabbits

Parameter	Major Findings
Mortality	No mitapivat-related mortality.
Clinical signs	HD: Few/absent feces.
Body weights	HD: At the high dose (62.5 mg/kg/BID) there were body weight losses in dams at several points during the dosing period. These led to a statistically significant absolute body weight loss (-15 g,) compared to control (+150 g) during the dosing period (GD 7 to GD 20). There were no statistically significant changes in net body weight and uterine weight in the treated groups compared to control at end of study. HD: Food consumption was concomitantly decreased.
Necropsy findings Cesarean section data	Mitapivat caused a decrease in male, female, and combined fetal mean weights (up to - 8.4%), which was below the historical control range.
Necropsy findings Offspring	Unremarkable.

Source: Nonclinical Reviewer.
Abbreviations: BID, twice daily; GD, gestation day; HD, high dose

13.1.1.6.4.3. An Oral (Gavage) Developmental and Perinatal/Postnatal Reproduction Study of mitapivat in Rats, Including a Postnatal Behavioral/Functional Evaluation/AG-348-N-099

Key Findings

- There was a dose-dependent increase in the incidence of mortality between GD 22 and 25 at ≥25 mg/kg/bid (13× MRHD, based on AUC). Mortality was attributed to mitapivat-induced prolongation of parturition and dystocia.
- Statistically significant decreases in body weight gain during gestation (-13%) and food consumption (-30%), persisting throughout lactation, were observed at the HD (63× MRHD).
- Significant increases in gestation length (+1.1 day), number of dams with stillborn pups (78%), number of dams with no live-born pups (44%), resulting in a significant decrease in liveborn pups, were observed at the HD. Lower pup weight was observed at the HD on postnatal day 4, but not thereafter.

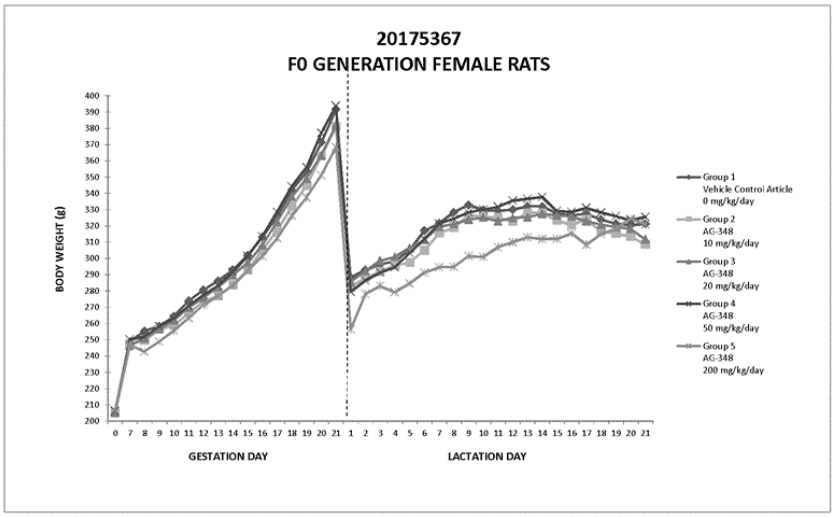
- There were no effects on growth and development, sperm motility and density, and reproductive performance of the F1 generation up to 13× MRHD.
- Mitapivat concentration in lactating rats was not measured.
- NOAEL is 10 mg/kg/BID (3.5× MRHD) for maternal toxicity and 25 mg/kg/BID (exposure 13× MRHD, based on AUC) for postnatal development.

Table 90. Methods of the Pre- and Postnatal Study in Rats

Parameter	Method Details
GLP compliance	Yes
Dose and frequency of dosing:	0 (vehicle), 5, 10, 25, and 100 mg/kg BID, GD 7 to LD 20
Route of administration:	Oral gavage
Formulation/vehicle:	0.5% methylcellulose, 400 cps/5% povidone (PVP) K30 in deionized water
Species/strain:	Time mated female rats/Sprague-Dawley (b) (4)
Number/sex/group:	22/group
Satellite groups:	None
Study design:	Standard Time mated pregnant female (F0 generation) were administered orally twice daily (12 h apart) from GD 7 through LD 20. Litters were not culled during the lactation period. Dams were euthanized on LD 21. Due to mortality in high-dose F0 females, no F1 pups from high-dose females were assigned for further evaluation.
Deviation from study protocol affecting interpretation of results:	Deviations reported to not have affected the interpretation of the study findings.

Source: Nonclinical Reviewer.
Abbreviations: BID, twice daily; GD, gestation day; LD, lactation day

Table 91. Observations and Results of the Pre- and Postnatal Study in Rats (F0-Generation Dams)

Parameter	Major Findings
Mortality	At 25 and 100 mg/kg/BID, mitapivat caused mortality in one and nine pregnant rats, respectively, due to prolonged parturition/dystocia around GD 22-25.
Clinical signs	Animals with dystocia exhibited adverse clinical signs including loss of righting reflex, decreased motor activity, pale appearance, cold to the touch, suspected dehydration (based on skin turgor), piloerection, hunched posture, chromodacryorrhea, ungroomed fur, excess salivation, bradypnea, and a red perivaginal substance. Although dystocia is not uncommon in rats, the dose-dependent nature and increased incidence at the highest dose indicate that dystocia was mitapivat-related.
Body weights	Gestation At 100 mg/kg/BID, there was mitapivat-related statistically significant decrease in body weight gain at GD 7 to 9, GD 15 to 18, and GD 18 to 21 (decreased by 80%, 16%, and 22% compared to vehicle control, respectively) leading to a 13% decrease in weight gain compared to vehicle control when the entire gestation period was considered. The decrease in body weight was associated with adverse clinical symptoms and dystocia. Lactation At 100 mg/kg/BID, there was a statistically significant decrease in body weight on LD 1 (-11%), which did not affect overall weight gain over the entire lactation period. Decreased food consumption at high dose correlated with changes in weight gain. **Figure 20. F0-Generation Female Rats**  Source: Excerpted from the Applicant's study report.
Natural delivery and litter observations	Mitapivat-related adverse effects on parturition were observed at 25 and 100 mg/kg/BID in a dose-dependent manner, but an effect on pup viability was observed only at 100 mg/kg/BID. Duration of gestation at 100 mg/kg/BID was significantly increased (23.3 days) versus both vehicle control (22.2 days) and historical control (23 days). There was a significant increase in percentage of stillborn pups at this dose, resulting in a significant decrease in mean number and total percent of liveborn pups. Of the 10 pregnant dams that delivered litters at 100 mg/kg/BID, 4 litters had no liveborn pups, resulting in a significantly decreased gestation index. Number of pups alive at PND 1 and 4 was significantly decreased at 100 mg/kg/BID, resulting in a statistically significant decrease in viability index (37.5% compared to 94.8% in controls) and lactation index (66.7% compared to 99.6% in controls). Lower pup weight was observed on PND 4.

Source: Nonclinical Reviewer.
Abbreviations: BID, twice daily, GD, gestational day; LD, lactation day; PND, postnatal day

Table 92. Observations and Results of the Pre- and Postnatal Study in Rats (F1 Generation)

Parameter	Major Findings
Mortality	No mitapivat mortality was observed in F1 rats during the postweaning period at any dose level.
Clinical signs	Surviving F1 pups from high-dose F0 females presented with dehydration and thinning of body. No mitapivat-related clinical signs were observed in F1 rats from any other dose group
Body weights	Unremarkable at any dose level.
Behavioral, sensory, and developmental evaluations	Unremarkable at any dose level.
Motor activity and learning and memory	Unremarkable at any dose level.
Sexual maturation	Unremarkable at any dose level.
Reproductive performance [mating, fertility, and fecundity indices, etc.]	Unremarkable at any dose level.
Uterine parameters	Unremarkable at any dose level.

Source: Nonclinical Reviewer.

13.1.1.6.5. Other Toxicology/Special Toxicology Studies

13.1.1.6.5.1. Neutral Red Uptake Phototoxicity Assay of Mitapivat (AG-348) in BALB/c 3T3 Mouse Fibroblasts/ AG-348-N-087

The phototoxic potential of mitapivat was evaluated in Balb/c 3T3 mouse fibroblasts by assessing the effect of mitapivat on their viability in the presence of ultraviolet (UV) radiation. Promethazine, a known phototoxic agent, was used as the positive control at up to 178 µg/mL. A geometric series of dilutions of mitapivat up to 100 µg/mL was added to Balb/c 3T3 fibroblasts, incubated for 90 minutes, and subjected to UV irradiation. Photo irritancy factor and mean photo effect were analyzed using Phototox software. The study was valid and under the conditions of the assay, mitapivat was negative for phototoxicity.

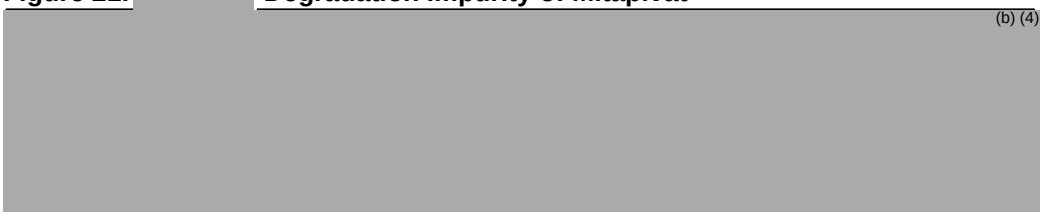
13.1.1.6.5.2. Updated Quantitative Structure-Activity Relationship DEREK and Leadscope Model Applier Assessments to Meet the Requirement of International Conference on Harmonisation M7 for Process Impurities in Mitapivat

For the mitapivat drug substance, the starting materials, intermediates, organic reagents, and observed or potential related impurities were evaluated for potential genotoxicity by performing a literature search as well as expert review- and statistics-based quantitative structure-activity relationship methods. There were several impurities that were potentially genotoxic. The process-related genotoxic impurities are well controlled (b) (4) and are not expected to be present in the drug product at toxicologically significant levels.

The chemistry, manufacturing, and controls reviewer communicated to nonclinical that forced degradation studies of the drug product identified (b) (4) degradation product, (b) (4). This degradation product did not present structural alerts for potential genotoxicity and is present at levels markedly lower than the International Conference on Harmonisation Q3B identification threshold of 0.1% at the MRHD. (b) (4) In addition, the (b) (4)

degradation product is not likely to be stable in the gastrointestinal (GI) environment in the tablet form. Therefore, the presence of extremely low levels of (b) (4) as a degradation product in the oral formulation is not likely a toxicologic concern.

Figure 21. (b) (4) Degradation Impurity of Mitapivat



Source: Excerpted from the Applicant's study report.

13.2. Individual Reviews of Studies Submitted to the New Drug Application

All nonclinical studies were also submitted to IND 119825 and were reviewed under the IND.

14. Clinical Pharmacology: Additional Information and Assessment

14.1. In Vitro Studies

14.1.1. Plasma Protein Binding

Mitapivat plasma protein binding was determined in human plasma at mitapivat concentrations of 0.2, 1.0, and 10μM using an ultracentrifugation method. The plasma protein binding of mitapivat was 97.7% and it was concentration independent.

14.1.2. Distribution in Red Blood Cells

Partitioning of 1.0μM mitapivat between RBC and plasma was determined in human blood. The RBC partitioning coefficient for mitapivat in human blood was 0.37.

14.1.3. Metabolism Studies

Mitapivat as Substrate

The metabolism of mitapivat was studied in human liver microsomes (HLM) with and without CYP enzyme–specific chemical inhibitors, human hepatocytes, and recombinant CYP enzymes. The metabolic pathways of mitapivat included N-dealkylation of the cyclopropyl-methyl moiety (AGI-8702), piperazine ring scission (M370, M424), and oxidation (M412, M452, M466a, M466b, and M466c).

In HLM incubations, [^{14}C]mitapivat was extensively metabolized to several Phase 1 metabolites: M370, AGI-8702, M412, M424, M452, M466b, and M466c. The N-dealkylated metabolite, AGI-8702, was the predominant metabolite (>50% of total radioactivity) observed in HLM. The other metabolites each represented <10% of the total radioactivity. The estimated hepatic

clearance of mitapivat was 1.05 L/hour/kg resulting in a hepatic extraction ratio of 0.85 in HLM, suggesting that mitapivat hepatic metabolism clearance is high. The formation of AGI-8702 in HLM was inhibited by 94.1% by the CYP3A4/5 inhibitor ketoconazole, and by 93.0% by the CYP3A4 inhibitor CYP3cide. There was no substantial inhibition observed during incubation with the other CYP enzyme inhibitors.

In human hepatocytes, [^{14}C]mitapivat was metabolized to one predominant metabolite, AGI-8702 (~33%), and four minor metabolites (<5% each), M466a, M466c, M452, and M424.

Incubation of mitapivat with recombinant CYP enzymes suggested that AGI-8702 is mainly formed by CYP3A4/5. CYP1A2 and CYP2D6 also formed trace amounts of AGI-8702. CYP2C8, CYP2C9, and CYP2C19 did not form any of the identified metabolites. In addition to AGI-8702, CYP3A4/5 formed metabolites M466b, M466c, M424, M412, and M370, whereas CYP1A2 also formed trace amounts of M466b, M466c, and M424. CYP2D6 and CYP2B6 formed a trace amount of M466c.

These results suggested that CYP3A4/5 is the major enzyme responsible for the metabolism of mitapivat in HLM. The other CYP enzymes (CYP1A2, CYP2B6, and CYP2D6) appeared to contribute only minimally to the metabolism of mitapivat.

Potential for CYP and UGT Enzyme Inhibition

Mitapivat was evaluated as a direct, time-dependent, and metabolism-dependent inhibitor of CYP enzymes over the concentration range of 0.1 to 100μM in HLM. Under the experimental conditions used, mitapivat was not a direct or time-dependent inhibitor of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, or CYP3A4/5 (IC_{50} >100μM).

IC_{50} shift experiments indicated the potential for metabolism-based inhibition of CYP2C19 and CYP3A4/5. The reversibility of the inhibition was determined by assessing microsomal activity after washing/re-isolation and upon the addition of potassium ferricyanide. The metabolism-dependent inhibition of CYP2C19 by mitapivat was largely reversible, and the metabolism-based inhibition of CYP3A4/5 was largely irreversible. The inactivation parameters of K_I (inactivator concentration at 50% the maximal rate of enzyme inactivation) and k_{inact} (maximal rate of enzyme inactivation at saturating concentrations of inhibitor) were therefore determined for CYP3A4/5 using testosterone and midazolam as substrates.

The k_{inact}/K_I for testosterone 6β-hydroxylation was determined to be 0.55 $min^{-1}mM^{-1}$ and the k_{inact}/K_I for midazolam 1′-hydroxylation was determined to be 0.64 $min^{-1}mM^{-1}$. In comparison, the CYP3A4/5 metabolism-dependent inhibitors erythromycin and ritonavir have k_{inact}/K_I values of approximately 3.6 and 1200 $min^{-1}mM^{-1}$, respectively for midazolam; and 4.0 and 1,500 $min^{-1}mM^{-1}$ for testosterone) (Obach et al. 2007). These data suggest that the metabolism-based inhibition of CYP3A4/5 by mitapivat is weaker compared to that caused by known metabolism-based inhibitors.

Mitapivat was evaluated as an inhibitor of UGT enzymes using recombinant human UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A9, or UGT2B6 and corresponding substrates at concentrations of 0.1 to 100μM. Mitapivat did not inhibit UGT1A1, UGT1A6, or UGT2B6 (IC_{50} >100μM) and was a weak inhibitor of UGT1A3, UGT1A4, and UGT1A9, with IC_{50} values of 15.4, 60.6, and 22.6μM, respectively.

Potential for CYP and UGT Enzyme Induction

Mitapivat was evaluated as an inducer of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, and CYP3A4 and UGT1A1expression in human hepatocytes from three separate human donor livers. Treatment of cultured human hepatocytes with up to 100μM mitapivat caused concentration-dependent increases in CYP2B6, CYP2C8, CYP2C9, CYP2C19, and CYP3A4 enzyme activity and mRNA expression of CYP2B6, CYP2C8, CYP2C9, CYP3A4, and UGT1A1. Calculated maximum drug-induced effect (E_{max}) and concentration of drug that achieved half-maximal effect (EC_{50}) values for induction of CYP enzyme activity and mRNA expression are summarized in Table 93. Based on these in vitro data, mitapivat is an inducer of CYP3A4, CYP2B6, CYP2C8, CYP2C9, CYP2C19, and UGT1A1.

Table 93. Mitapivat E_{max} and EC_{50} Associated With the Induction of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP3A4/5, and UGT1A1 Enzyme Activity and mRNA Expression

Enzyme Activity							
Activity Parameter	**CYP1A2**	**CYP2B6**	**CYP2C8**	**CYP2C9**	**CYP2C19**	**CYP3A4/5**	**UGT1A1**
Activity E_{max} (fold increase)	NC	5.0-12	2.5-14	2.0-2.1	3.9-4.2	1.3-3.0	NA
Activity EC_{50} (μM)	NC	3.3-7.2	0.2-7.6	0.4-0.6	2.0-3.3	1.1-1.5	NA
Maximal induction relative to the positive control	NC	109%	93.3%	73.3%	41.5%	60.7%	NA
Enzyme mRNA Expression							
Expression Parameter	**CYP1A2**	**CYP2B6**	**CYP2C8**	**CYP2C9**	**CYP2C19**	**CYP3A4/5**	**UGT1A1**
mRNA E_{max} (fold increase)	NC	3.5-5.6	3.1-5.0	2.6-5.1	NC	7.2-13	2.8-7.9
mRNA EC_{50} (μM)	NC	1.3-3.8	2.2-5.8	1.5-13	NC	1.6-22	1.6-12
Maximal induction relative to the positive control	NC	92.4%	125%	92.1%	NC	134%	86.2%

Source: Clinical Pharmacology Reviewers' table.
Abbreviations: E_{max}, maximal effect at high concentration; EC_{50}, half maximal effective concentration

14.1.4. Transporter Characterization

Mitapivat as Substrate

To determine if mitapivat is a substrate of P-gp and breast cancer resistance protein (BCRP), the bidirectional permeability of mitapivat at two concentrations (4 and 16μM) across Caco-2 cells was measured. To further evaluate if mitapivat is a substrate of P-gp and BCRP, the bidirectional permeability of mitapivat (5μM) across MDCKII-MDR1 and MDCKII-BCRP cells was measured in the presence and absence of the P-gp inhibitors, valspodar and verapamil, and the BCRP inhibitor, Ko143.

The bidirectional permeability of mitapivat was measured in a Caco-2 cell Transwell® system. The apparent permeability of mitapivat (4 and 16μM) in the B-to-A direction (67.2 and 73.7×10^{-6} cm/sec, respectively) was greater than in the A-to-B direction (18.2 and 24.2×10^{-6} cm/sec, respectively). The resulting B-to-A/A-to-B efflux ratios were >2, indicating that mitapivat is actively transported across Caco-2 cells.

Since the efflux ratio of mitapivat across Caco-2 cells was >2, the bidirectional permeability of 5μM mitapivat was evaluated in MDCKII-MDR1 and BCRP cells in the absence and presence of inhibitors. The bidirectional permeability of mitapivat across MDCKII-MDR1 and control cells in the absence of inhibitor resulted in a net efflux ratio of 7.30. In the presence of valspodar and verapamil, the net efflux ratio was reduced by 92% and 98%, respectively. The results show that mitapivat is actively transported across MDCKII-MDR1 cells, confirming that mitapivat is a

substrate of P-gp. In the human absorption, distribution, metabolism, and excretion (ADME) study (Study AG-348-009), the fraction absorbed (f_a) × fraction escaping gut metabolism (f_g) was calculated to be >0.8, suggesting that the risk of clinically relevant drug-drug interactions (DDIs) with mitapivat by P-gp inhibitors is low. The bidirectional permeability of 5µM mitapivat across MDCKII-BCRP cells was similar to that in control cells, resulting in a net efflux ratio of 0.943. In the presence of Ko143, the net efflux ratio was similar (0.971). These results show that mitapivat is not actively transported across MDCKII-BCRP cells.

The uptake ratio of mitapivat in OATP1B1- and OATP1B3-expressing cells was <2, and it was neither time- nor concentration-dependent, suggesting that mitapivat is not a substrate of OATP1B1 or OATP1B3.

Potential Transporter Inhibition

The ability of mitapivat to inhibit human efflux transporters, namely, P-gp (MDR1/ABCB1) and BCRP (ABCG2) was evaluated by measuring the bidirectional permeability of a probe substrate, i.e., digoxin or prazosin, respectively, across a monolayer of Caco-2 and MDCKII-BCRP cells, in the presence of two concentrations of mitapivat (41 and 411µM). The ability of mitapivat at six concentrations ranging from 1 to 500µM to inhibit P-gp and BCRP was evaluated to determine an IC_{50}.

The bidirectional permeability of digoxin was reduced in the presence of 41 and 411µM mitapivat by 91% and 99%, respectively. The bidirectional permeability of digoxin in the presence of mitapivat (1 to 500µM) was reduced in a dose-dependent manner and the calculated IC_{50} value was 12.8µM. Based on the static model (I_{gut} [intestinal concentration] ÷ IC_{50} >10), there is a potential for DDIs with P-gp substrates. The bidirectional permeability of prazosin was reduced in the presence of 41 and 411µM mitapivat by 66 and 85%, respectively. The bidirectional permeability of prazosin in the presence of mitapivat (1 to 500µM) was evaluated. The highest concentration precipitated out of solution and the remaining concentrations (1 to 100µM) showed that inhibition was not concentration-dependent. These data suggest that mitapivat is not a BCRP inhibitor at the concentrations evaluated (IC_{50} >100µM).

Mitapivat (0.1 to 100µM) was evaluated as an inhibitor of the human ATP-binding cassette transporters BSEP, MRP2, and MRP3 and the solute carrier transporters OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1, and MATE2-K by measuring the accumulation of probe substrates after incubation with transporter-expressing membrane vesicles or HEK293 cells. The results suggest that mitapivat does not inhibit MRP2, MRP3, OATP1B3, OAT1, or MATE2-K (IC_{50} >100µM) but does inhibit BSEP, OATP1B1, OAT3, OCT2, and MATE1, with IC_{50} values of 22.0, 29.0, 12.1, 7.76, and 7.17µM, respectively, under the conditions tested.

The clinically relevant effect of mitapivat on the systemic exposures of selected transporter substrate drugs for OATP1B1/OATP1B3, P-gp, MATE1, OCT2 and OAT3 was investigated via physiologically based pharmacokinetic modeling and simulation (details in Section 14.4).

14.2. In Vivo Studies

Study AG-348-C-001 A Phase 1, Randomized, Double-Blind, Placebo-Controlled, Single Ascending Dose, Safety, Pharmacokinetic, and Pharmacodynamic Study of Orally Administered AG-348 (Mitapivat) In Healthy Volunteers

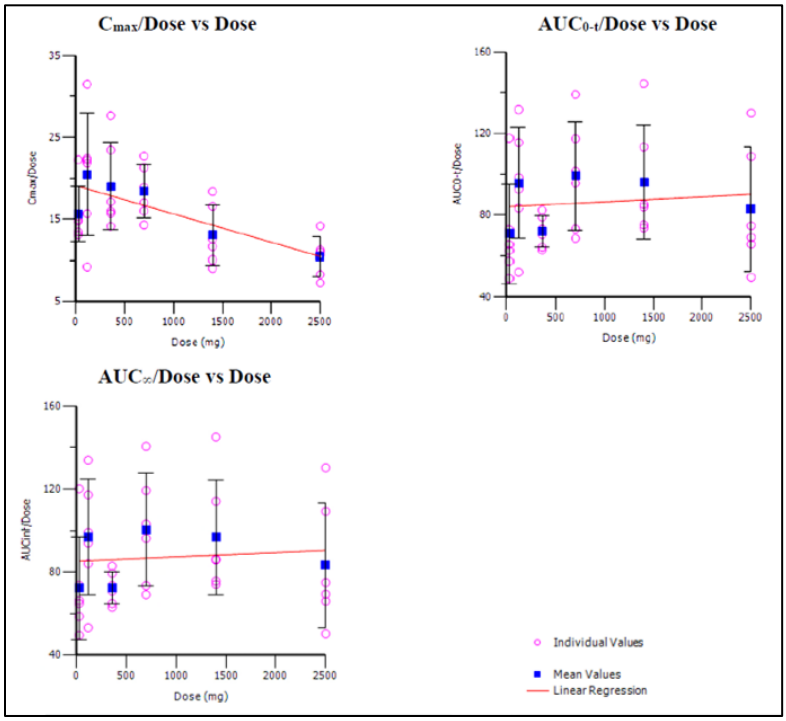
Study Design

Six cohorts of eight subjects each were randomized to receive a single dose of either mitapivat (n=6) or placebo (n=2). The doses of mitapivat studied were 30, 120, 360, 700, 1400, and 2500 mg. All doses were administered after a 10-hour fast, and subjects continued to fast until 4 hours postdose. Blood samples for analysis of mitapivat and its major metabolite (AGI-8702, pharmacologically nonactive N-dealkylation of mitapivat) in plasma were collected predose and up to 72 hours postdose for the 30 to 360 mg dose levels, and up to 120 hours postdose for the 700 to 2500 mg dose levels. Urine for analysis of mitapivat and AGI-8702 concentrations was collected continuously from predose up to 48 hours postdose. Blood samples for 2,3-bisphosphoglyceric acid (2,3-DPG) and ATP concentrations were collected at screening, Day -1, predose on Day 1 (-1 and 0 hours), and at intervals up to 120 hours postdose.

Results

A total of 48 subjects was randomized in the study, and all subjects completed the fasted period. The absorption phase was prolonged at doses >360 mg. Median t_{max} ranged from 0.77 hours at 30 to 360 mg, and up to 4.07 hours at 2500 mg. After the peak, mean concentrations declined in a multiexponential manner with a steeper initial decline phase and remained above the lower limit of quantitation (0.500 ng/mL) for up to 72 hours postdose. Based on this observation in the early dose cohorts, the blood sampling time was extended from 72 hours to 120 hours for the 700, 1400, and 2500 mg dose levels. Mean $t_{½}$ was shorter after doses of 30 to 360 mg mitapivat (17.8 to 20.4 hours) compared with doses ≥700 mg (50.3 to 79.3 hours). Geometric mean of apparent clearance (CL/F) ranged from 10.3 to 14.4 L/hour over the 30 to 2500 mg dose range. The fraction of mitapivat excreted in urine ranged from 1.45% to 2.33%. Individual and mean dose-normalized plasma mitapivat pharmacokinetics parameters (C_{max}, AUC from 0 to the last quantifiable concentration [AUC_{0-t}], and AUC_{∞}) were plotted against mitapivat dose to assess trends across the dose range studied (Figure 22). Dose-normalized mitapivat C_{max} decreased with increasing mitapivat doses, suggesting less than dose-proportional increases in C_{max} with increasing doses, which were more pronounced at >700 mg. Similarly, dose-normalized mitapivat AUC parameters showed a slightly decreasing trend after a single 1500 mg dose of mitapivat (Figure 22).

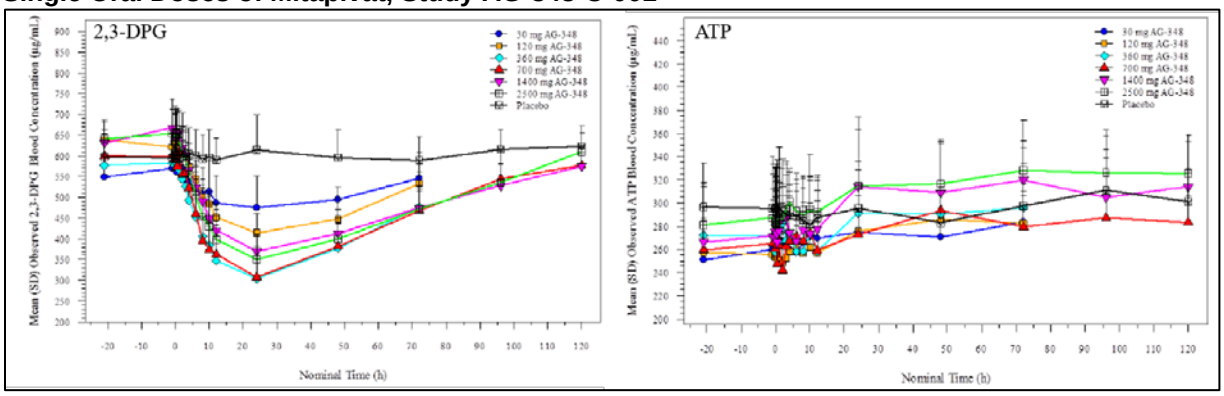
Figure 22. Plasma Mitapivat Dose-Normalized C_{max}, AUC_{0-t}, and AUC_{∞} Versus Dose, Study AG-348-C-001



Source: Applicant's analysis.
Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum serum concentration

A summary of the 2,3-DPG and ATP pharmacodynamics (PD) parameters after oral administration of single doses of mitapivat and placebo under fasted conditions is presented in Table 94, and the mean blood concentration-time profiles are presented in Figure 23. A dose-dependent decrease in mean 2,3-DPG concentrations was observed across the mitapivat dose range studied (30 to 2500 mg). The median t_{min} for 2,3-DPG was 24 hours for all mitapivat dose levels. Mean 2,3-DPG concentrations returned to baseline at approximately 120 hours postdose after doses of 360 to 2500 mg mitapivat.

Figure 23. Mean (SD) of Observed Blood Concentrations of 2,3-DPG and ATP Versus Time After Single Oral Doses of Mitapivat, Study AG-348-C-001



Source: Applicant's analysis.
Abbreviations: ATP, adenosine triphosphate; 2,3-DPG, 2,3-bisphosphoglyceric acid; SD, standard deviation

Table 94. Pharmacodynamics Parameters of 2,3-DPG and ATP After Single Oral Doses of Mitapivat and Placebo, Study AG-348-C-001

2,3-DPG

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)

ATP

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: Applicant's analysis.
Abbreviations: ATP, adenosine triphosphate; AUC, area under the concentration-time curve; AUC_Net_B, AUC_Above_B – AUC_Below_B; B, baseline; BR_{max}, maximum percentage increase from baseline response value; BR_{min}, maximum percentage decrease from baseline response value; 2,3-DPG, 2,3-bisphosphoglyceric acid; N, total number of subjects; SD, standard deviation; t_{max}, time to reach maximum concentration

Mean 2,3-DPG concentrations remained relatively constant after dosing with placebo, with high intersubject variability. Mean maximum percentage decrease from baseline response values ($\%BR_{min}$) in 2,3-DPG concentrations ranged from -18% after 30 mg mitapivat to -49% after 700 mg mitapivat. Dose-dependent decreases in mean 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) for 2,3-DPG were -4590 hour µg/mL after 30 mg to -21,684 hour µg/mL after 2500 mg mitapivat.

Mean ATP concentrations remained relatively constant after dosing with placebo, with high intersubject variablity. Increases from baseline in ATP blood concentrations were observed within 24 hours after mitapivat administration at all doses studied (30 to 2500 mg), with median t_{max} for the increase in ATP from baseline after mitapivat of 18.15 to 96.03 hours. Mean ATP concentrations remained higher than baseline at 120 hours postdose (the last sampling time). Mean maximum percentage increases from baseline response value ($\%BR_{max}$) in ATP concentrations ranged from 12% after 30 mg to 31% after 1400 mg mitapivat. Mean AUC_Net_$B_{0\text{-}t}$ for ATP values increased with increasing mitapivat dose from 748 hour µg/mL at 30 mg to 4125 hour µg/mL at 2500 mg mitapivat.

<u>Reviewer comment</u>: In general, mitapivat was rapidly absorbed, reaching t_{max} within 1 hour for a single dose of 30 mg to 360 mg mitapivat, and t_{max} of 1.5 to 4.1 hours for 700 mg to 2500 mg. Terminal $t_{1/2}$ ranged from 18 to 20 hours for a single dose of 30 mg to 360 mg mitapivat, which was prolonged to 50 to 79 hours for doses of 700 mg to 2500 mg, probably due to the multiple phases of terminal decline. C_{max} increased proportionally within 30 mg to 700 mg, but increased less-than-dose-proportionally from 700 mg to 2500 mg. Such nonlinearity at higher doses was also evidenced by a less-than-dose-proportional increase of AUC from 1400 mg to 2500 mg

single doses. In summary, dose-proportional pharmacokinetics of mitapivat was only seen from 30 mg to 700 mg single doses, and less-than-dose-proportional pharmacokinetics was observed at doses >700 mg, as evidenced by the less-than-dose-proportional increases of exposure metrics (C_{max} and AUC).

For PD assessment of mitapivat, compared to the placebo group, a concentration-dependent decrease of 2,3-DPG from baseline was observed after single-dose administration of mitapivat at 30 mg to 2500 mg. A slight increase of ATP level from baseline was also observed in the mitapivat dosing groups compared to the placebo group (Figure 23, Table 94). Both 2,3-DPG and ATP changes are consistent with the mechanism of action of mitapivat.

Study AG-348-C-002 a Phase 1, Randomized, Double-Blind, Placebo-Controlled Study Multiple Ascending Dose, Safety, Tolerability, Pharmacokinetic, and Pharmacodynamics of Orally Administered AG-348 (Mitapivat) In Healthy Volunteers

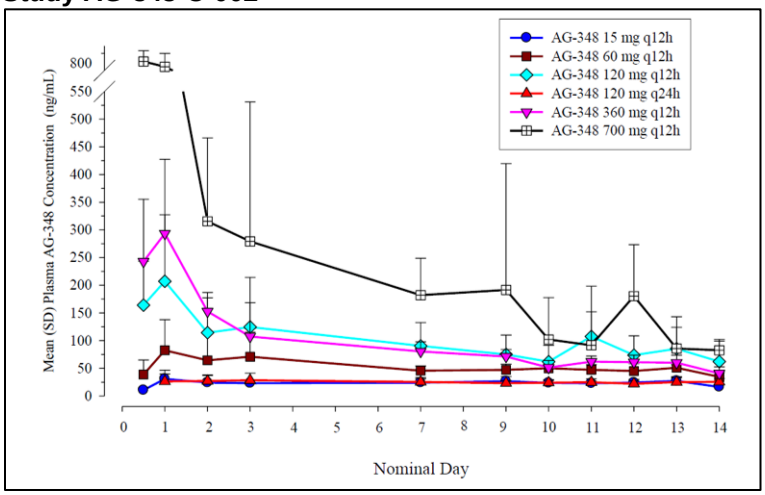
Study Design

Six cohorts of eight subjects each were randomized 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 once daily (QD), 60 mg BID, and 15 mg BID for 14 days. For both QD and BID regimens, the final dose was taken on the morning of Day 14. Subjects were required to fast for at least 10 hours before all morning doses and at least 2 hours before and after all evening doses. Serial blood samples for analysis of mitapivat and AGI-8702 plasma concentrations were collected predose and up to 12 hours postdose on Day 1, predose and up to 72 hours postdose on Day 14 for the 120 and 360 mg BID regimens, and up to 120 hours postdose on Day 14 for all other dose regimens. Pre–morning dose samples were also collected at intervals across the study. Blood samples for 2,3-DPG/ATP concentrations were collected at the same times as those for mitapivat except there was no pre–morning dose sample on Day 3. Because the recommended dosing regimen for mitapivat is BID, this section focuses on the BID data only.

Results

A total of 48 subjects was randomized in the study, and 44 subjects completed all scheduled study dosing through Day 14. Median t_{max} ranged from 0.76 to 1.53 hours after a single dose, and from 0.50 to 1.02 hours after multiple doses. After repeated BID dosing, mitapivat exposures were comparable on Days 1 and 14 at the lower dose levels (15 mg and 60 mg BID) but were lower on Day 14 compared with Day 1 at the higher dose levels (120 mg to 700 mg BID). For the 15 and 60 mg BID dose levels, mean R_{AUC} was 1.20 and 1.03, respectively, and mean R_{Cmax} was 1.07 and 1.17, respectively. Mean R_{AUC} ranged from 0.65 for 120 mg BID to 0.32 for 700 mg BID. Mean R_{Cmax} ranged from 0.77 for 120 mg BID to 0.49 for 700 mg BID. Geometric mean plasma concentration at the end of the dosing interval (C_{trough}) values decreased with repeated dosing, especially at doses >60 mg BID (Figure 24). Based on plasma C_{trough} values, mitapivat reached steady state by approximately 7 to 10 days of BID dosing. Geometric mean CL/F also increased after multiple BID dosing, i.e., from 16.0 and 17.9 L/hour after 15 mg BID and 60 mg BID, respectively, to 38.4 L/hour after 700 mg BID. Overall, these data suggest that autoinduction of mitapivat metabolism occurs with repeated dosing.

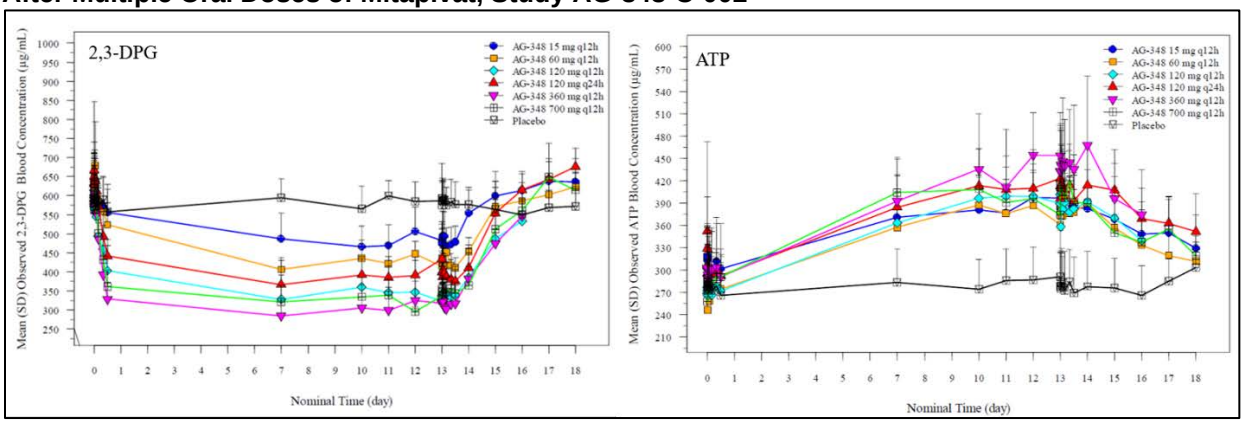
Figure 24. Mean (SD) Trough Level of Mitapivat (C_{trough}) After 14 days of Repeated Oral Doses, Study AG-348-C-002



Source: Applicant's analysis.
Abbreviations: C_{trough}, predose trough concentration; SD, standard deviation

A summary of the 2,3-bisphosphoglyceric acid (2,3-DPG) and ATP PD parameters after oral administration of single and multiple doses of mitapivat is presented in Table 95, and the mean trough blood concentration–time profiles are presented in Figure 25.

Figure 25. Mean (SD) Observed Trough Blood Concentrations of 2,3-DPG and ATP Versus Time After Multiple Oral Doses of Mitapivat, Study AG-348-C-002



Source: Applicant's analysis.
Abbreviations: ATP, adenosine triphosphate; 2,3-DPG, 2,3-bisphosphoglyceric acid; SD, standard deviation

Table 95. Pharmacodynamics Parameters of 2,3-DPG and ATP After Single (Day 1) and Multiple (Day 14) Oral Doses of Mitapivat, Study AG-348-C-002

2,3-DPG

Day	Pharmacodynamic Parameters	Placebo (N=12)	Mitapivat Dose 15 mg BID (N=6)	60 mg BID (N=5)	120 mg QD (N=6)	120 mg BID (N=6)	360 mg BID (N=6)	700 mg BID (N=6)
1	n	12	6	6	6	6	6	6
	Baseline	588 (9.8)	636 (9.5)	641 (3.8)	650 (9.6)	578 (10.3)	594 (7.8)	605 (5.6)
	AUC_Net_B$_{0\text{-}12hr}$ (hr·µg/mL)	-109 (374.2)	-724 (62.3)	-784 (27.3)	-1,350 (31.8)	-1,128 (22.4)	-1,736 (4.1)	-1,559 (14.9)
	BR_{min} (µg/mL)	-65.3 (82.6)	-106 (45.7)	-125 (26.3)	-208 (24.7)	-175 (13.5)	-266 (2.4)	-243 (8.2)
	%BR_{min} (%)	-10.7 (85.5)	-16.3 (37.8)	-19.7 (28.7)	-31.8 (19.5)	-30.6 (17.8)	-44.9 (7.2)	-40.3 (11.4)
	t_{min} (h)	1.53 (0.22, 12.05)	7.98 (1.00, 12.02)	11.95 (4.02, 11.98)	12.01 (7.97, 12.03)	11.95 (11.92, 11.98)	11.95 (11.95, 12.00)	11.98 (11.93, 12.07)
14	n	12	6	5	6	6	6	3
	AUC_Net_B$_{0\text{-}12hr}$ (hr·µg/mL)	-77.3 (488.0)	-1,909 (20.7)	-2,490 (6.0)	-3,139 (22.3)	-2,883 (9.4)	-3,350 (14.6)	-3,249 (6.9)
	AUC_Net_B$_{0\text{-}24hr}$ (hr·µg/mL)	-1,064 (80.5)[1]	NA	NA	-6,228 (23.4)	NA	NA	NA
	BR_{min} (µg/mL)	-47.6 (83.5)	-185 (25.2)	-244 (10.0)	-289 (21.9)	-280 (6.7)	-301 (14.8)	-290 (8.8)
	%BR_{min} (%)	-7.93 (81.9)	-28.8 (17.7)	-38.4 (10.5)	-44.1 (15.5)	-48.8 (10.5)	-50.6 (11.7)	-46.7 (8.2)
	t_{min} (h)	3.00 (0.00, 12.03)	1.52 (0.25, 8.10)	1.98 (1.00, 12.05)	10.03 (0.50, 12.02)	1.27 (0.25, 8.15)	4.03 (2.02, 12.02)	2.00 (0.25, 8.00)

ATP

Day	Pharmacodynamic Parameters	Placebo (N=12)	Mitapivat Dose 15 mg BID (N=6)	60 mg BID (N=5)	120 mg QD (N=6)	120 mg BID (N=6)	360 mg BID (N=6)	700 mg BID (N=6)
1	n	12	6	6	6	6	6	6
	Baseline	283 (16.6)	316 (12.8)	269 (14.0)	329 (21.1)	272 (5.6)	301 (19.6)	280 (10.2)
	AUC_Net_B$_{0\text{-}12hr}$ (hr·µg/mL)	-117 (209.7)	-173 (107.8)	45.3 (545.1)	-384 (175.0)	-0.20 (33,448)	-27.2 (269.9)	68.5 (115.2)
	BR_{max} (µg/mL)	18.4 (96.4)	14.5 (80.7)	32.9 (80.0)	24.4 (145.6)	17.5 (44.9)	16.9 (80.8)	31.5 (43.0)
	%BR_{max} (%)	6.91 (106.4)	4.78 (80.3)	13.3 (91.0)	6.49 (120.4)	6.34 (40.8)	5.92 (91.7)	11.7 (51.7)
	t_{max} (h)	0.40 (0.00, 8.00)	6.00 (0.00, 11.98)	0.54 (0.28, 11.97)	0.42 (0.00, 7.98)	5.70 (3.24, 9.95)	5.00 (0.25, 11.95)	6.04 (1.00, 12.00)
14	n	12	6	5	6	6	6	3
	AUC_Net_B$_{0\text{-}12hr}$ (hr·µg/mL)	-70.5 (213.7)	793 (31.1)	1,474 (22.6)	925 (64.2)	1,322 (15.8)	1,665 (14.7)	1,631 (11.3)
	AUC_Net_B$_{0\text{-}24hr}$ (hr·µg/mL)	-482 (130.8)[1]	NA	NA	1,823 (65.7)	NA	NA	NA
	BR_{max} (µg/mL)	13.8 (80.0)	91.2 (19.9)	145 (23.3)	101 (49.5)	142 (16.6)	176 (23.4)	160 (6.0)
	%BR_{max} (%)	5.22 (81.9)	29.0 (19.7)	56.6 (24.3)	32.7 (48.0)	52.2 (15.9)	59.9 (30.7)	59.8 (12.6)
	t_{max} (h)	3.03 (0.25, 8.05)	0.83 (0.25, 12.10)	1.02 (0.25, 8.00)	1.00 (0.28, 24.00)	1.01 (0.25, 1.95)	2.04 (0.50, 12.00)	0.55 (0.25, 8.00)

Source: Applicant's analysis.
Abbreviations: ATP, adenosine triphosphate; AUC, area under the concentration-time curve; AUC_Net_B, AUC_Above_B – AUC_Below_B; B, baseline; BID, twice daily; BR_{max}, maximum percentage increase from baseline response value; BR_{min}, maximum percentage decrease from baseline response value; 2,3-DPG, 2,3-bisphosphoglyceric acid; N, total number of subjects; n, number of subjects in each category; NA, not applicable; QD, once daily; SD, standard deviation; t_{max}, time to reach maximum concentration

Mean 2,3-DPG concentrations remained relatively constant after dosing with placebo on Days 1 and 14, with high intersubject variablity. After the Day 1 dose, dose-dependent decreases in mean 2,3-DPG concentrations and AUC_Net_B$_{0\text{-}12hr}$ were observed after single and multiple does of mitapivat up to 360 mg and plateaued thereafter (Table 95). Likewise, a dose-dependent increase in %BR_{min} with increasing doses of mitapivat were observed up to 360 mg mitapivat

with a reduction in 2,3-DPG concentration from baseline of 16.3% after 15 mg mitapivat to 44.9% after 360 mg mitapivat. The median t_{min} for 2,3-DPG was 8 to 12 hours after the Day 1 dose (Table 95).

After the Day 14 doses, similarly to Day 1, dose-dependent decreases in mean AUC_Net_$B_{0\text{-}12hr}$ and increases in $\%BR_{min}$ for 2,3-DPG with increasing mitapivat dose were observed up to 360 mg BID mitapivat (Table 95). Median t_{min} ranged from 1.27 hours to 4.03 hours across the mitapivat BID dose groups (Table 95). On Day 14, predose 2,3-DPG concentrations in the mitapivat dose groups were lower compared with Day 1 baseline values and were similar to predose concentrations on Day 8 (Figure 25). These data suggest a sustained effect of mitapivat after multiple BID dosing that achieved steady-state within 7 days of dose initiation. Based on the similarity of the highest concentration of 2,3-DPG (R_{max} range 336 to 506 µg/mL) and lowest concentration of 2,3-DPG (R_{min} range 294 to 451 µg/mL) after mitapivat administration over the dosing interval on Day 14, the 2,3-DPG blood concentrations fluctuated little between doses at steady-state. Mean 2,3-DPG concentrations returned to levels close to baseline within approximately 72 hours after the final dose of mitapivat.

For ATP, in placebo-treated subjects, mean blood ATP trough concentrations remained close to baseline values from Day 1 to Day 19, with high intersubject variablity. After the Day 1 dose of mitapivat, minimal changes in ATP blood concentrations were observed across the tested mitapivat dose levels. After multiple doses, a dose-dependent increase in mean AUC_Net_$B_{0\text{-}12hr}$ and an increase in $\%BR_{max}$ for 2,3-DPG with increasing mitapivat dose were observed up to 360 mg BID mitapivat (Table 95). Median t_{max} for ATP ranged from 0.55 hours to 2.04 hours across the mitapivat BID dose groups.

Mean ATP trough concentrations in blood increased from baseline after multiple doses of mitapivat; however, no obvious dose-dependent increases were observed (Figure 25). After multiple-dose administration of mitapivat BID for 14 days, mean ATP trough blood concentrations on Day 14 were similar to the trough concentrations observed on Day 10, suggesting that a steady-state effect was achieved by Day 10. Based on the similarity of the highest concentration of ATP (R_{max} range 402 to 476 µg/mL) and lowest concentration of ATP (R_{min} range 345 to 413 µg/mL) over the dosing interval on Day 14, the ATPblood concentrations fluctuated little between doses at steady state. Mean ATP concentrations decreased after dosing cessation (Day 14), and returned to close to the baseline value on Day 18.

Reviewer comment: After multiple doses of mitapivat, limited exposure accumulations were observed from 15 mg to 60 mg BID with accumulation ratios (R_{AUC}) of 1.20 to 1.03. Less-than-dose-proportional increases in exposure were observed at higher doses of mitapivat from 120 mg to 700 mg BID with R_{AUC} of 0.65 to 0.32, which suggest autoinduction of mitapivat metabolism. Based on the trough level of mitapivat, mitapivat approached steady-state after 7 to 10 days.

For PD parameters, after single- and multiple-dose administrations of mitapivat ranged from 15 mg to 700 mg BID, an increase in mitapivat dose was associated with a decrease in 2,3-DPG concentrations with limited fluctuation at steady-state. Minimal changes in ATP were observed after single doses of mitapivat, but ATP concentrations increased after multiple doses of mitapivat with limited fluctuation at steady-state.

Study AG-348-C-009 A Phase 1, Open-Label Study to Evaluate the Absorption, Distribution, Metabolism, and Excretion and to Assess the Absolute Bioavailability of AG-348 in Healthy Male Subjects Following Administration of a Single Oral Dose of [^{14}C]AG-348 and Concomitant Single Intravenous Microdose of [$^{13}C_6$]AG-348

Study Design

Study AG-348-C-009 (Study 009) was an open-label study to evaluate the ADME, as well as absolute bioavailability of oral mitapivat. Healthy male subjects received a single 120 mg oral dose of [^{14}C]mitapivat (containing approximately 100 μCi) followed 1 hour later by a single IV microdose of approximately 0.1 mg of [$^{13}C_6$]mitapivat. Oral doses were administered after a 10-hour fast, and subjects remained fasted for a minimum of 2 hours post–oral dose. Blood samples for mitapivat and AGI-8702 plasma concentrations were collected from predose to 72 hours post–oral dose, and blood samples for [$^{13}C_6$]mitapivat were collected from 1 hour post–oral dose (i.e., immediately before the IV dose) through 72 hours after the IV dose. Blood samples for total radioactivity and metabolite profiling and identification were collected from pre–oral dose through 240 hours post–oral dose. Urine and feces for mitapivat concentrations, total radioactivity, and metabolite profiling and identification were also collected from pre–oral dose to 240 hours post–oral dose. The absolute bioavailability of mitapivat was estimated.

Results

Eight subjects were enrolled and received mitapivat (oral and IV). Seven subjects completed the study and were included in the pharmacokinetics analysis set. After oral dosing, total radioactivity appeared rapidly in plasma and whole blood with a median t_{max} of 1.5 to 2.0 hours. The geometric mean AUC ratio of total radioactivity in whole blood to plasma was 0.646, indicating minimal association of radioactivity with RBCs.

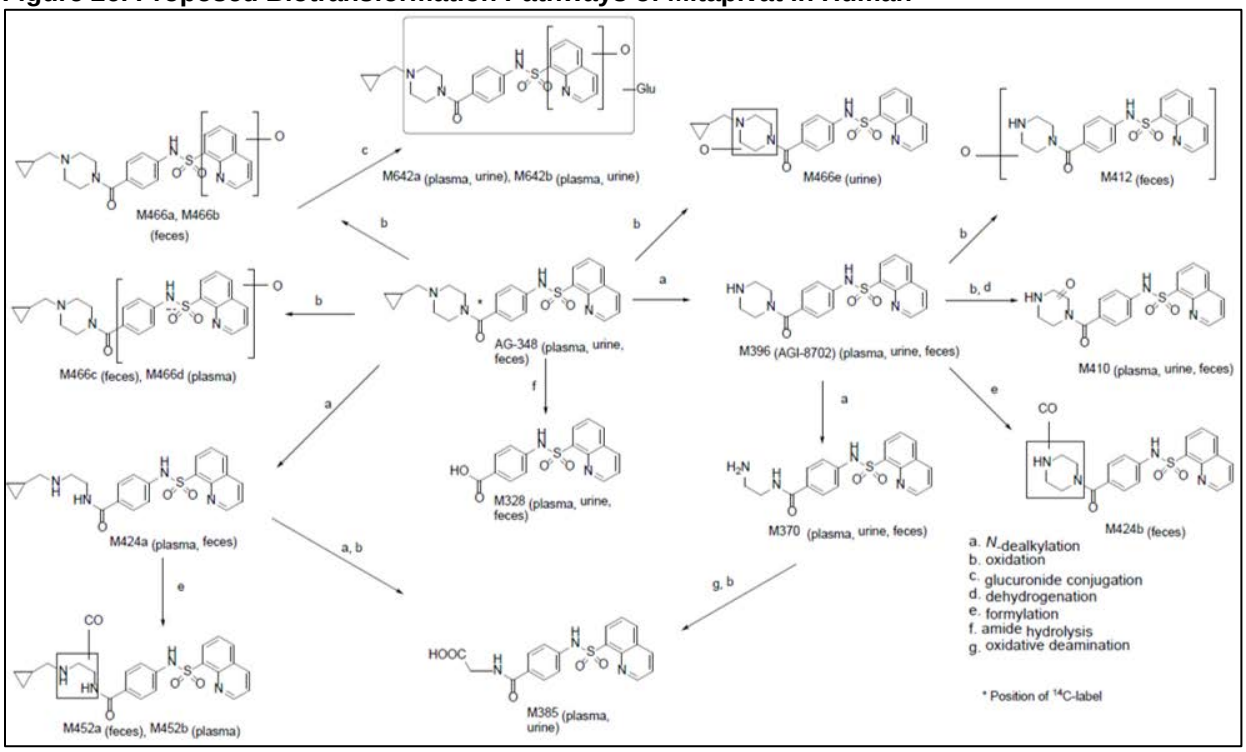
After IV dosing, [$^{13}C_6$]mitapivat plasma concentrations declined in a multiexponential manner with a mean $t_{1/2}$ of 16.3 hours. The mean clearance (CL) of [$^{13}C_6$]mitapivat was 9.53 L/hour. Mean V_{ss} after IV administration was 42.5 L, indicating extensive distribution. The mean volume of distribution in the terminal elimination phase after IV administration value of 135 L was higher and variable. Between-subject variability for the IV formulation was moderate, with coefficients of variation (CV%) for C_{max} and AUC parameters ranging from 29% to 34%.

The overall mean recovery of administered radioactivity was 89.1% over the 240-hour collection period. Approximately 40% of the administered radioactivity was recovered in feces and 50% was recovered in urine. Unchanged mitapivat was present in urine at a mean of 2.62% of the dose, suggesting that most urinary radioactivity was related to mitapivat metabolites. Mean mitapivat CL_R was 0.335 L/hour and accounted for 3.4% of total clearance of [$^{13}C_6$]mitapivat. The absolute oral bioavailability of mitapivat was 72.7%, and $f_a \times f_g$ was calculated to be >0.8.

[^{14}C]Mitapivat is extensively metabolized in humans after a single oral dose, as <3% and <1% of the dose was excreted unchanged in urine and feces, respectively. A total of 17 metabolites was characterized/identified in plasma, urine, and feces (Figure 26). The metabolites AGI-8702 (M396), M410, M466e, M424b, M466b, M385, and M370 were the most abundant metabolites in urine and feces. Unchanged mitapivat accounted for approximately 57% and 34% of the total plasma radioactivity in $AUC_{0\text{-}24hr}$ and $AUC_{0\text{-}72hr}$ pooled plasma samples, respectively. The

remaining radioactivity was due to several metabolites, each representing <10% of the total radioactivity in $AUC_{0\text{-}72hr}$ pooled plasma samples. Based on the metabolite structures, the primary metabolic pathways for [^{14}C]mitapivat in human subjects involved oxidation, N-dealkylation of the methylcyclopropane moiety, piperazine ring cleavage, and hydrolysis of the piperazine amide (Figure 26). The other metabolites were formed by a combination of these primary pathways followed by glucuronidation and formylation.

Figure 26. Proposed Biotransformation Pathways of Mitapivat in Human



Source: Applicant's analysis.

Reviewer's comment: Oral [^{14}C]Mitapivat-derived radioactivity undergoes significant fecal (~40%) excretion with less than 1% of unchanged mitapivat in feces, indicating mitapivat is well absorbed. The urinary excretion was approximately 50% with only 2.6% as unchanged mitapivat. The systemic clearance of mitapivat after IV dosing was 9.53 L/hour with a renal CL of 0.335 L/hour. Mitapivat was extensively metabolized, and 17 metabolites were identified, where each metabolite representing <10% of the total radioactivity in $AUC_{0\text{-}72hr}$ pooled plasma samples. Unchanged mitapivat was the major circulating entity. These data indicate that mitapivat undergoes extensive hepatic metabolism, and renal excretion is not the major elimination pathway for unchanged mitapivat. Intravenous volume of distribution parameters suggested extensive distribution into tissues. The absolute oral bioavailability of mitapivat was 72.7%.

Study AG-348-C-005 A Phase 1, Randomized, Open-Label, Two-Period Crossover Study Evaluating the Relative Bioavailability and Safety of the AG-348 Tablet and Capsule Formulations After Single-Dose Administration in Healthy Adults

Study Design

A total of 26 subjects in two treatment sequences was planned, with a washout period of 7 days. One dose was administered as a tablet formulation and the other as a capsule formulation of 50 mg mitapivat. Subjects were required to take each dose of mitapivat with approximately 240 mL of noncarbonated, room temperature water while in the fasted state, i.e., having fasted for a minimum of 10 hours predose and a minimum of 2 hours postdose. Plasma samples for pharmacokinetics analysis of mitapivat were collected up to 72 hours.

Results

Following a single 50 mg dose of mitapivat as one 50 mg tablet and two 25 mg capsules, the plasma concentration versus time profiles were characterized by a rapid absorption phase, with an approximately 19% higher geometric mean C_{max} observed for the tablet formulation. In addition, for the tablet formulation, t_{max} occurred earlier than the capsule formulation, with observed respective tablet and capsule median t_{max} values of 0.75 and 1.50 hours. After reaching C_{max}, the disposition of mitapivat appeared to be multiphasic, with a similar arithmetic mean $t_{1/2}$ for each formulation, 24.2 and 23.9 hours for the tablet and capsule formulations, respectively. Geometric mean CL/F (12 and 13 L/hour) and V_z/F (412 and 427 L/hour) values were also similar between the two formulations.

In this relative bioavailability study comparing tablet and capsule formulations, the exposures of the two formulations were similar (Table 96). Between-subject variability was considered moderate for $AUC_{0\text{-last}}$, $AUC_{0\text{-}\infty}$, and C_{max} for each formulation, with values ranging from 23.3% to 28.6%. Within-subject variability was considered low with a within-subject coefficient of variation (CV_W) values less than 12.9%.

Table 96. Statistical Analysis of Relative Bioavailability of Mitapivat Administered as a Tablet Versus Capsule, Study AG-348-C-005

Comparison	Pharmacokinetic Parameter	% Ratio of Least Squares Means	90% Confidence Interval	Within Subject CV%
50 mg mitapivat (tablet) versus 50 mg mitapivat (capsule)	$AUC_{0\text{-last}}$	105	101, 109	7.89
	AUC_{∞}	105	101, 109	8.12
	C_{max}	119	112, 127	12.9

Source: Applicant's analysis.
Abbreviations: AUC, area under the concentration-time curve; C_{max}, maximum serum concentration; CV, coefficient of variation

Reviewer's comment: Bioequivalence was established between the capsule formulation of mitapivat used in early development phases, and the tablet formulation of mitapivat used in other clinical studies including the pivotal Phase 3 studies. The geometric least squares mean ratios and 90% confidence intervals (CIs) of $AUC_{0\text{-last}}$ and AUC_{∞} were within the (b) (4) *%*

bioequivalence range. The upper limit of the 90% CI for C_{max} was 127%, which is slightly beyond the (b) (4)% bioequivalence criterion but a significant clinical difference is not expected.

Study AG-348-C-003 A Phase 2, Open-Label, Randomized, Dose-Ranging, Safety, Efficacy, Pharmacokinetic and Pharmacodynamic Study of AG-348 in Adult Patients with Pyruvate Kinase Deficiency

Study Design

Study AG-348-C-003 (Study 003) was a Phase 2, randomized, open-label, two-arm dose-ranging study designed to assess the safety, efficacy, pharmacokinetics, and PD of multiple oral doses of mitapivat administered using a capsule formulation in subjects with PKD. During the Extension Period of the study, subjects were switched to a tablet formulation after reviewing results from a relative bioavailability study (Study AG-348-C-005 [Study 005]). The study was divided into a 24-week Core Period and an Extension Period.

During the Core Period, approximately 50 subjects were randomized 1:1 into either Arm 1 (300 mg BID) or Arm 2 (50 mg BID) and received mitapivat for up to 24 weeks. For pharmacokinetics (mitapivat and AGI-8702) and PD (ATP and 2,3-DPG) evaluation, 12 subjects from the Core Period followed an intensive blood sampling schedule on Day 1 (predose and up to 12 hours postdose) and Day 15 (predose and up to 8 hours postdose). Also, for these subjects, predose samples were collected on Days 22, 43, 64, 85, 113, 141, and 169. For the remainder of the subjects, predose samples were collected on Days 1, 15, 22, 43, 64, 85, 113, 141, and 169.

Subjects who were eligible then entered the Extension Period. All subjects who received mitapivat doses >25 mg BID underwent an individual and gradual dose-taper regimen to identify their individual optimal maintenance dose. The minimum target dose was 25 mg BID. During the Extension Period, predose pharmacokinetics and PD samples were drawn for trough concentrations at each study visit (every 3 months). Pharmacokinetics and PD samples were collected for approximately 3 years in the Extension Period.

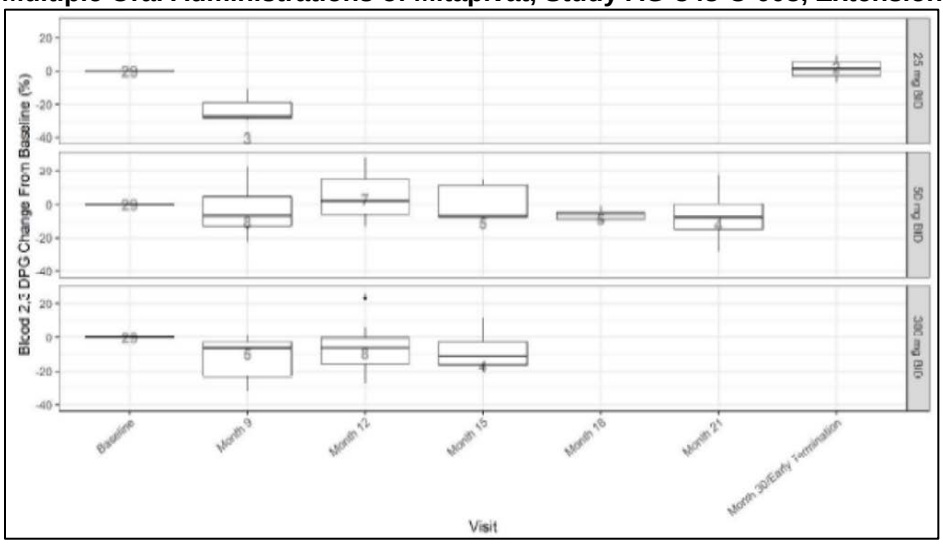
Results

Mitapivat was readily absorbed with a median t_{max} around 2 hours after a single dose (Day 1), and t_{max} of 1 hour after multiple doses (Day 15) for both dose levels (50 mg BID and 300 mg BID). Mean accumulation ratios for $AUC_{0-\tau}$ and C_{max} were 0.47 and 0.80 at the 300 mg dose level and 1.26 and 1.14 at the 50 mg dose level, suggesting lower exposure to mitapivat after repeated 300 mg BID dosing and little to no accumulation after repeated 50 mg BID dosing. The lower exposures on Day 15 compared with Day 1 at the 300 mg BID dose level suggest that mitapivat exhibits autoinduction, consistent with the time-dependent pharmacokinetics observed in healthy subjects at the higher dose levels (>60 mg BID) after multiple-dose administration. For the Extension Period, A total of 30 subjects was included in the pharmacokinetics/pharmacodynamics (pharmacokinetics-PD) population. The arithmetic mean plasma mitapivat predose concentrations (C_{trough}) were similar to those in the Core Period.

At the two tested mitapivat dose levels (50 mg BID and 300 mg BID), mean 2,3-DPG concentrations decreased numerically from baseline after the first dose (Day 1) and after multiple dosing (Day 15) in the Core Period. However, high intersubject variability was observed for both single and multiple doses, The $\%BR_{min}$ was -10.6 (% relative SD =87) and 15.3 (% relative

SD =56) after Day 1 and Day 15 of 50 mg BID dosing, and 11.2 (% relative SD =106) and 14.9 (% relative SD =151) after Day 1 and Day 15 for 300 mg BID dosing. In the Extension Period, the average percent change from baseline in 2,3-DPG ranged from -6.57% to 4.84% for 50 mg BID and -12.61% to -6.41% for 300 mg BID. These results suggest that blood 2,3-DPG concentrations remained close to baseline values (Figure 27).

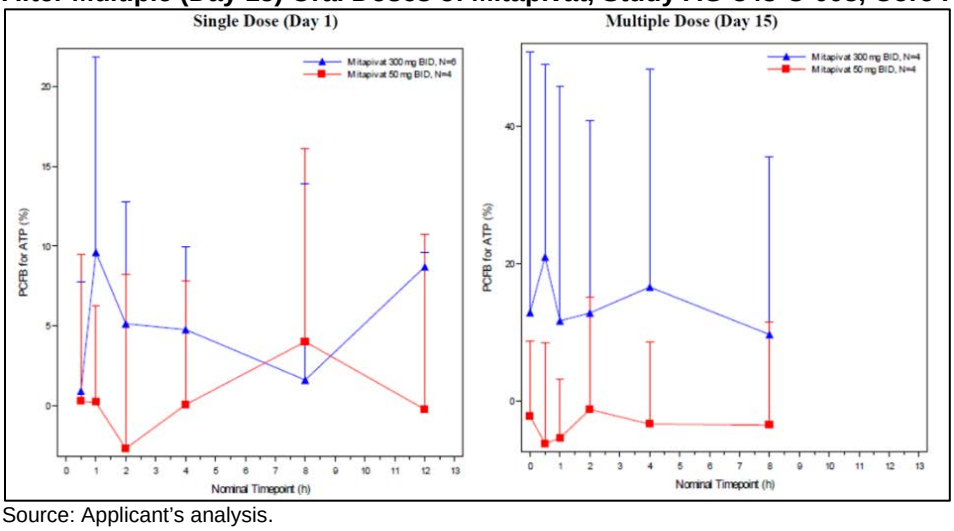
Figure 27. Blood 2,3-DPG Predose Concentration Percentage Changes From Baseline After Multiple Oral Administrations of Mitapivat, Study AG-348-C-003, Extension Period



Source: Applicant's analysis.
Abbreviations: BID, twice daily; 2,3-DPG, 2,3-bisphosphoglyceric acid

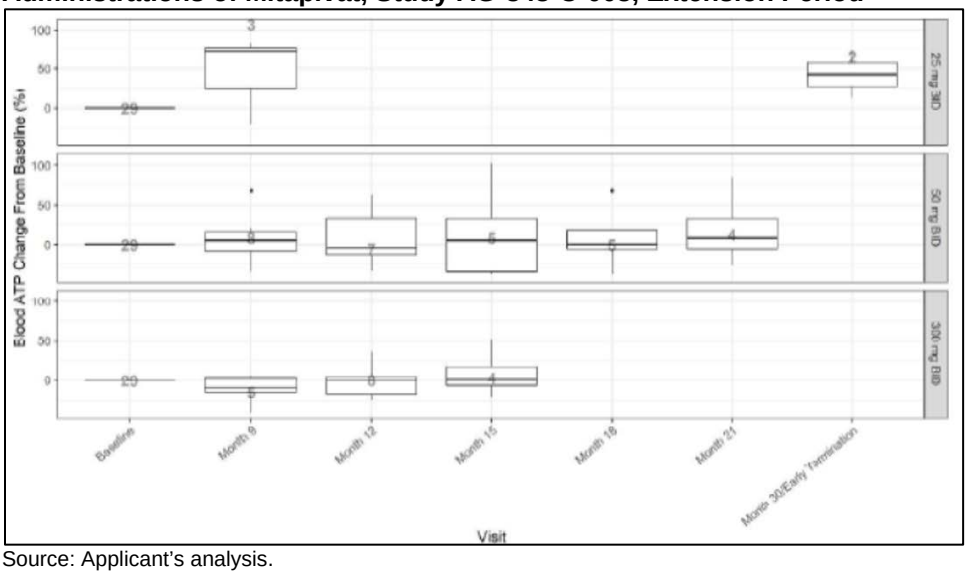
At both tested mitapivat dose levels (50 mg BID and 300 mg BID), minimal changes in ATP and large intersubject variability were observed after the first dose (Day 1) and multiple dosing (Day 15) in the Core Period (Figure 28). In the Extension Period, the average percentage increase from baseline in ATP ranged from 7.6% to 18.4% for 50 mg BID and -11.1% (i.e., a decrease) to 8.9% for 300 mg BID (Figure 29).

Figure 28. Mean (SD) Percentage Change From Baseline Blood Concentration of ATP Versus Time After Multiple (Day 15) Oral Doses of Mitapivat, Study AG-348-C-003, Core Period



Source: Applicant's analysis.
Abbreviations: ATP, adenosine triphosphate; BID, twice daily; N, total number of subjects; PCFB, percent change from baseline; SD, standard deviation

Figure 29. ATP Predose Concentration Percentage Changes From Baseline After Multiple Oral Administrations of Mitapivat, Study AG-348-C-003, Extension Period



Source: Applicant's analysis.
Abbreviations: ATP, adenosine triphosphate; BID, twice daily

Reviewer's comment: Steady-state exposures of mitapivat in patients with PK deficiency was lower on Day 15 compared with Day 1 at 300 mg BID, with little to no accumulation at 50 mg BID, consistent with the previously observed dose-dependent autoinduction of mitapivat in healthy subjects. However, in patients with PK deficiency, 2,3-DPG and ATP concentrations remained close to baseline values with 50 mg BID mitapivat, probably due to the large intersubject variability.

Study AG-348-C-004 A Phase 1, Single-Dose, Open-Label Study to Characterize and Compare the Pharmacokinetics, Safety, and Effect on QTc Interval of AG-348 in Healthy Subjects of Japanese Origin and Healthy Subjects of NonAsian Origin

Study Design

Subjects were enrolled into one of three cohorts (20 subjects each; 10 Japanese and 10 non-Asian) and received a single dose of either 5, 50, or 200 mg mitapivat. All doses were administered after a 10-hour fast, and subjects remained fasted for a minimum of 2 hours postdose. Blood samples for mitapivat and AGI-8702 plasma concentrations were collected from predose through 72 hours postdose. The plasma pharmacokinetics parameters for Japanese (test) and non-Asian (reference) subjects were analyzed using analysis of variance to determine the effect of race.

Results

A total of 60 subjects (30 Japanese and 30 non-Asian) was enrolled, and all subjects completed the study. After single doses of mitapivat, mitapivat exposure as defined by C_{max} and AUC increased with increasing doses. Maximum concentrations occurred at similar times with a median t_{max} of 0.88 to 1.50 hours for both groups and across the studied dose range. Apparent total body clearance was comparable for both groups, which was approximately 16 L/hour for

5 mg mitapivat, to 11 to 13 L/hour for 50 mg and 200 mg mitapivat. Statistical analysis of C_{max} and AUC parameters suggested that mitapivat exposure was comparable between the two groups, with geometric mean ratios close to 1.00 for all dose levels (Table 97).

Table 97. Statistical Analysis of the Effect of Race on Mitapivat Pharmacokinetics, Study AG-348-C-004

Comparison	Pharmacokinetic Parameter	Ratio of Least Squares Means	90% CI
5 mg mitapivat: Japanese versus non-Asian	AUC_{0-t}	1.01	0.83, 1.24
	AUC_{∞}	1.01	0.83, 1.23
	C_{max}	1.07	0.91, 1.26
50 mg mitapivat: Japanese versus non-Asian	AUC_{0-t}	0.96	0.79, 1.18
	AUC_{∞}	0.96	0.79, 1.17
	C_{max}	1.15	0.98, 1.36
200 mg mitapivat: Japanese versus non-Asian	AUC_{0-t}	1.20	0.98, 1.47
	AUC_{∞}	1.20	0.98, 1.47
	C_{max}	1.30	1.11, 1.53
Overall (dose-normalized) mitapivat: Japanese versus non-Asian	AUC_{0-t}	1.06	0.94, 1.19
	AUC_{∞}	1.05	0.94, 1.18
	C_{max}	1.17	1.07, 1.29

Source: Applicant's analysis.
Abbreviations: AUC, area under the concentration-time curve; CI, confidence interval; C_{max}, maximum serum concentration

Reviewer's comment: Geometric mean ratios for AUC_{0-t}, AUC_{∞}, and C_{max} between Japanese and non-Asian subjects were close to 1.0 with 90% CIs of 0.79 to 1.36 for a single dose of 5 mg or 50 mg mitapivat. Statistical analyses for AUC_{0-t}, AUC_{∞}, and C_{max} indicate the exposure of mitapivat is not affected by race within therapeutic relevant dose range of 5 mg to 50 mg, tested in Japanese and non-Asian subjects.

Study AG-348-C-014 A Phase 1, Randomized, Four-Period Crossover Food-Effect Study With Mitapivat Sulfate in Healthy Adult Subjects

Study Design

Study AG-348-C-014 was a Phase 1, randomized, double-blind, single-dose, four-period crossover study to evaluate the pharmacokinetics, safety, and tolerability of mitapivat in healthy subjects under fasted and fed (high-fat meal) conditions. The study also evaluated the effect of mitapivat on electrocardiogram (ECG) parameters, including a concentration-QTc analysis (fasted arms only). Subjects received the following treatments in random order, each separated by a minimum of 7 days: placebo, mitapivat 100 mg (fasted), mitapivat 100 mg (fed), and mitapivat 300 mg (fasted). Mitapivat was administered as a tablet formulation (50 mg mitapivat and/or matched placebo). For the fasted condition, subjects fasted overnight (nothing to eat or drink except water) for at least 10 hours before each study drug administration and remained fasted for 4 hours after dosing. For the fed condition, subjects fasted overnight (nothing to eat or drink except water) for at least 10 hours and received a high-fat (approximately 50% of total caloric content of the meal) and high-calorie (approximately 800 to 1000 calories) breakfast approximately 30 minutes before dose administration. Subjects consumed the meal in 30 minutes or less. Blood samples for pharmacokinetics analysis were collected predose and up to 120 hours postdose in each period.

Results

The ratio of geometric least-squares means of AUC_{0-t} and AUC_{0-inf} under fed versus fasted conditions were 0.98 and 1.00, respectively (Table 98). The 90% confidence intervals of these parameters were contained within the 0.8 and 1.25 limits, indicating that the effect of a high-fat, high-calorie meal on exposure (AUC) of mitapivat was not statistically significant. The ratio of geometric least-squares means of C_{max} under fed versus fasted conditions was 0.58. The 90% confidence interval of C_{max} was not contained within the 0.8 and 1.25 limits, indicating that the effect of a high-fat, high-calorie meal on C_{max} of mitapivat was statistically significant. t_{max} was delayed under fed conditions (high-fat, high-calorie meal) compared to fasted conditions, with a median difference in t_{max} under fed and fasted conditions of 1.88 hours ($p<0.0001$).

Table 98. Statistical Analysis of Plasma Pharmacokinetic Parameters of 100 mg Mitapivat Under Fasting and Fed Conditions, Study AG-348-C-014

Comparison	Pharmacokinetic Parameter	Ratio of Least Squares Means	90% CI
100 mg mitapivat (fed) versus 100 mg mitapivat (fasted)	AUC_{0-t}	0.98	0.93, 1.03
	AUC_{∞}	1.00	0.94, 1.05
	C_{max}	0.58	0.52, 0.65
		Median Difference (Fed-Fasted)	**90% CI**
	t_{max} (hr)	1.88	1.48, 2.27

Source: Applicant's analysis.
Abbreviations: AUC, area under the concentration-time curve; CI, confidence interval; C_{max}, maximum serum concentration; t_{max}, time for maximum drug concentration

Reviewer's comment: The design of this food effect study is acceptable with a standard high-fat/high-calorie meal and sufficiently long washout period. The tablet formulation used in this food effect study is the same as used in the pivotal Phase 3 studies. This tablet formulation is the intended final commercial formulation, with the only change for the commercial market being the addition of a cosmetic unique product identifier of ink-printed characters. There was no food effect on the total systemic exposure of mitapivat with a single oral dose of 100 mg mitapivat. However, the absorption of mitapivat was delayed by a high-fat/high-calorie meal, in which C_{max} decreased by 42% under fed compared with fasted conditions, and t_{max} was delayed by a median of 1.9 hours under the fed condition. For a chronically administered drug like mitapivat which works by allosterically binding to pyruvate kinase enzyme to increase its activity in red blood cell, total systemic exposures i.e., AUC, may be more important from an efficacy perspective than peak concentration. In addition, mitapivat is titrated based on tolerability and hemoglobin response. Taking these into consideration, the 42% decrease in C_{max} and 1.9 hours delay in t_{max} are not expected to produce clinical meaningful impact over chronic use of mitapivat, given the fact that the AUC of mitapivat was not affected by high-fat food. Mitapivat is proposed to be used with or without food, and the review team agrees with the Applicant's proposal.

Study AG-348-C-012: A Two-Part, Open-Label, Fixed-Sequence Study to Evaluate the Effect of Multiple Doses of Itraconazole and Rifampin on Single-Dose Pharmacokinetics of Mitapivat Sulfate (AG-348) in Healthy Adult Subjects

Study Design

In Part 1, subjects received a single oral dose of 20 mg mitapivat on Day 1 of Treatment Period 1. In Treatment Period 2, subjects received oral itraconazole (200 mg QD) from Day 1 through Day 9, with a single oral dose of 20 mg mitapivat on Day 5.

In Part 2, subjects received a single oral dose of 50 mg mitapivat on Day 1 of Treatment Period 1. In Treatment Period 2, subjects received oral rifampin (600 mg QD) from Day 1 through Day 12, with a single oral dose of 50 mg mitapivat on Day 8.

In both parts, the treatment periods were separated by a washout period of 7 days. All mitapivat doses were administered after a 10-hour fast, and subjects remained fasted for 4 hours postdose. On all other dosing days, itraconazole or rifampin was administered after a predose fast of at least 4 hours, and subjects remained fasted for at least 2 hours postdose. Because mitapivat has the potential to induce CYP3A4/5 and itraconazole is metabolized via CYP3A4/5, the study was designed as a single-dose study with a prospective plan to use results from this study to simulate multiple-dose scenarios by PBPK modeling. Serial blood samples for plasma concentrations of mitapivat and AGI-8702 were collected from predose to 120 hours after each mitapivat dose.

Results

A total of 14 subjects was enrolled in Part 1; all completed the study. Statistical analysis showed that mitapivat $AUC_{0\text{-}t}$, AUC_{∞}, and C_{max} increased by 4.7-, 4.9- and 1.7-fold, respectively in the presence of itraconazole compared with mitapivat alone (Table 99). Nonparametric statistical comparisons of mitapivat t_{max} indicated a statistically significant difference ($p<0.05$) between the two treatments with a median t_{max} approximately 0.5 hours later when mitapivat was coadministered with itraconazole compared with mitapivat alone. Mean CL/F was lower when mitapivat was coadministered with itraconazole (2.1 L/hour) compared with mitapivat alone (10.5 L/hour). Intrasubject variability was low, with CVs of approximately 11% and 15% for C_{max} and AUC, respectively. Intersubject variability was moderate with geometric CVs for C_{max} and AUC parameters of <30% for mitapivat with and without itraconazole.

Table 99. Statistical Analysis of the Effect of Itraconazole on Mitapivat Pharmacokinetics, Study AG-348-C-012

Comparison	Pharmacokinetic Parameter	Ratio of Least Squares Means	90% CI
20 mg mitapivat + itraconazole versus 20 mg mitapivat alone	AUC_{0-t}	4.70	4.25, 5.19
	AUC_{∞}	4.88	4.42, 5.39
	C_{max}	1.71	1.58, 1.84
		Median Difference	**90% CI**
	t_{max}	0.50	0.01, 1.25

Source: Applicant's analysis.
Abbreviations: AUC, area under the concentration-time curve; CI, confidence interval; C_{max}, maximum serum concentration; t_{max}, time for maximum drug concentration

A total of 14 subjects was enrolled in Part 2; all completed the study. Mean mitapivat concentrations were lower and peaked earlier when administered with rifampin (median t_{max} 0.5 hours) compared with mitapivat administered alone (median t_{max} 1.0 hour). After the peak, plasma concentrations declined in a multiexponential manner for both periods, although the decline was more rapid after coadministration with rifampin (mean $t_{½}$ 11.5 hours) compared with mitapivat administration alone (mean $t_{½}$ 50.1 hours). Statistical analysis showed that mitapivat AUC_{0-t}, AUC_{∞}, and C_{max} decreased by 91%, 91%, and 77%, respectively, when coadministered with rifampin compared with administration alone (Table 100). Nonparametric statistical comparisons of mitapivat t_{max} indicated a non–statistically significant difference ($p>0.05$) between the two treatments, with a median t_{max} approximately 0.24 hours earlier when mitapivat was coadministered with rifampin (106.2 L/hour) compared with mitapivat alone (9.6 L/hour). Mean CL/F was higher when mitapivat was coadministered with rifampin compared with mitapivat alone.

Table 100. Statistical Analysis of the Effect of Rifampin on Mitapivat Pharmacokinetics, Study AG-348-C-012

Comparison	Pharmacokinetic Parameter	Ratio of Least Squares Means	90% CI
50 mg mitapivat + rifampin versus 50 mg mitapivat alone	AUC_{0-t}	0.09	0.07, 0.11
	AUC_{∞}	0.09	0.07, 0.11
	C_{max}	0.23	0.19, 0.28
		Median Difference	**90% CI**
	t_{max}	-0.24	-0.77, 0.04

Source: Applicant's analysis.
Abbreviations: AUC, area under the concentration-time curve; CI, confidence interval; C_{max}, maximum serum concentration; t_{max}, time for maximum drug concentration

Reviewer's comment: The strong DDI potential between mitapivat and strong CYP3A inhibitors was confirmed by itraconazole, a strong CYP3A and P-gp inhibitor, which increased the mitapivat AUC_{∞} and C_{max} by 4.9- and 1.7-fold, respectively. The strong DDI potential between mitapivat and strong CYP3A inducers was confirmed by rifampin, a strong CYP3A and P-pg inducer, which decreased the mitapivat AUC_{∞} and C_{max} by 91% and 77%, respectively. Avoidance of concomitant use of mitapivat with strong CYP3A inhibitors and inducers was proposed by the Applicant and agreed to by the review team.

14.3. Pharmacometrics Review

14.3.1. Summary of Applicant's Population PK Analysis

Data were available from 186 healthy subjects and 155 patients with PKD. A total of 4913 pharmacokinetic samples from 341 subjects was available and included in the NONMEM dataset. Samples with abnormal concentrations, samples with below the limit of quantification concentrations, and samples with incomplete dosing/sample collection times were flagged in the dataset and excluded from modeling. In total, there were 4686 (95.4% of the total) samples from 341 subjects in the final model. Table 101 lists the baseline continuous covariates by population for the evaluable subjects. Table 102 lists the baseline categorical covariates by population for the evaluable subjects.

Table 101. Summary of Baseline Continuous Covariates Stratified by Population

Covariate Statistic	Healthy Subjects (N=186)	Subjects With Pyruvate Kinase Deficiency (N=155)	Overall (N=341)
Age (years)			
Mean (SD)	38.4 (10.4)	36.1 (14.1)	37.4 (12.2)
Median [Min, Max]	38.0 [18.0, 60.0]	34.0 [18.0, 78.0]	36.0 [18.0, 78.0]
Body weight (kg)			
Mean (SD)	76.6 (12.8)	69.0 (14.9)	73.1 (14.3)
Median [Min, Max]	77.0 [47.8, 110]	67.8 [41.4, 135]	72.4 [41.4, 135]
Body mass index (kg/m^2)			
Mean (SD)	25.8 (3.01)	24.1 (4.93)	25.0 (4.08)
Median [Min, Max]	26.0 [19.0, 31.6]	23.4 [14.7, 54.4]	24.9 [14.7, 54.4]
Missing	0 (0%)	1 (0.6%)	1 (0.3%)
Albumin (g/dL)			
Mean (SD)	4.29 (0.348)	4.65 (0.285)	4.45 (0.368)
Median [Min, Max]	4.30 [3.20, 5.20]	4.60 [3.80, 5.30]	4.50 [3.20, 5.30]
Alkaline phosphatase (U/L)			
Mean (SD)	65.5 (17.4)	76.8 (25.8)	70.6 (22.4)
Median [Min, Max]	65.0 [31.0, 130]	70.0 [39.0, 179]	67.0 [31.0, 179]
Alanine aminotransferase (U/L)			
Mean (SD)	22.3 (8.85)	27.3 (17.3)	24.6 (13.6)
Median [Min, Max]	21.0 [6.00, 51.0]	22.0 [7.00, 104]	22.0 [6.00, 104]
Aspartate aminotransferase (U/L)			
Mean (SD)	19.5 (4.66)	30.3 (17.6)	24.4 (13.5)
Median [Min, Max]	19.0 [9.00, 37.5]	26.0 [12.0, 142]	21.0 [9.00, 142]
Bilirubin (mg/dL)			
Mean (SD)	0.567 (0.254)	5.58 (3.79)	2.87 (3.59)
Median [Min, Max]	0.500 [0.200, 1.60]	4.52 [0.842, 23.3]	0.900 [0.200, 23.3]
Missing	3 (1.6%)	0 (0%)	3 (0.9%)
Blood urea nitrogen (mg/dL)			
Mean (SD)	12.7 (3.54)	14.8 (4.30)	13.7 (4.04)
Median [Min, Max]	12.0 [3.00, 22.0]	14.0 [7.00, 29.1]	13.0 [3.00, 29.1]

Covariate Statistic	Healthy Subjects (N=186)	Subjects With Pyruvate Kinase Deficiency (N=155)	Overall (N=341)
Creatinine (mg/dL)			
Mean (SD)	0.868 (0.203)	0.671 (0.187)	0.779 (0.219)
Median [Min, Max]	0.880 [0.400, 1.38]	0.650 [0.284, 1.54]	0.770 [0.284, 1.54]
Estimated glomerular filtration rate (mL/min/1.73 m²)			
Mean (SD)	104 (24.0)	126 (37.8)	114 (32.9)
Median [Min, Max]	99.0 [63.7, 187]	121 [44.8, 268]	108 [44.8, 268]
Hemoglobin (g/dL)			
Mean (SD)	14.2 (1.34)	8.85 (1.23)	11.7 (2.94)
Median [Min, Max]	14.2 [10.1, 17.4]	8.83 [5.90, 12.3]	12.3 [5.90, 17.4]
Hematocrit (%)			
Mean (SD)	41.9 (3.56)	28.4 (3.92)	35.8 (7.68)
Median [Min, Max]	41.7 [30.8, 52.4]	28.4 [19.4, 39.2]	37.5 [19.4, 52.4]

Source: data.summary.exploratory.r
Abbreviations: Max = maximum; Min = minimum; N = number of subjects; SD = standard deviation.
Source: ag348-pmx-001-poppk-analysis-report, Table 3.

Table 102. Summary of Baseline Categorical Covariates Stratified by Population

Covariate Category	Healthy Subjects (N=186)	Subjects With Pyruvate Kinase Deficiency (N=155)	Overall (N=341)
Sex, n (%)			
Male	128 (68.8)	70 (45.2)	198 (58.1)
Female	58 (31.2)	85 (54.8)	143 (41.9)
Race, n (%)			
White	77 (41.4)	120 (77.4)	197 (57.8)
Black or African American	77 (41.4)	0	77 (22.6)
Asian	31 (16.7)	14 (9.0)	45 (13.2)
Native Hawaiian or other Pacific Islander	1 (0.5)	1 (0.6)	2 (0.6)
Other	0	4 (2.6)	4 (1.2)
Not reported	0	16 (10.3)	16 (4.7)

Covariate Category	Healthy Subjects (N=186)	Subjects With Pyruvate Kinase Deficiency (N=155)	Overall (N=341)
Ethnicity, n (%)			
Hispanic or Latino	45 (24.2)	3 (1.9)	48 (14.1)
Not Hispanic or Latino	141 (75.8)	126 (81.3)	267 (78.3)
Not reported	0	26 (16.8)	26 (7.6)
Geographic region, n (%)			
North America	0	55 (35.5)	55 (16.1)
Western Europe	0	88 (56.8)	88 (25.8)
Rest of the world[1]	0	12 (7.7)	12 (3.5)
Missing	186 (100)	0	186 (54.5)
Mutation type, n (%)			
Missense/missense	0	103 (66.5)	103 (30.2)
Missense/non-missense	0	42 (27.1)	42 (12.3)
Non-missense/non-missense	0	10 (6.5)	10 (2.9)
Missing	186 (100)	0	186 (54.5)
Fasting condition, n (%)			
Fasted	211 (97.7)	0	211 (56.9)
With/without food[2]	0	155 (100)	155 (41.8)
High-fat meal	5 (2.3)	0	5 (1.3)
Formulation, n (%)			
Capsule	102 (47.2)	52 (33.5)	154 (41.5)
Tablet	114 (52.8)	103 (66.5)	217 (58.5)

Source: data.summary.exploratory.r
Abbreviations: N = total number of subjects; n = number of subjects in the category.
Note: Counts are reported as number of subjects (%). Some subjects could have different food status or formulations in the different study periods.
[1] Excluding North America and Western Europe.
[2] Drug may be taken with or without food. Food status was not captured in the Phase 2/3 studies.
Source: ag348-pmx-001-poppk-analysis-report, Table 4.

The final mitapivat population pharmacokinetics model was a three-compartment model with first-order absorption. Clearance of mitapivat was modeled as exponentially increasing to steady state to account for the autoinduction observed after multiple doses. CL is a function of time (DAY) and DOSE and is given below:

Figure 30. Clearance Function in Final Mitapivat Population Pharmacokinetics Model

$$TVCL = CL0 + dCLss \times \left(1 - e^{\left(-\frac{\ln(2)}{THCL} \times DAY\right)}\right)$$

$$dCLss = dCLss100 \times \left(\frac{DOSE}{100}\right)^{Theta13}$$

$$THCL = THCL100 \times \left(\frac{DOSE}{100}\right)^{-Theta14}$$

Source: ag348-pmx-001-poppk-analysis-report, Section 5.2.2, page 41.
Note: CL is the clearance on a certain DAY from the beginning of the treatment; CL0 is the clearance on the first day of the treatment (DAY =0); dCLss is an increase in CL from the start of dosing to steady state due to autoinduction, expressed as a function of DOSE; and THCL is the time to reach 50% of the dCLss (in days). dCLss100 and THCL100 are the dCLss and THCL values, respectively, at a dose of 100 mg. Exponential coefficients were estimated to descr be the effect of DOSE (mg) given twice daily on dCLss and THCL (Theta13 and Theta14, respectively).

The following covariate effects were identified in the final model: volumes of distribution (V2, V3, and V4) increased with body weight; females had a slightly lower V2 relative to males; and CL increased with hematocrit (HCT) level; CL in patients with PKD was generally lower than in healthy subjects. Other covariates tested, including age, race, ethnicity, estimated glomerular filtration rate, and PKD mutation type, were not identified as statistically significant covariates on mitapivat pharmacokinetic parameters. The parameter estimates for the final model are presented in Table 103.

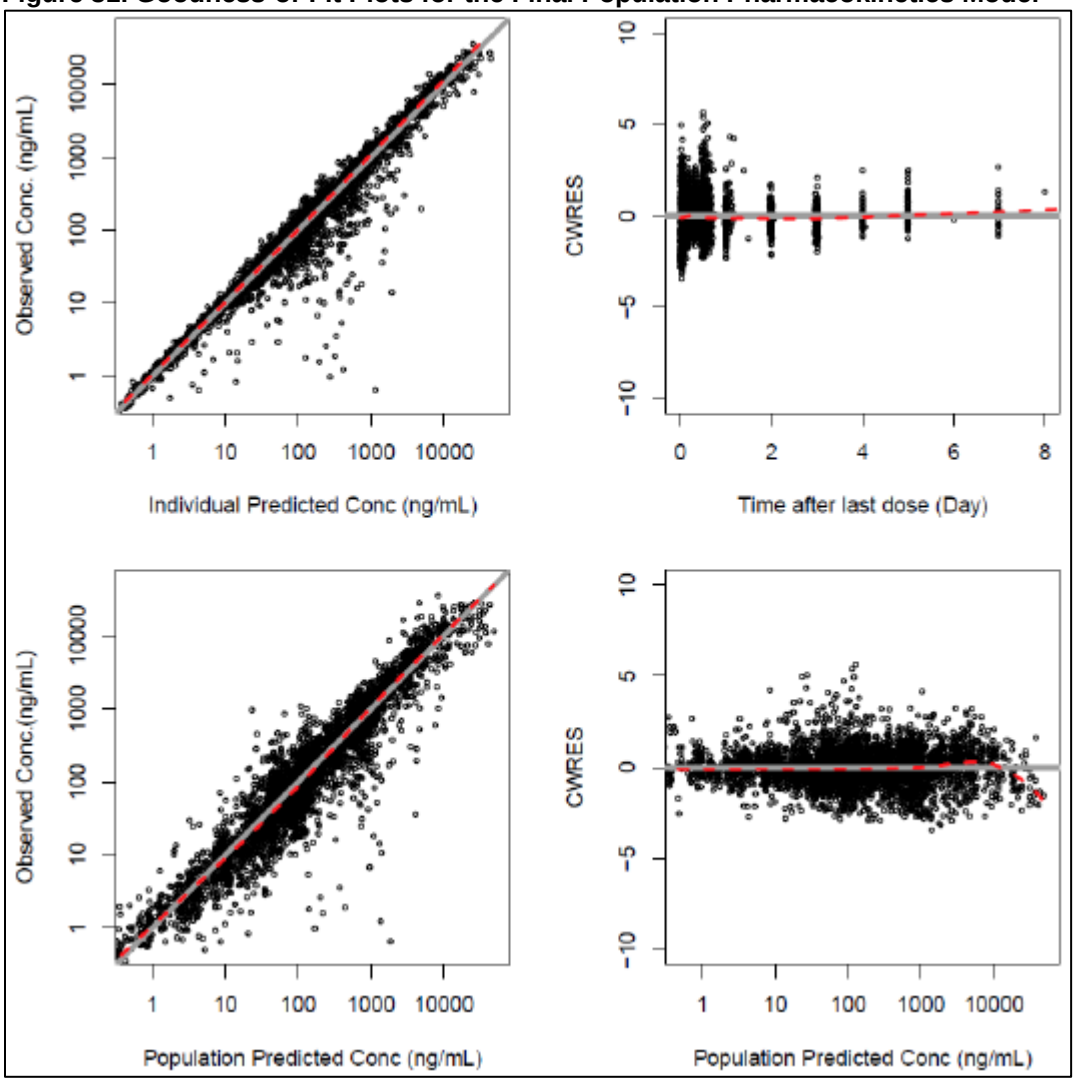
Table 103. Final Population PK Model Parameter Estimates

Parameter	Estimate	RSE (%)	BSV		Shrinkage (%)
			CV%	RSE (%)	
Fixed effects					
CL/F (L/hr) on Day 1	10.6	3.55	29.0	11.2	6.6
V_2/F (L)	43.1	3.18	21.2	17.3	34.9
Q_3/F (L/hr)	1.48	6.41	71.8	19	20.4
V_3/F (L)	105	6.47	46.6	25.5	52.1
Q_4/F (L/hr)	1.76	10.4	--	--	--
V_4/F (L)	10.1	5.67	--	--	--
ALAG1 for tablet formulation (hr)	0.249 (fixed)	--	--	--	--
ALAG1 for capsule formulation (hr)	0.227 (fixed)	--	--	--	--
Ka (1/hr)	3.19	7.67	89.8	12.8	21.1
CL increase for 100 mg, dCLss/F (L/hr)	5.66	8.68	--	--	--
Time to reach 50% of the dCLss for 100 mg, THCL (day)	6.4	25.7	--	--	--
Dose on dCLss/F	0.653	11.2	--	--	--
Dose on THCL	-0.511	34.4	--	--	--
WT on V_2/F, V_3/F, and V_4/F	0.646	18.4	--	--	--
HCT on CL/F	0.364	26.2	--	--	--
Sex female on V_2/F	-0.114	31.5	--	--	--
Interoccasion variability					
ω^2 of IOV on Ka	1.85	25.9	--	--	--
Residuals					
δ of proportional error (healthy subjects)	0.277	3.82	--	--	9.1
δ of proportional error (subjects with pyruvate kinase deficiency)	0.425	4.64	--	--	9.1

Source: final.model.r
Abbreviations: ω^2 = variance; δ = standard deviation; ALAG1 = absorption lag time; BSV = between-subject variability; CL = clearance; CL/F = apparent clearance; CV = coefficient of variation; dCLss = increase in CL from start of dosing to steady state due to autoinduction; dCLss/F = apparent dCLss; HCT = hematocrit; IOV = interoccasion variability; Ka = first-order absorption rate constant; PK = pharmacokinetic; Q_3/F, Q_4/F = apparent intercompartmental clearance; RSE = relative standard error; THCL = time to reach 50% of the dCLss; V_2/F= apparent central volume of distribution; V_3/F, V_4/F = apparent peripheral volume of distribution; WT = body weight.
Source: ag348-pmx-001-poppk-analysis-report, Table 8.

Diagnostic plots for the final population pharmacokinetics model are presented in Figure 31 and demonstrate an adequate model fit. Moderate to high between-subject variability was estimated in the model, with CV% of 29.0%, 21.2%, 71.8%, 46.6%, and 89.8% for CL/F, V2/F, Q3/F, V3/F, and Ka, respectively. Shrinkage was 6.6% for CL/F and ranged from 20.4% to 52% for V2/F, Q3/F, and V3/F.

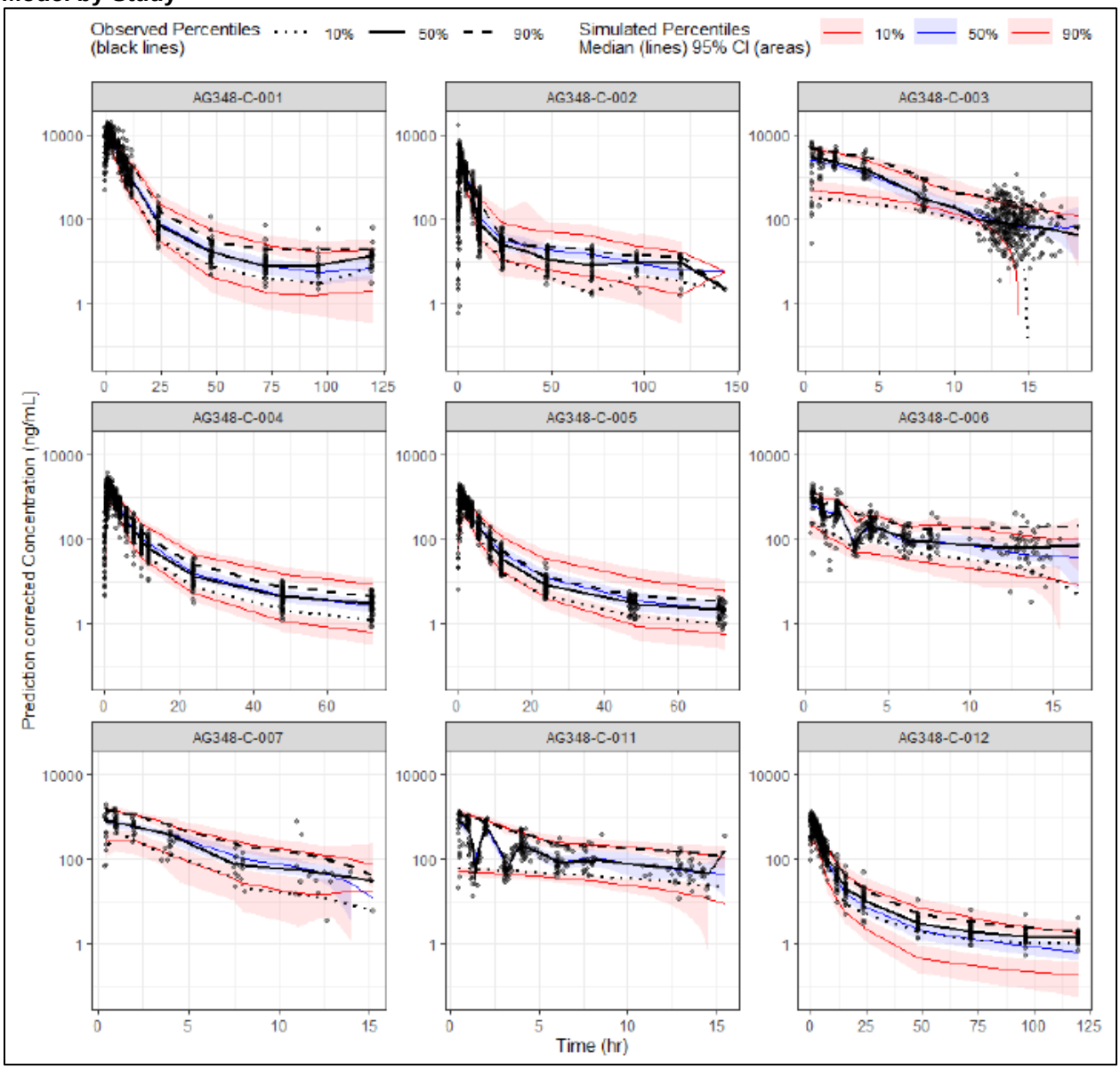
Figure 31. Goodness-of-Fit Plots for the Final Population Pharmacokinetics Model



Source: ag348-pmx-001-poppk-analysis-report, Figure 7.
Abbreviations: conc, concentration; CWRES, conditional weighted residuals

Prediction-corrected visual prediction checks are presented in Figure 32 for each study. Each figure is based on 1000 replicate simulations, accounting for subject covariates. The overall concurrence of the simulation results (5th, 50th, and 95th percentiles) with percentiles of the observed data indicates that the final model well described the central tendency and the spread of the mitapivat pharmacokinetic profiles.

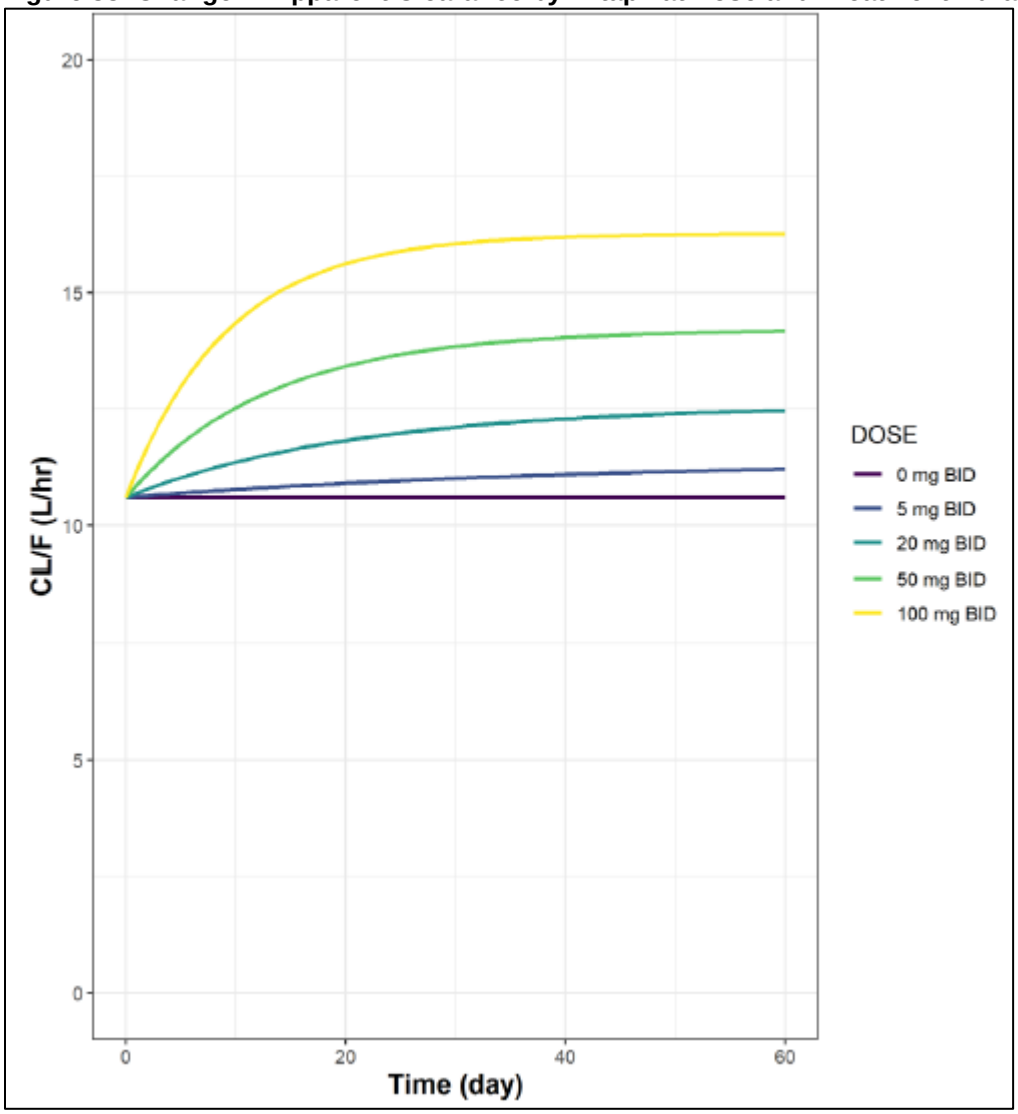
Figure 32. Prediction-Corrected Visual Predictive Check for the Final Population Pharmacokinetics Model by Study



Source: ag348-pmx-001-poppk-analysis-report, Figure 8.
Abbreviation: CI, confidence interval

Due to autoinduction of clearance, CL/F of mitapivat increases in a dose- and time-dependent manner. Figure 33 illustrates the CL/F versus time curves at different doses for a typical patient with pyruvate kinase deficiency. Steady-state clearance increased at higher dose levels due to autoinduction. Median and 5th to 95th percentiles of the mitapivat CL/F and exposure metrics (C_{max}, Ctrough, and AUC) at steady state for different dose regimens were simulated using the individual pharmacokinetic parameters of patients with PKD; the results are presented in Figure 34.

Figure 33. Change in Apparent Clearance by Mitapivat Dose and Treatment Duration



Source: ag348-pmx-001-poppk-analysis-report, Figure 9.
Abbreviations: BID, twice daily; CL/F, apparent clearance

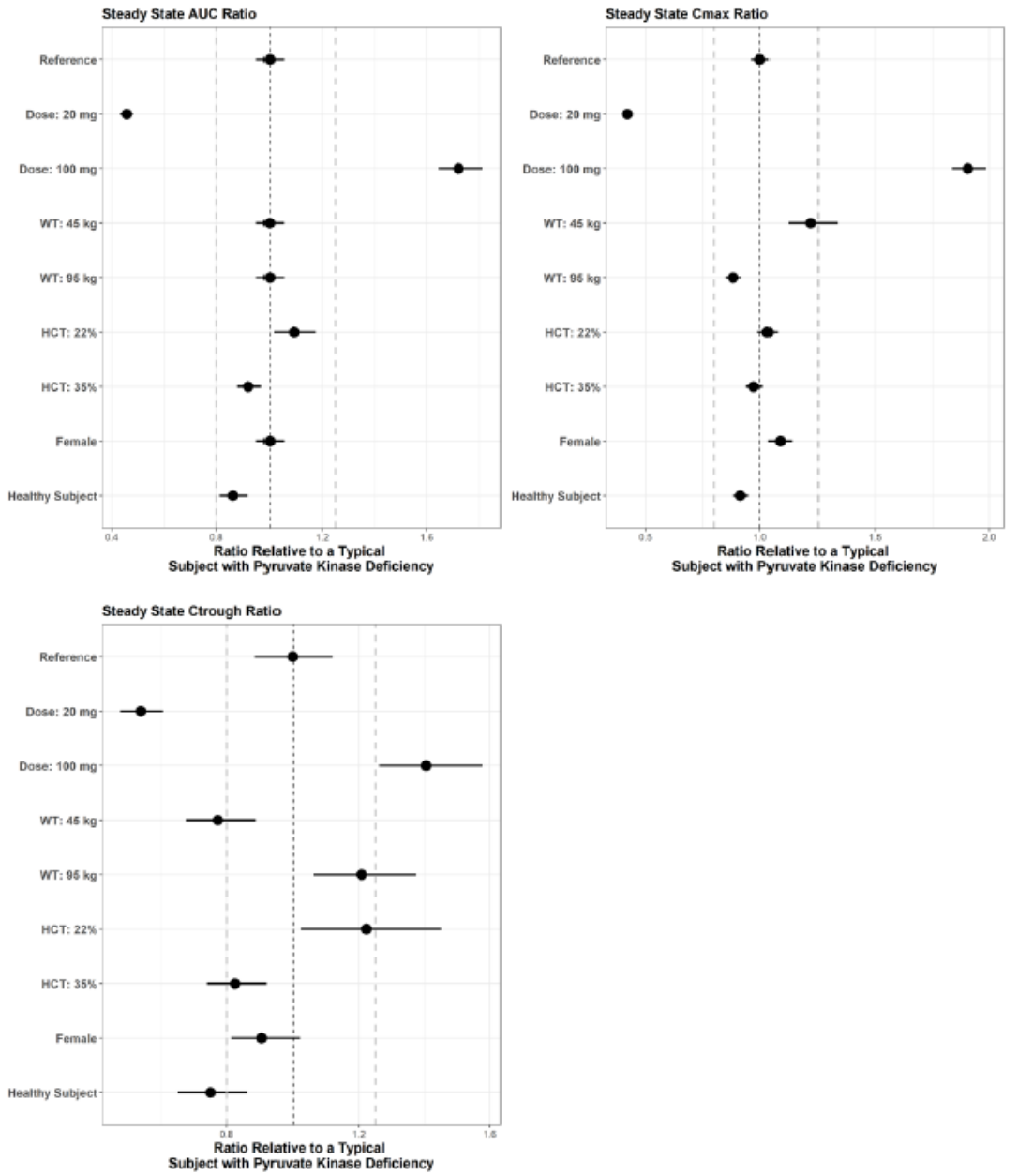
Figure 34. Steady-State Mitapivat Exposure and Clearance in Patients With Pyruvate Kinase Deficiency

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: ag348-pmx-001-poppk-analysis-report, Table 6.
Abbreviations: AUC, area under the concentration-time curve; BID, twice daily; CL/F, apparent clearance; C_{max}, maximum serum concentration; C_{trough}, predose trough concentration

The effects of mitapivat dose and covariates on the exposure (AUC, C_{max}, and C_{trough}) at steady state is shown in Figure 35, where the exposures relative to the reference typical patients with PKD are expressed as ratios for various doses and covariate settings. The reference exposure was simulated for a patient with typical covariate values (male, body weight of 70 kg, and baseline HCT of 28%) treated with mitapivat 50 mg BID; the 90% CIs of the exposure were obtained from 1000 simulations by sampling the uncertainty of the model parameters. Dose or covariate values were changed one at a time for different scenarios, and values were compared to the reference. The 90% CIs of the ratios for the impact of body weight and HCT on AUC and C_{max} were contained within the range 0.8 to 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 HCT on C_{trough} were slightly outside the window of 0.8 to 1.25; the point estimate of the C_{trough} ratio for a 45 kg body weight was less than 0.8.

Figure 35. Impact of Dose and Covariates on Steady-State Mitapivat Exposure in Patients With Pyruvate Kinase Deficiency



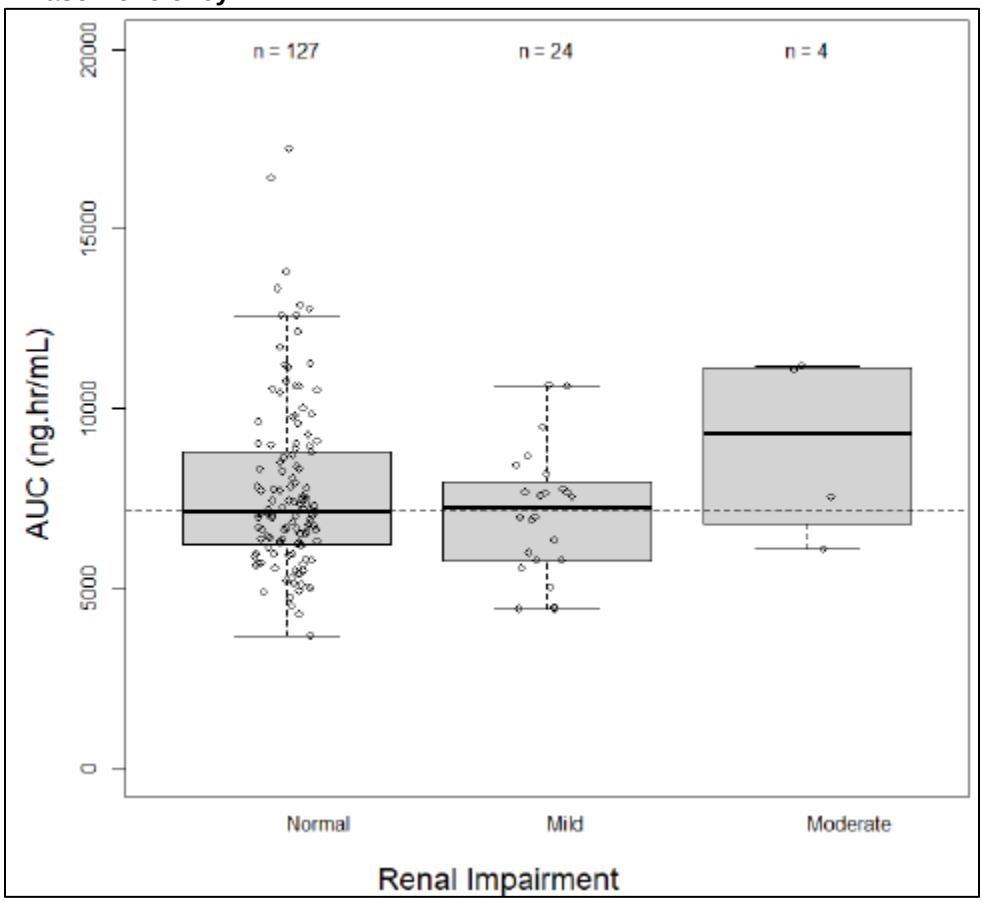
Source: pk.sim.r.
Abbreviations: AUC = area under the concentration-time curve; BID = twice daily; Cmax = maximum concentration; Ctrough = trough concentration; HCT = hematocrit; PK = pharmacokinetic; WT = body weight.
Note: Vertical black central dashed line indicates the reference steady-state exposure of a typical subject with pyruvate kinase deficiency (male, WT=70 kg, and HCT=28%) receiving mitapivat 50 mg BID. Vertical gray dashed lines indicate the 0.8 and 1.25 ratio relative to the reference. Error bars indicate the 90% confidence intervals of the ratio estimated from 1000 simulations based on the typical value and uncertainty of PK parameters and the dose or covariate values. Healthy subject was simulated as a male with WT=77 kg and HCT=42%.

Source: ag348-pmx-001-poppk-analysis-report, Figure 13.

Figure 36 compares the steady-state AUC among patients with PKD according to renal category. Most patients in the dataset had normal renal function (N=127, 81.9%), whereas 24 (15.5%) patients had mild renal impairment and 4 (2.6%) patients had moderate renal impairment.

Steady-state AUC was similar between patients with normal renal function and those with mild renal impairment. The steady-state AUC of patients with moderate renal impairment was numerically higher but still within the range in patients with normal renal function. The sample size of patients with moderate renal impairment in the analysis was small (N=4).

Figure 36. Impact of Renal Function on Steady-State Mitapivat Exposure in Patients With Pyruvate Kinase Deficiency



Source: ag348-pmx-001-poppk-analysis-report, Figure 15.
Circles represent individual subjects. Boxplots show the median (solid bold line), the interquartile range (boxes), and 1.5 times the interquartile range (whiskers). Points are jittered in the x-axis direction for clarity. Dashed horizontal line indicates zero value.
Abbreviation: AUC, area under the concentration-time curve

Reviewer's comment: The reviewer reproduced the Applicant's final population pharmacokinetics model analysis described in Study Report ag348-pmx-001-poppk-analysis-report. The population pharmacokinetics model-derived geometric mean of mitapivat pharmacokinetic parameters at steady state of 5 mg, 20 mg, and 50 mg BID are presented in Table 104, and recommended to be included in the final labeling.

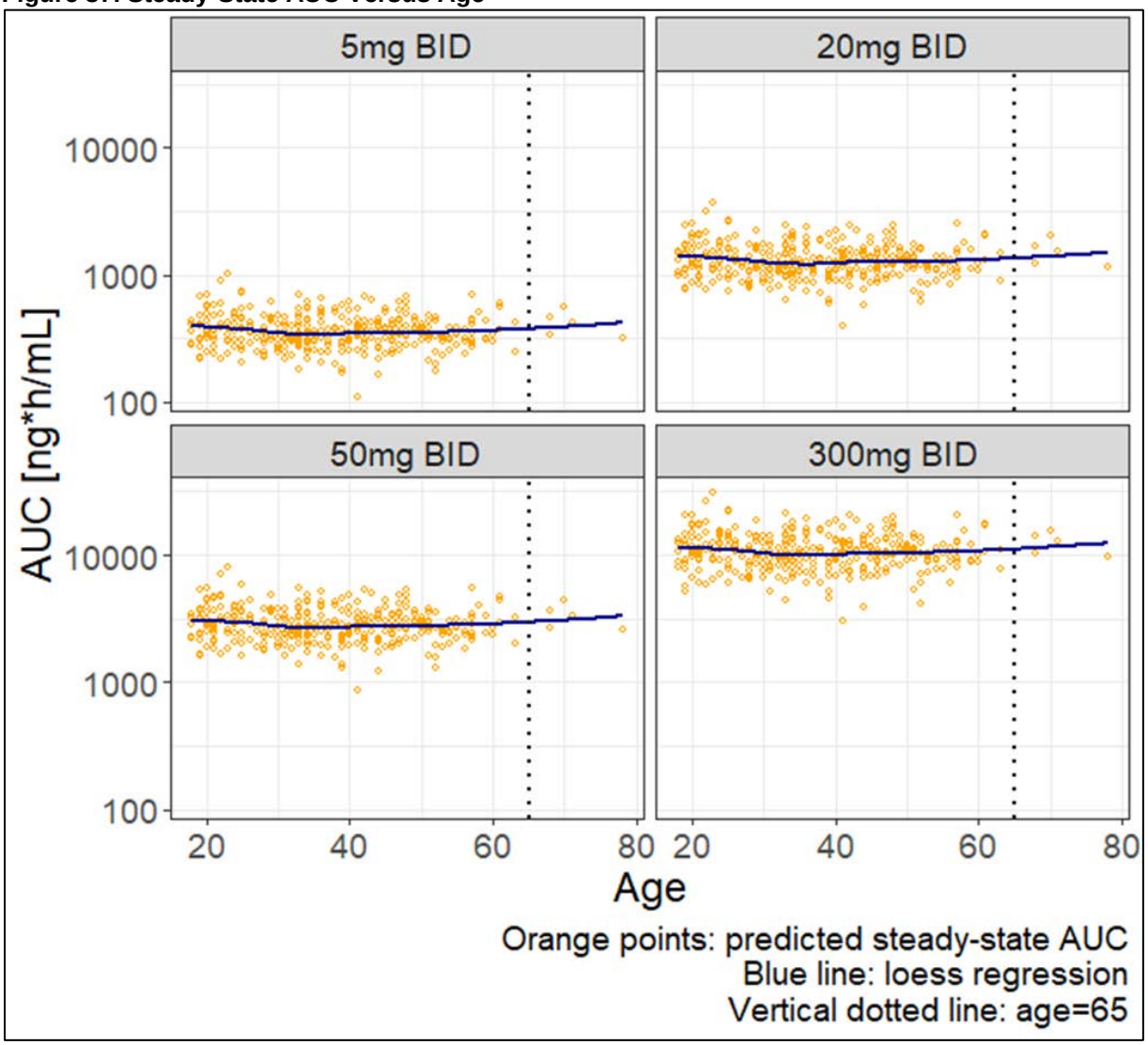
Table 104. Population Pharmacokinetics-Derived Pharmacokinetic Parameters at Steady State

Mitapivat Dosage	CL (L/h)	Cmax (ng/mL)	Ctrough (ng/mL)	AUCss (ng*h/mL)	Accumulation Ratio
5 mg BID	11.2 (28.3%)	131 (27.3%)	1.97 (237%)	440 (31.5%)	1.21 (21.4%)
20 mg BID	12.3 (28.3%)	499 (27.3%)	4.94 (486%)	1582 (31.6%)	1.09 (21.7%)
50 mg BID	13.9 (28.3%)	1178 (28.3%)	8.52 (783%)	3482 (32.1%)	0.96 (22%)

Source: Reviewer's analysis.
The pharmacokinetic parameters (expressed as geometric means [CV%]) were derived from population pharmacokinetics simulations. The simulations were performed until 100 days after the first dose. The interval of the last 12 hours was selected for steady-state pharmacokinetic parameter calculation.
Abbreviations: AUC_{ss}, area under the concentration-time curve at steady state; BID, twice daily; CL, drug clearance; C_{max}, maximum serum concentration; C_{trough}, predose trough concentration

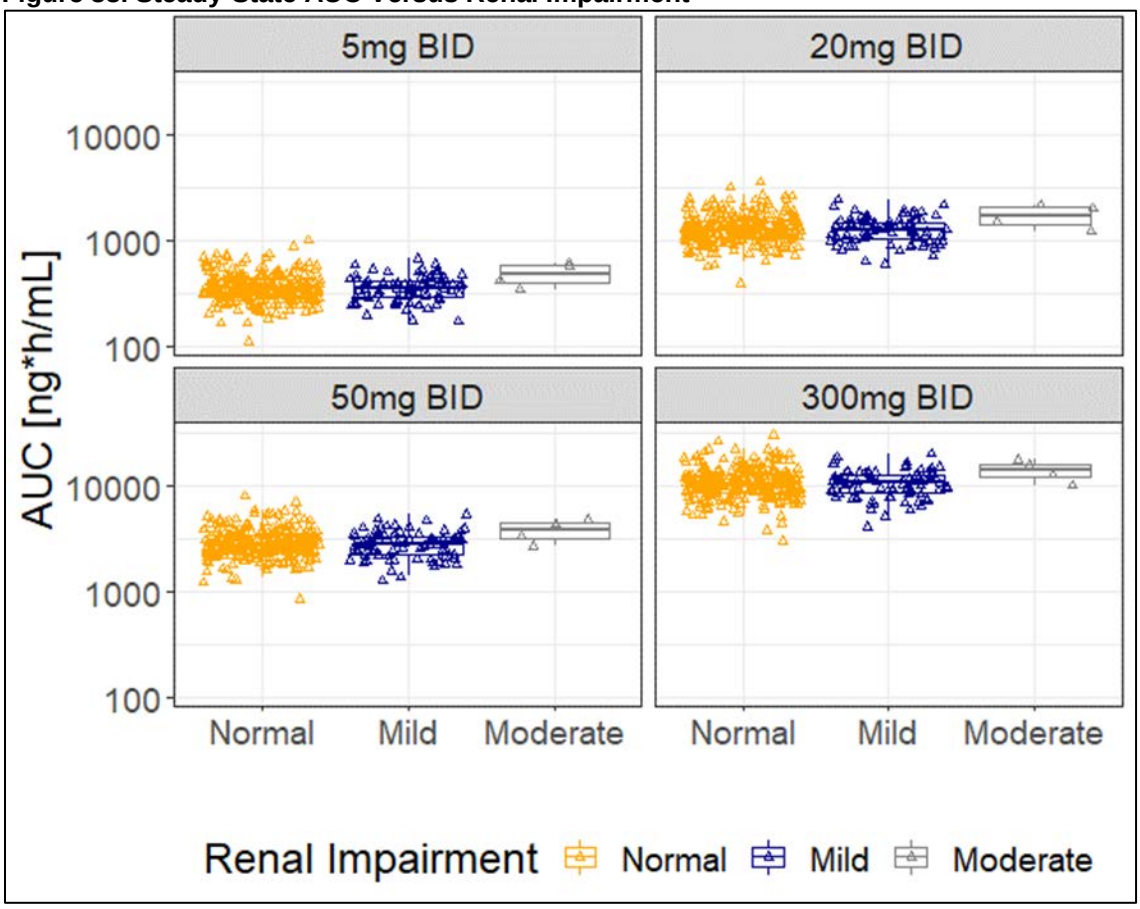
The steady-state AUC derived from population-pharmacokinetics-estimated individual pharmacokinetic parameters showed no trend with increasing age (Figure 37). The simulated mean steady-state AUC in patients with moderate renal impairment is numerically higher than that in patients with normal and mild renal impairment (Figure 38). However, the sample size and the number of pharmacokinetic samples were too small to inform dose adjustment for patients with moderate renal impairment. Refer to Section II.8.1 for further discussion regarding dose adjustment for patients with moderate and severe renal impairment. Overall, the Reviewers agree with the Applicant's labeling statement regarding mitapivat pharmacokinetics in specific populations, i.e., no clinically meaningful effects on the pharmacokinetics of mitapivat were observed based on age, sex, race, body weight, and mild renal impairment.

Figure 37. Steady-State AUC Versus Age



Source: Reviewer's analysis.
Abbreviations: AUC, area under the concentration-time curve; BID, twice daily

Figure 38. Steady-State AUC Versus Renal Impairment



Source: Reviewer's analysis.
Triangle dots represent individual values. Box represents 25th, 50th, and 75th percentile of observations. Error bars represent the smallest value within 1.5 times interquantile range below 25th percentile and largest value within 1.5 times interquantile range above 75th percentile.
Abbreviations: AUC, area under the concentration-time curve; BID, twice daily

14.3.2. Summary of Applicant's Exposure-Response Analysis

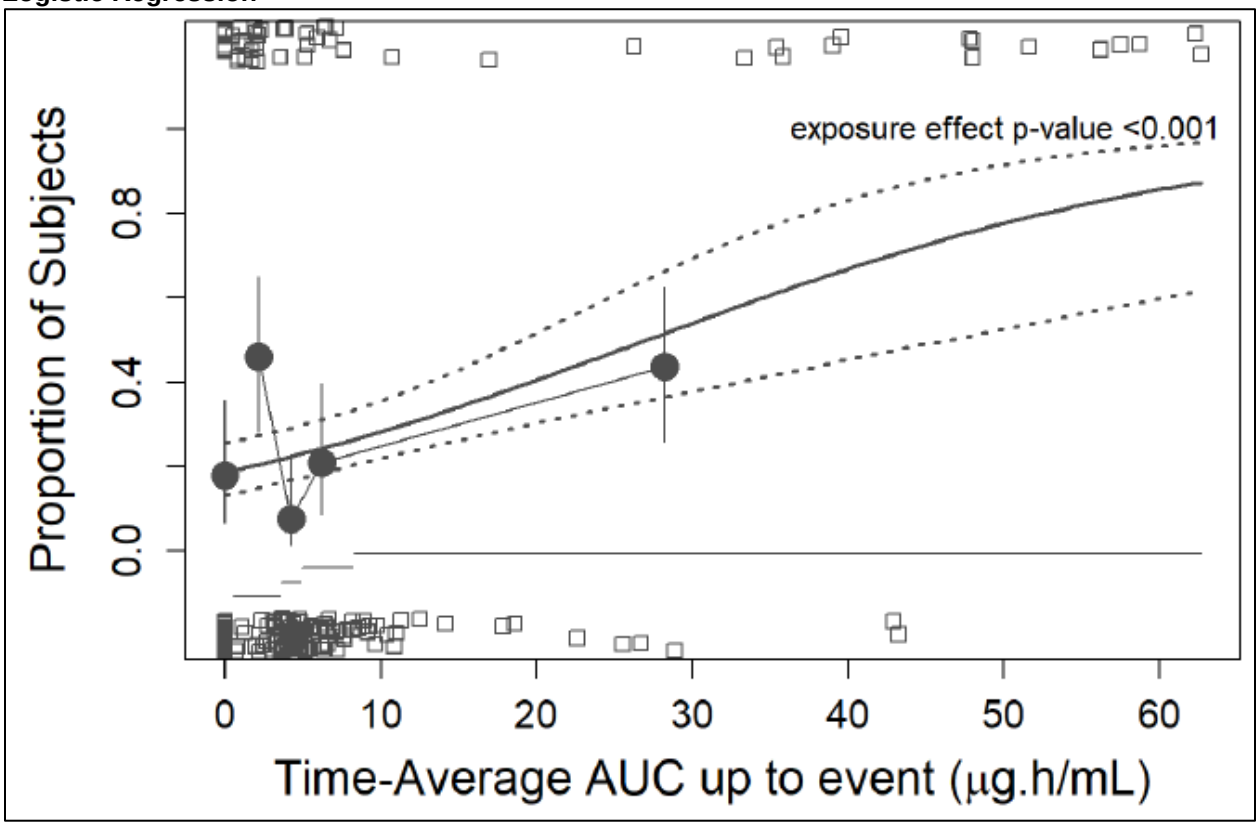
14.3.2.1. Exposure-Response for Safety

Data from 155 patients treated with mitapivat from 5 to 300 mg BID in Studies 003, AG-348-C-006 (Study 006), AG-348-C-007 (Study 007), and AG-348-C-011 (Study 011), were used to perform an exposure-response (E-R) analysis for treatment-emergent adverse events (TEAEs). The exposure metric was the time-averaged exposure up to the day of the event or, in case of no event, the time-averaged exposure up to Day 168 (Week 24). No E-R relationships were identified based on logistic regression for all-grade alanine aminotransferase (ALT) elevation (p=0.34), all-grade aspartate aminotransferase (AST) events (p=0.67), or all-grade triglycerides events (p=0.77). Linear regression analysis suggested no significant E-R relationship for change from baseline in adjusted spine dual-energy x-ray absorptiometry (DXA), T-score (p=0.27), or change from baseline in femoral total DXA T-score (p=0.44).

All-grade insomnia event occurrence data were available for 155 mitapivat- and 39 placebo-treated patients. To explore exposure trends, the number of patients with an event was stratified

by exposure quartile. A logistic regression applied to the individual data confirmed a statistically significant exposure effect across the observed exposure range. The incidence of all-grade insomnia events increases with increasing exposure (Figure 39). Since the exposure effect was significant and showed an increasing frequency with increasing exposure, covariate evaluation was performed. No covariate met the p<0.05 significance level for forward inclusion. No covariate was included in the all-grade insomnia regression model. The predicted probability of all-grade insomnia was 19.9% for 5 mg BID, 22.1% for 20 mg BID, and 26.0% for 50 mg BID.

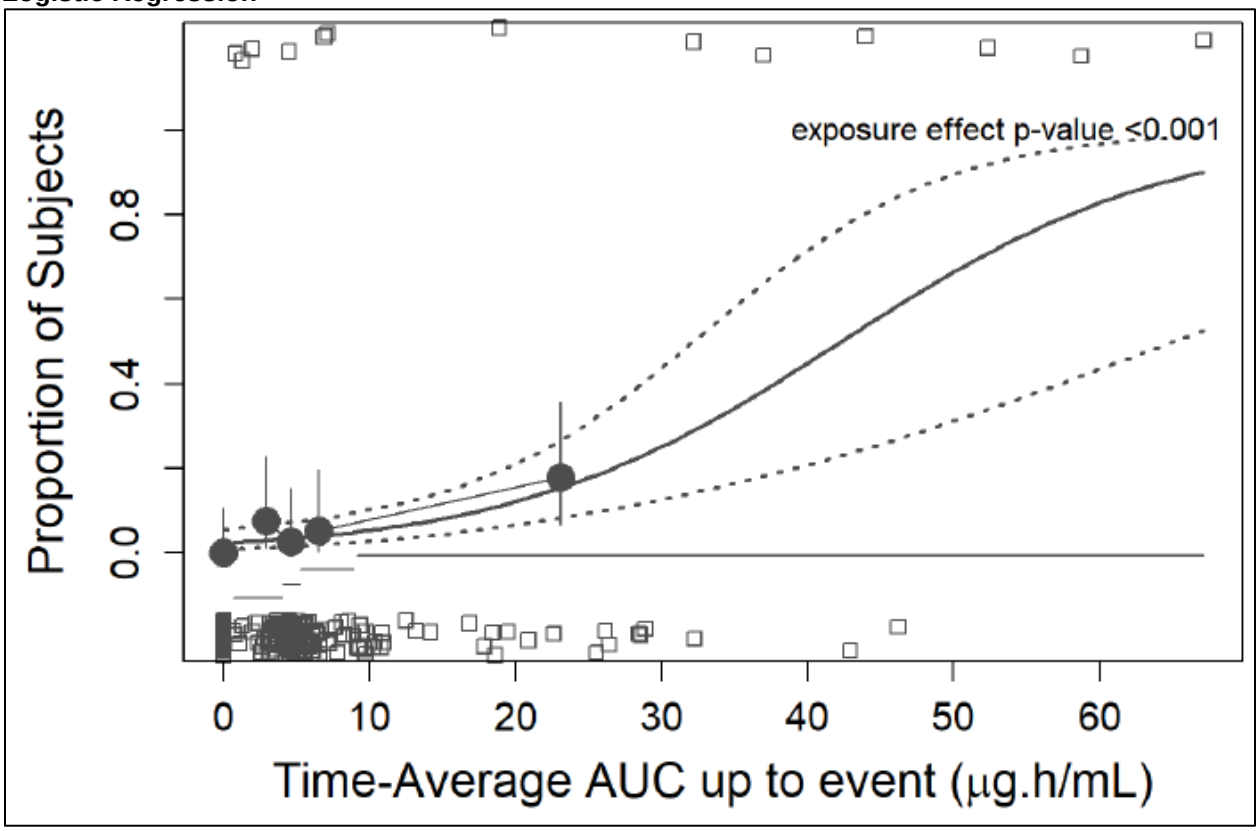
Figure 39. Quantile Plot for Exposure Versus All-Grade Insomnia Event Occurrence and a Linear Logistic Regression



Source: ag348-pmx-002, Figure 20.
Open squares represent individual values. Solid dots and vertical lines represent the incidence and 95% CI of observation within a quantile. The solid line represents the logistic regression fit of the data in the form of logit(Prob[Response])~Intercept+Exposure×Slope. Dashed lines represent the 95% CI from logistic regression. Horizontal lines indicate the range of the exposure quartiles
Abbreviation: AUC, area under the concentration-time curve

All-grade hot flush event occurrence data were available for 155 mitapivat-treated patients. Events were observed in 0 (0%) placebo-treated patients and in 13 (8.4%) mitapivat-treated patients. For exploration of exposure trends, the number of patients with an event was stratified by exposure quartiles. A logistic regression applied to the individual data to further investigate the exposure effect confirmed the presence of a statistically significant exposure effect across the observed exposure range. The incidence of hot flush events increases with increasing exposure (Figure 40). Since the exposure effect was significant and showed an increasing frequency with increasing exposure, covariate evaluation was performed. No covariate met the required P<0.05 significance level for forward inclusion. No covariate was included in the all-grade hot flush regression model. The predicted probability of all-grade hot flush was 3.4% for 5 mg BID, 4.0% for 20 mg BID, and 5.5% for 50 mg BID.

Figure 40. Quantile Plot for Exposure Versus All-Grade Hot Flush Event Occurrence and a Linear Logistic Regression

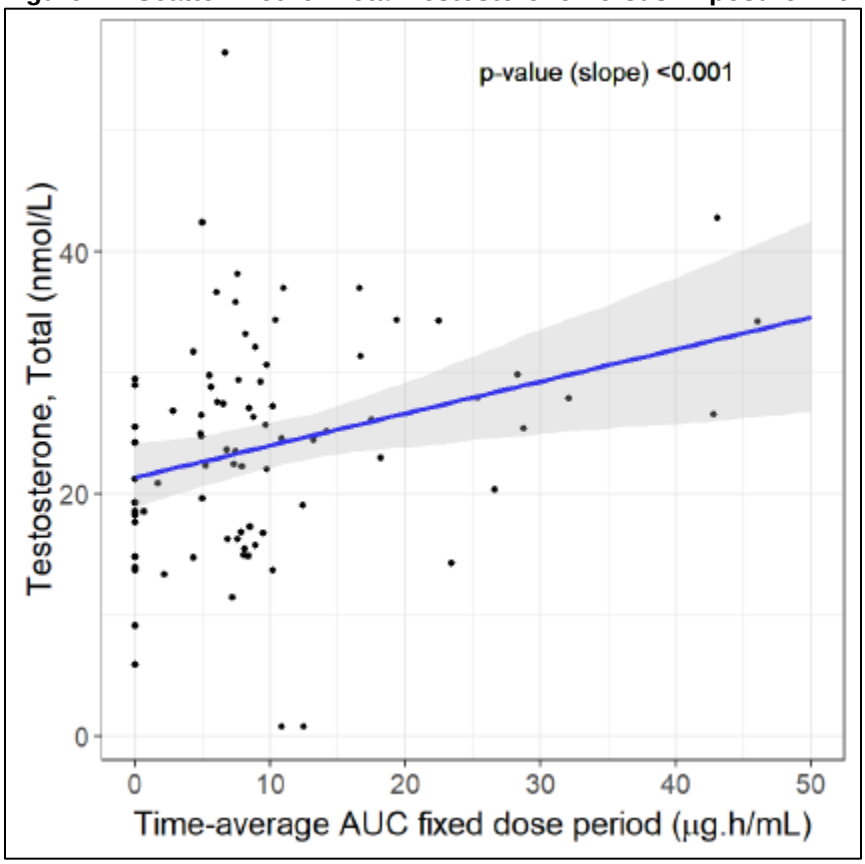


Source: ag348-pmx-002, Figure 21.
Open squares represent individual values. Solid dots and vertical lines represent the incidence and 95% CI of observation within a quantile. The solid line represents the logistic regression fit of the data in the form of logit(Prob[Response])~Intercept+Exposure×Slope. Dashed lines represent the 95% CI from logistic regression. Horizontal lines indicate the range of the exposure quartiles.
Abbreviation: AUC, area under the concentration-time curve

Data from 68 male patients treated with mitapivat (Study 003: 32 patients; Study 006: 15 patients; Study 007: 7 patients; and Study 011: 14 patients) and 15 male patients treated with placebo (Study 006) were available for sex hormone E-R analysis.

Total testosterone levels were higher in male mitapivat-treated patients compared with patients on placebo. Linear regression was applied to the individual data confirmed the presence of a statistically significant exposure effect across the observed exposure range ($p<0.001$). Baseline total testosterone was the only covariate retained in the model, both on the intercept and the slope.

Figure 41. Scatter Plot for Total Testosterone Versus Exposure With Model Fit in Male Patients

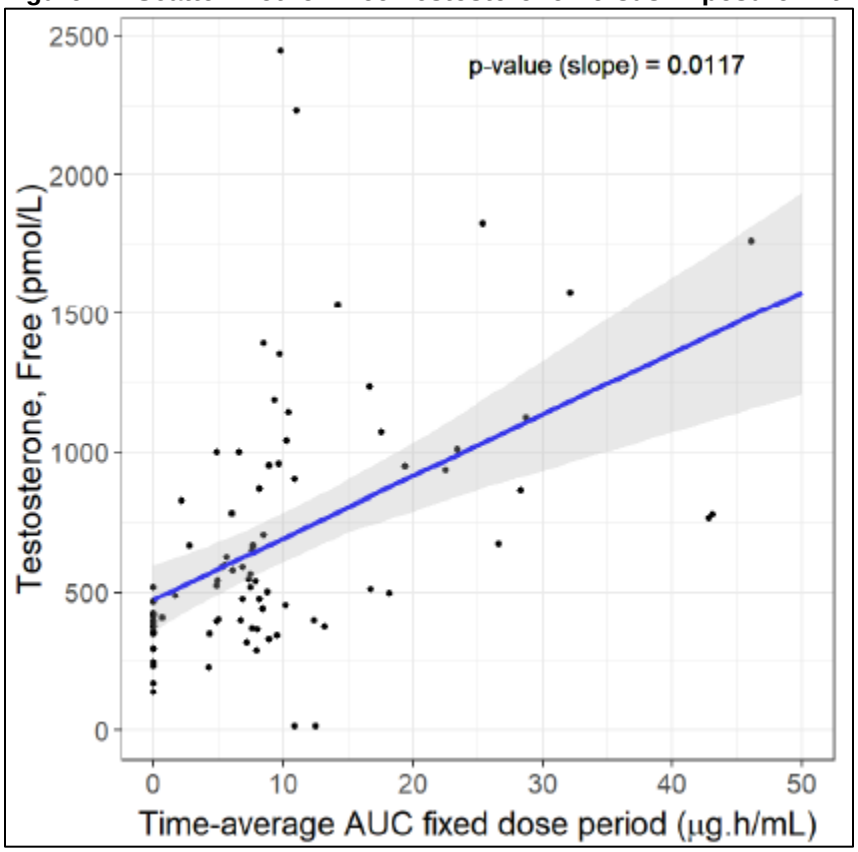


Source: ag348-pmx-002, Figure 23.
Dots represent individual values. Solid lines and gray areas represent the model fit and 95% confidence interval.
Abbreviation: AUC, area under the concentration-time curve

Free testosterone levels were higher in male mitapivat-treated patients compared with patients on placebo. A weak trend of increasing levels with increasing exposure was present (p=0.012). The effect of exposure was estimated based on the placebo- and mitapivat-treated patients. Baseline free testosterone was the only covariate retained in the model, both on the intercept and the slope.

For 50 mg BID, dose-response for total testosterone and free testosterone in male patients showed an upward trend, but changes were modest (<30% change compared to placebo).

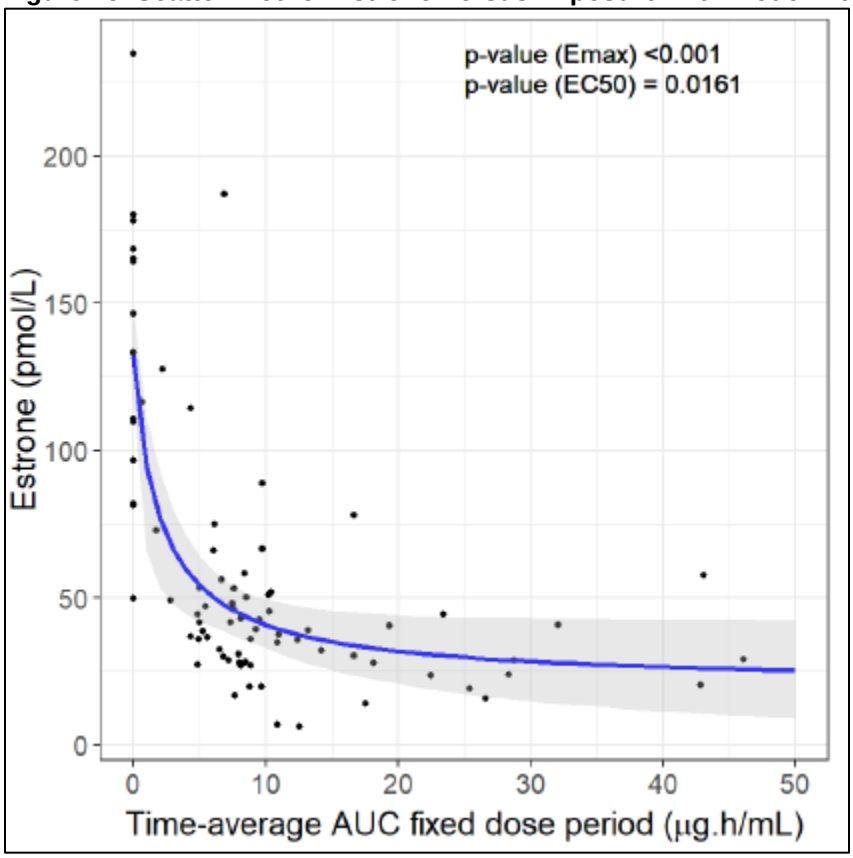
Figure 42. Scatter Plot for Free Testosterone Versus Exposure With Model Fit in Male Patients



Source: ag348-pmx-002, Figure 24.
Dots represent individual values. Solid lines and gray areas represent the model fit and 95% confidence interval.
Abbreviation: AUC, area under the concentration-time curve

Estrone levels were lower in male mitapivat-treated patients compared with patients on placebo. There was a clear trend of decreased estrone level with increasing exposure (E_{max} $p<0.001$; EC_{50} $p=0.016$). The E_{max} model best described the data. Baseline estrone was the only covariate retained in the model, both on the intercept and the maximum effect. For 50 mg mitapivat BID, estrone levels in males were predicted to decrease compared to placebo from 137 to 43.4 (68.2% compared to placebo); however, the vast majority (88.4%) of patients had estrone levels in the normal range (33 to 133 pmol/L), and 11.6% had estrone levels below the lower limit of normal.

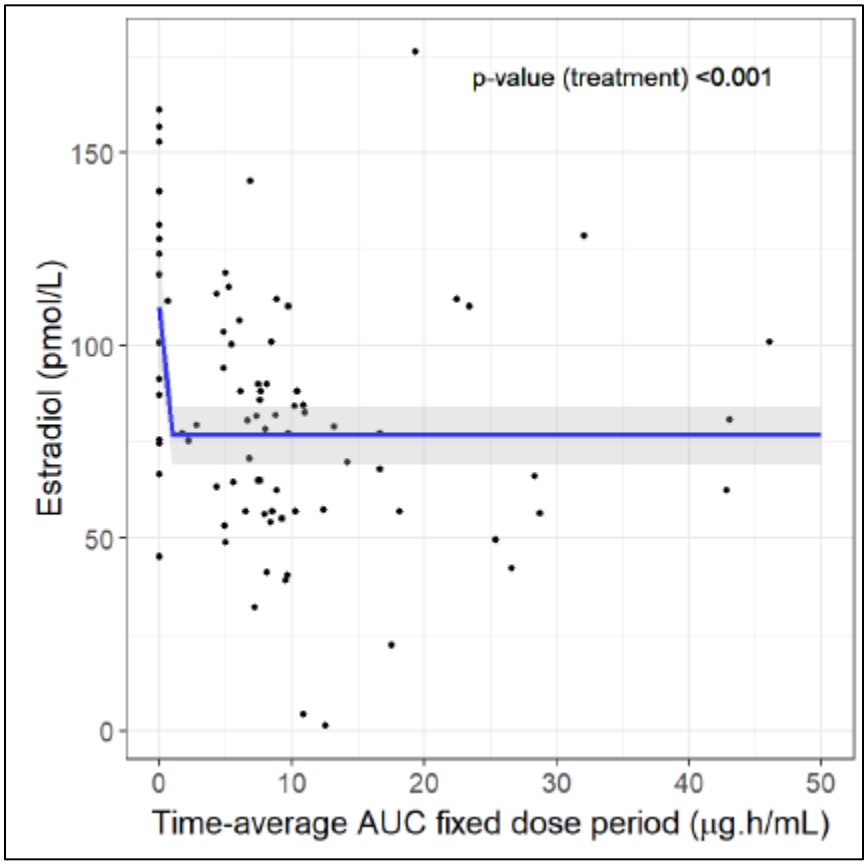
Figure 43. Scatter Plot for Estrone Versus Exposure With Model Fit in Male Patients



Source: ag348-pmx-002, Figure 25.
Dots represent individual values. Solid lines and gray areas represent the model fit and 95% confidence interval.
Abbreviation: AUC, area under the concentration-time curve

Estradiol levels were lower in male mitapivat-treated patients compared with patients on placebo. However, no consistent trend with exposure was observed across the exposure quartiles. Levels were similar across the exposure quartiles. The effect of exposure was evaluated based on the placebo- and mitapivat-treated male patients. The best fit of the data was obtained with a treatment effect model ($p<0.001$).

Figure 44. Scatter Plot for Observed Estradiol Versus Exposure With Model Predictions in Male Patients



Source: ag348-pmx-002, Figure 26.
Dots represent individual values. Solid lines and gray areas represent the model fit and 95% confidence interval.
Abbreviation: AUC, area under the concentration-time curve

Reviewer's comment: The reviewer has reproduced the E-R analysis for the above-mentioned safety measures. Increased risk with increasing mitapivat exposure was identified for all-grade insomnia and all-grade hot flush. However, the increase was considered minor. Significant correlations were confirmed for male total and free testosterone, estrone, and estradiol. However, the predicted fluctuation of the male hormones was largely within the normal range. In summary, there is no substantial safety concern for up to 50 mg BID as an individual maintenance dose.

14.3.2.2. Exposure-Response for Efficacy

Data from 57 mitapivat-treated patients (Study 006: 40 patients; Study 011: 17 patients), and 39 placebo-treated patients (Study 006) were included. The time-averaged AUC across the Fixed-Dose Period was used as the exposure metric. To explore exposure trends, the number of Hgb responders, defined as a ≥1.5 g/dL increase in Hgb concentration from baseline that was sustained at two or more scheduled assessments in Study 006 and Study 011, was stratified by exposure tertile. There were no Hgb responders with placebo treatment, whereas after mitapivat treatment at least 30% of the patients were Hgb responders (Table 105). The number and percentage of responders across the exposure tertiles were similar for responders and nonresponders; no significant trends with exposure were apparent (Table 105). A logistic regression applied to the individual data to investigate the exposure effect confirmed the absence

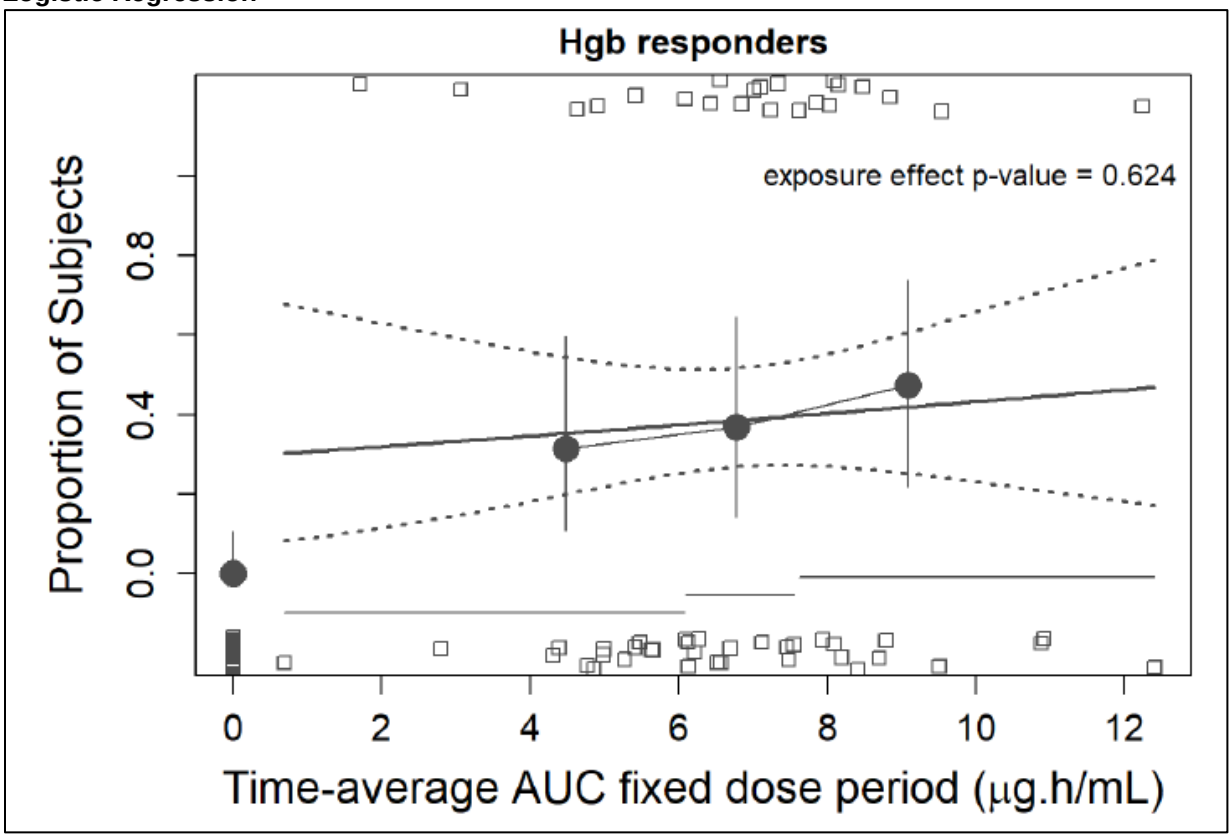
of a statistically significant exposure effect across the observed exposure range (Table 105 and Figure 45). The lack of an exposure-efficacy relationship could be a result of the dose titration algorithm. Patients who were more sensitive to mitapivat might achieve response at lower doses, whereas patients who were less sensitive might require higher doses to achieve desired response.

Table 105. Number and Percentage of Hemoglobin Responders by Exposure Group, Studies 006 and 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.
Source: Report ag348-pmx-002, Table 7.

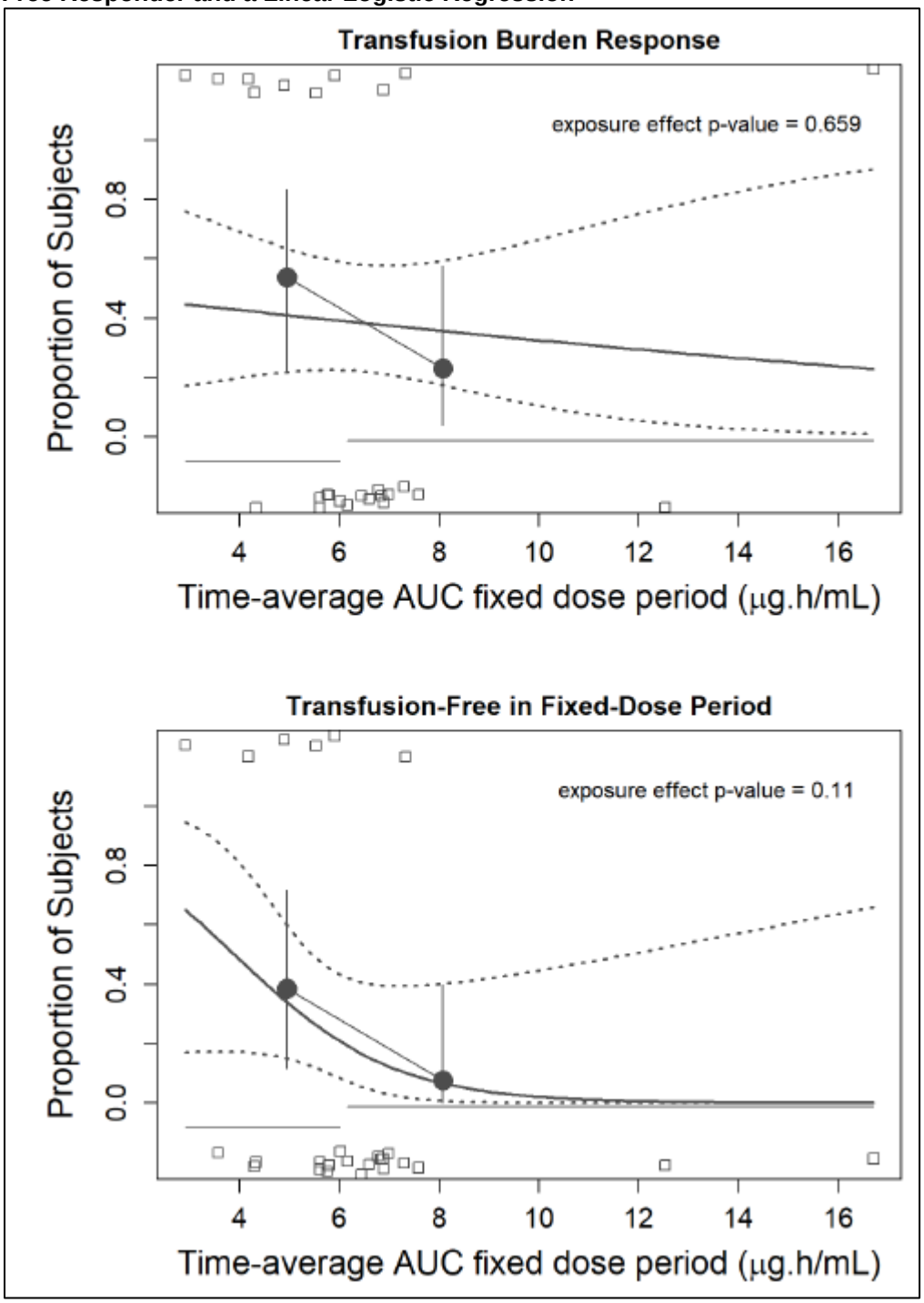
Figure 45. Quantile Plot for Exposure Versus Hemoglobin Responder Incidence and a Linear Logistic Regression



Source: Report ag348-pmx-002, Figure 7.
Open squares represent individual values. Solid dots and vertical lines represent the incidence and 95% CI of observation within a quantile. The solid line represents the logistic regression fit of the data in the form of logit(Prob[Response])~Intercept+Exposure×Slope. The dashed lines represent the 95% CI from logistic regression. Horizontal lines indicate the range of the exposure quartiles.
Abbreviation: AUC, area under the concentration-time curve

In Study 007, all 26 efficacy evaluable patients had mitapivat exposure estimates. 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 standardized to 24 weeks, and transfusion free, defined as zero transfusions administered through the 24 weeks of the fixed-dose period in Study 007, were available from 26 mitapivat-treated patients. A logistic regression applied to the individual data to investigate the exposure effect illustrated a trend but indicated that the exposure effect was not statistically significant (Figure 46).

Figure 46. Quantile Plot for Exposure Versus Incidence of Transfusion Burden and Transfusion-Free Responder and a Linear Logistic Regression



Source: Report ag348-pmx-002, Figure 10.
Open squares represent individual values; solid dots and vertical lines represent the incidence and 95% CI of observation within a quartile, respectively; the solid line represents the logistic regression fit of the data in the form logit(Prob[Response])~Intercept Exposure×Slope; dashed lines represent 95% CI from logistic regression; horizontal lines represent the range of exposure quartiles.Abbreviation: AUC, area under the concentration-time curve

<u>Reviewer's comment</u>: The reviewer has reproduced the Applicant's E-R analysis for efficacy. For Hgb responders, the mitapivat-treated patients showed a higher responder rate compared to

patients in the placebo group. Among the patients exposed to mitapivat, no significant correlation was identified between mitapivat time-averaged AUC and responder rate. For transfusion burden response and transfusion-free responder rate, exposure effects were not significantly correlated. The lack of an E-R correlation is probably due to dose titration. In summary, the E-R for efficacy results provided evidence to support an individualized maintenance dose (5 mg, 20 mg, and 50 mg BID).

14.3.2.3. Pharmacokinetics-PD Analysis

An indirect-effect pharmacokinetics-PD model was developed for Hgb changes with time using individual pharmacokinetic parameters and predicted concentration profiles from the mitapivat population pharmacokinetics model. Hemoglobin data from 168 patients with PKD who are not regularly transfused were used to evaluate the pharmacodynamic response. The developed pharmacokinetics-PD model was used for simulation. The Base Case Scenario mimics the proposed titration regimen. The Fast Response scenario evaluated whether efficacy could be accelerated by increasing the dose every 2 weeks. The Super Aggressive scenarios evaluate whether patients could start at a higher dose, and in so doing, reduce the time to achieve efficacy. The down-titration margin was 2 g/dL below the upper limit of normal (ULN) of Hgb. The simulation results indicated that the proposed titration algorithm led to a similar probability of response and the lowest dose reduction due to Hgb overshooting (Table 8).

Reviewer comment: The Applicant's population pharmacokinetics-PD modeling and simulations support the proposed dose titration algorithm.

14.4. Physiologically Based Pharmacokinetic Analyses Review

Executive Summary

The objective of this review is to evaluate the adequacy of the Applicant's PBPK analyses to predict the following:

- The DDI effect of strong and moderate CYP3A4 inhibitors on the exposure of mitapivat at 5, 20, and 50 mg BID.
- The DDI effect of strong and moderate CYP3A4 inducers on the exposure of mitapivat at 5, 20, and 50 mg BID.
- The effect of elevated gastric pH on mitapivat exposure.
- The DDI potential of mitapivat on the exposure of substrates of CYP2B6 (bupropion), CYP3A (midazolam), CYP2C8 (repaglinide), CYP2C9 (warfarin), and CYP2C19 (omeprazole).
- The DDI potential of mitapivat on the exposure of substrates of the transporters P-gp (digoxin), OATP1B (rosuvastatin), OAT3 (methotrexate), and MATE1/OCT2 (metformin).

The Division of Pharmacometrics has reviewed the PBPK submission (Report AG-48-PBPK-001, and model files), and responses to FDA's request for information to conclude the following:

- PBPK analyses were adequate to predict the interaction effect of CYP3A inhibitors on mitapivat exposure at steady state. Coadministration of a strong CYP3A inhibitor

increased mitapivat AUC_{tau} from 3.5- to 5-fold. Coadministration of a moderate CYP3A inhibitor increased mitapivat AUC_{tau} from 2.6-to 3.3-fold.

- PBPK analyses were adequate to predict the interaction effect of CYP3A inducers on mitapivat exposure at steady state. Coadministration of a strong CYP3A inducer decreased mitapivat AUC_{tau} by approximately 95%; while a moderate CYP3A inducer decreased AUC_{tau} by approximately 55 to 60%.
- The PBPK analysis indicated a moderate induction effect of mitapivat (50 mg BID) with a sensitive substrate of CYP3A.
- The induction effect of mitapivat on CYP2B6, CYP2C8, CYP2C9, and CYP2C19 could not be predicted by PBPK analysis. The potential for DDI of mitapivat with substrates of CYP2B6 and CYP2C isoforms cannot be excluded.
- The PBPK analyses were inadequate to confirm a negative DDI potential of mitapivat on the pharmacokinetics of substrates of the transporters OATP1B, MATE2, and OCT1. However, the static models indicated no DDI liability of mitapivat with a substrate of OATP1B1, MATE1, or OCT2.
- The PBPK analysis and static model indicated no DDI liability of mitapivat with a substrate of OAT3.
- The mitapivat advance dissolution, absorption, and metabolism (ADAM) PBPK model, along with the in vitro solubility and human absorption data, was adequate to evaluate the effect of elevated gastric pH on the exposure of mitapivat. Increase in gastric pH due to coadministration of an acid-reducing agent is unlikely to be clinically meaningful.

Background

The proposed mitapivat dosing regimen is a total daily dose of 100 mg taken as 50 mg BID (twice daily) without regard to food. The starting dose is 5 mg BID. Mitapivat exhibited dose proportional increase in AUC after single doses of 30 to 2,500 mg mitapivat (Study AG-348-C-001). A less than dose-proportional increase in mitapivat exposure over the dosing interval (AUC_{tau}) was observed after multiple doses (Study AG-348-C-002).The median T_{max} was around 0.5 to 1 hour. The absorption of mitapivat was not affected by food (high-fat meal). Population pharmacokinetics analysis of clinical pharmacokinetics data (n=186 healthy, n=155 subjects with PKD) showed that mitapivat clearance increased in a time- and dose-dependent manner after multiple doses. At steady state, the population pharmacokinetics estimates of median CL/F were 11.2, 12.3, and 14 L/h for mitapivat dosages of 5, 20, and 50 mg BID, respectively. There was a less than dose-proportional increases in AUC and C_{trough}, but minimal impact on C_{max}. There was no apparent difference in the pharmacokinetics of mitapivat in patients compared to healthy subjects (Report AG-348-PMX-001). No major plasma metabolite of mitapivat was observed (less than 10% of total drug-related AUC). The inactive metabolite AGI-8702 showed metabolite to parent ratios for AUC and C_{max} of $\leq$0.155 and $\leq$0.39 after a single dose and multiple doses of mitapivat, respectively (Summary of Clinical Pharmacology Report). The 50 mg capsule and 50 mg tablet formulations demonstrated similar bioavailability (Study 005).

Human mass balance data (ADME Study 009) showed that mitapivat was extensively metabolized (recovery of unchanged mitapivat in feces and urine was less than 5%). In vitro studies suggested mitapivat was primarily metabolized by CYP3A4. A clinical DDI study in healthy volunteers evaluated the effect of coadministration of the strong CYP3A inhibitor

itraconazole and strong CYP3A inducer rifampicin on the pharmacokinetics of mitapivat single dose in healthy volunteers (Study AG-348-C-012 [Study 012]).

In vitro, mitapivat was determined to be an inducer of the CYP isoforms 2B6, 2C8, and 2C9 without apparent reversible and time-dependent inhibition towards these enzymes. Mitapivat was determined to be an inducer and time-dependent inhibitor of CYP3A, and an inducer and mixed-inhibitor of CYP2C19 (Reports AG-348-N-054-R1, AG-348-N-055, AG-348-N-103). Mitapivat was also determined to be an in vitro inhibitor of the transporters P-gp, OATP1B1, OAT3, OCT2, and MATE1 (Reports AG-348-N-089 and AG-348-N-097). In vitro, mitapivat was a substrate of P-gp, but not a substrate of BCRP, OATP1B1, or OATP1B3 (Reports AG-348-N-057-Amend-1 and AG-348-N-097).

Methods

The PBPK analyses were performed using the software Simcyp®. The absorption of mitapivat was described using a first-order absorption model. The model used the in vitro permeability data in Caco-2 cells (AG-348-N-057) to predict Qgut (nominal flow through gut model) and effective permeability ($P_{eff,man}=2.9\times10^{-4}$ cm/s). The absorption was assumed to be complete (fa=1), based on mass balance data (unchanged mitapivat accounted for <1% in faces). The absolute bioavailability (F) of mitapivat was 72.7% and the intravenous clearance was 9.53 L/h (Study 009). Based on these data, estimates for Fh and fa × Fg were calculated to be 0.841 and 0.865, respectively, assuming a fa of 1.0. In the absence of any information on active drug uptake into the enterocyte, fugut was initially set at a default value of 1. This value however did not recover the observed oral clearance of mitapivat. Using parameter sensitivity analysis for fugut over the range of 0.02 to 1, the value of 0.48 recovered the estimated Fg of 0.87. The first order absorption rate constant (Ka=6 L/h) with lag time (0.4 h) were optimized over a range of 2 to 6 L/h and 0 to 0.5 h, respectively, to recover the observed C_{max} and T_{max} values for mitapivat in Studies AG-348-C-001 and -C-002. Mitapivat was determined to be a substrate of P-gp in vitro (Report AG-348-N-057-R1). However, an intestinal P-gp component was not incorporated in the model due to the following reasons: (1) human mass balance data indicated that drug absorption is nearly complete (fa estimated as 0.99) at the dose level of 120 mg; (2) clinical single ascending dose data demonstrated linearity in mitapivat AUC and C_{max} over the dose range of 5 mg to 200 mg (Study AG-348-C-004 [Study 004]), suggesting that P-gp is saturated over this dose range and not limiting the extent and rate of absorption.

A mechanistic absorption model (ADAM) was subsequently developed to assess the impact of an increase in gastric pH on the pharmacokinetics of mitapivat. Formulation, solubility, and dissolution data were used as model inputs. The dissolution of mitapivat was predicted using the diffusion layer model. The in vitro aqueous solubility of mitapivat at differing pH values (PPD Study 074125-13-03) combined with mitapivat physicochemical parameters were analyzed, using the Simcyp In Vitro Analysis toolkit, to obtain estimates of the intrinsic solubility and a solubility factor 1 (SF1) for mitapivat. All other ADAM related parameters were set to default values.

The unbound fraction of mitapivat in plasma (fup) was 0.023 and dose-independent (Report AG-348-N-040-R1). The blood to plasma partitioning ratio was 0.641 (Report AG-348-N-039-R1). A minimal PBPK model with a single adjusting compartment was used to characterize the disposition of mitapivat. The volume of distribution at steady state (Vss=0.54 L/kg) was predicted using the Rodgers-Rowland method, and a global Kp scalar of 0.42 was optimized to

recover the clinical pharmacokinetics. The predicted Vss was comparable to the reported Vss value (0.53 L/kg) following intravenous administration of mitapivat (Study 009). The SAC-related parameters (Vsac, kin, and kout) were estimated from clinical single ascending dose data (Study AG-348-C-001).

In vitro data suggested no evidence for active uptake mechanism by OATP1B for mitapivat into human hepatocytes (Report AG-348-N-097). An in vitro study using recombinantly expressed CYP enzymes established CYP3A4 and CYP3A5 as the main enzymes responsible for CYP mediated hepatic metabolism of mitapivat by measuring the formation of metabolite AGI-8702 (Report AG-348-N-096). The relative contribution of CYP3A4 to the total clearance of mitapivat was assigned to 87% based on the clinical DDI with itraconazole (Study 012). The retrograde method was used to calculate the unbound intrinsic clearance value of CYP3A4 (CLint,u CYP3A4) based on the intravenous clearance of 9.53 L/h (Study 009). Additional intrinsic clearance in liver microsomes (HLM CLint,u) was assigned in the model (13% of total metabolic clearance). Renal clearance of mitapivat was minimal (CLr=0.335 L/h), based on urinary recovery of unchanged mitapivat (average of 3%) in the ADME study (Study 009). The in vitro inhibition parameters towards CYP3A, reversible (Ki=81μM) and time-dependent (KI=24μM and kinact=0.78 h^{-1}, using testosterone as the probe), were initially evaluated as model inputs (AG-348-N-054). Correction for nonspecific binding used a predicted fumic value of 0.983 (protein concentration 0.1 mg/mL, nonspecific binding was not measured). Preliminary simulations showed that the use of in vitro CYP3A inactivation parameters for mitapivat led to overprediction of the CYP3A inactivation effect. Further, the CYP3A inactivation parameters for erythromycin (used as positive control in the same in vitro study) were up to three-fold more potent than the default values in the library model. Next, a three-fold correction to the KI (72μM) of mitapivat was applied by calibration with erythromycin data. The Applicant rationale was based on literature reports showing that HLM data tend to overestimate CYP3A inactivation potential by a factor of two- to four-fold (Mao et al. 2016).

The in vitro induction parameters towards CYP3A4 (IndC50=9.23μM and Indmax 15-fold) were initially evaluated as model input (Report AG-348-N-055). These in vitro values were calibrated using rifampin data as a positive control. In the absence of a measured value of the fraction of mitapivat unbound in the hepatocyte incubation (fuinc), a predicted fuinc value of 0.75 was used. The Applicant assumed that autoinduction of CYP3A4 activity is the main explanation for the dose and time dependency of mitapivat pharmacokinetics following multiple dosing. The CYP3A4 induction data were optimized to recover the observed extent of autoinduction observed in the multiple ascending doses study of mitapivat (AG-348-C-002). An IndC50 value of 0.92μM (i.e., 10-fold reduction) was required to recover the observed accumulation ratio.

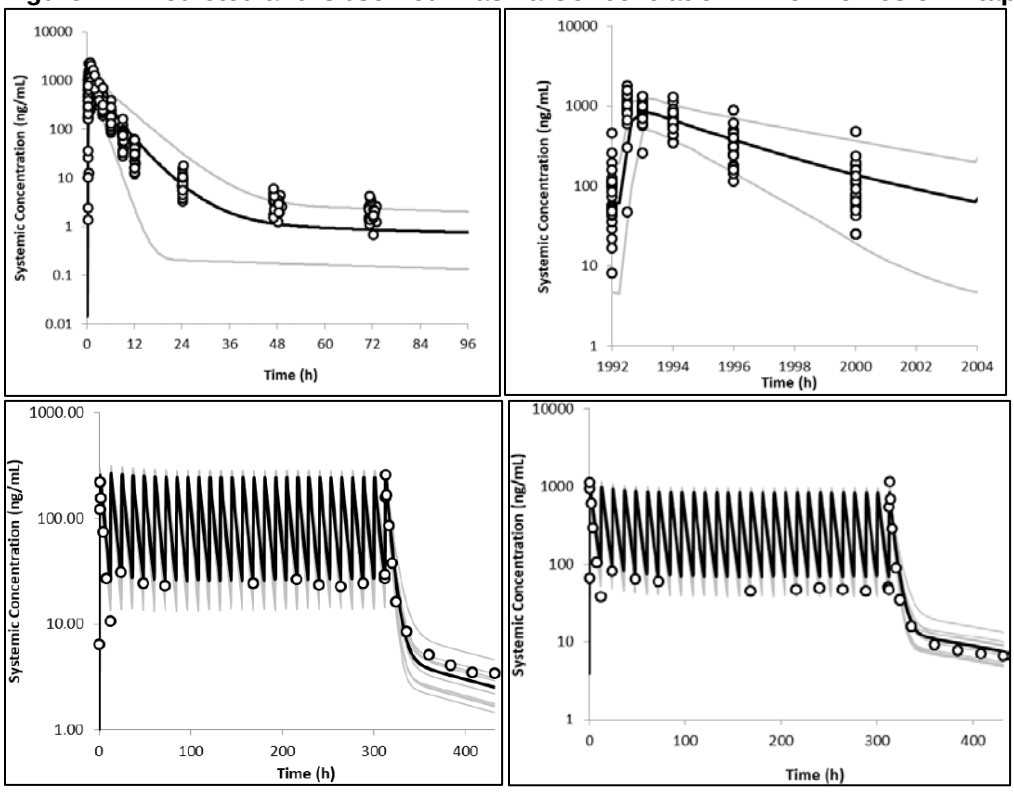
The in vitro induction parameters, IndC50 and Indmax (=E_{max} +1), towards the CYP isoforms 2B6 (IndC50=2.36μM and Indmax=5.73), 2C8 (IndC50=2.29μM and Indmax=4.1), 2C9 (IndC50=5.83μM and Indmax=3.8), and 2C19 (IndC50=2.62μM and Indmax=4.05) were based on mRNA expression, except for CYP2C9 which was based on CYP2C9 activity. The in vitro IC_{50} values for the transporters OATP1B1 (29μM), MATE1 (7.17μM), OCT2 (7.76μM), OAT3 (12.1μM) and P-gp (12.8μM) were used as model input (Report AG-348-N-057).

Results

Q1. Can the PBPK Model Adequately Describe the Pharmacokinetics Profiles of Mitapivat?

Yes, the PBPK model of mitapivat reasonably captured the observed pharmacokinetics in healthy subjects following oral administration of single-doses of 5 to 200 mg (Studies 004, 005, and 012-control group) and multiple-doses of 15 mg BID to 360 mg BID (Study AG-348-C-002). Likewise, the model adequately captured the observed pharmacokinetics of mitapivat in the target patient population following oral administration of 50 mg BID for 12 weeks (Studies 006 and -C011) and 24 weeks (Study 007) (Figure 47 and Table 106). Of note, the number of patients at dose levels of 5 mg BID and 20 mg BID was small. Thus, a direct comparison of model performance for these dose levels was not conducted.

Figure 47. Predicted and Observed Plasma Concentration-Time Profiles of Mitapivat



Source: Applicant's Response to Clinical Pharmacology Information Request and * Reviewer's analysis.
*(A): PK profile (log-linear scale) of a single oral dose of 50 mg mitapivat in healthy subjects. Individual data from Study AG-348-C-005 (circles, n=26). Black line: mean for the predicted population (N=260, 26 x 10 trials); grey lines: 5th-95th percentiles.
*(B): PK profile (log-linear scale) of 50 mg BID mitapivat on Day 84 (Week 12) in patients. Individual data from Study 006 (circles, n=30). Solid black line: mean for the predicted population (N=300, 30 x 10 trials); grey lines: 5th-95th percentiles.
(C): PK profile (log-linear scale) of 15 mg BID mitapivat in healthy subjects. Mean observed data from Study AG-348-C-002 (circles, mean of n=6 subjects). Black line: mean for the predicted population (N=100); grey lines: 5th-95th percentiles.
(D): PK profile (log-linear scale) of 60 mg BID mitapivat in healthy subjects. Mean observed data from Study AG-348-C-002 (circles, mean of n=6 subjects). Black line: mean for the predicted population (N=100); grey lines: 5th-95th percentiles.

Table 106. Predicted (Using the First-Order Absorption Model) and Observed Pharmacokinetic Parameters of Mitapivat Following Single and Multiple Doses

Mitapivat Dosage	C_{max} (ng/mL)			AUC (ng/mL*h)		
[Study]	Obs	Pred	%PE[1]	Obs	Pred	%PE[1]
*5 mg SD [C-004]	76	106	40	304	467	54
20 mg SD [C-012]	413	414	0	1942	1857	-4
50 mg SD [C-012]	1262	1029	-18	5681	4470	-21
*50 mg SD [C-004]	1005	1050	4	4007	4520	13
*50 mg SD [C-005]	1280	1032	-19	4160	4349	5
*200 mg SD [C-004]	4425	4190	-5	15,642	17,060	9
15 mg BID Day 1 [C-002]	248	258	4	778	1135	46
15 mg BID Day 14 [C-002]	255	245	-4	938	1074	15
60 mg BID Day 1 [C-002]	1287	1029	-20	3279	4454	36
60 mg BID Day 14 [C-002]	1322	827	-37	3358	3365	0
120 mg BID Day 14 [C-002]	1854	1505	-19	5769	5636	-2
120 mg QD Day 1 [C-002]	2806	2101	-25	10,034	9413	-6
120 mg QD Day 14 [C-002]	2094	1725	18	8461	66,731	-20
*50 mg BID Day 84 [C-006]	1033	837	-19	3162	2943	-7
*50 mg BID Day 84 [C-011]	994	819	-18	2952	2923	1
*50 mg BID Day 168 [C-007]	938	849	-9	2628	2937	12

Source: Applicant's response to a Clinical Pharmacology Information Request, and * Reviewer's analysis.
Pharmacokinetic parameters are geometric means. AUC values: AUC_{inf} for single dose (SD), $AUC_{12h,ss}$ for Day 14, $AUC_{8h,ss}$ for mitapivat administration ≥12 weeks. Simulated trials: 10 trials of 10 subjects (N=100) with age range and proportion of females matching the demographics of each study, and dosing cohort.
[1] %PE: = [(predicted value − observed value) ÷ observed value] × 100.
Abbreviations: AUC, area under the concentration-time curve; BID, twice daily; C_{max}, maximum serum concentration; Obs, observed; Pred, predicted; SD, single dose; QD, once daily

The predicted pharmacokinetic results showed that the model could reasonably estimate mitapivat plasma exposure following a single dose and multiple doses. Overall, the percentage prediction errors (%PE) for AUC and C_{max} were ≤±54% across the dose range simulated. Following oral administration, the predicted median T_{max} was around 1 hour and the reported median T_{max} was around 1 hour (range 0.5 to 1 hour) across studies. Following a 50 mg single dose, the predicted CL/F was 11.5 L/hour compared to the CL/F value of 12 L/hour reported in healthy subjects in Study 005. Following 50 mg BID, the predicted median CL/F was around 15.3 L/h compared to the median CL/F value of 14.4 L/hour estimated by population pharmacokinetics analysis. The predicted mean trough concentration was around 56 ng/mL compared to around 44 to 70 L/hour in patients in Studies 006, 007, and 011. The predicted accumulations of AUC and C_{max} following 15 mg BID and 60 mg BID dosages were comparable with observed values (%PE ≤25).

Q2. Can PBPK Analyses Predict the Effect of a CYP3A Modulator on the Pharmacokinetics of Mitapivat?

Yes, the PBPK analysis was considered adequate to estimate the interaction effects of CYP3A modulators on the pharmacokinetics of mitapivat following multiple doses of 5, 20, and 50 mg BID.

The relative contribution of the CYP3A4 pathway to the total clearance of mitapivat (e.g., fmCYP3A4 value) was optimized using clinical DDI data (Study 012) with the strong CYP3A inhibitor itraconazole. The fmCYP3A4 value was then tested against the clinical DDI with the strong CYP3A inducer rifampin (Table 107).

Table 107. Predicted and Observed C_{max} and AUC Values of Mitapivat in the Absence and Presence of CYP3A Modulators

	C_{max} (ng/mL)		AUC_{inf} (ng/mL.h)		C_{max} Ratio (90%CI)		AUC_{inf} Ratio (90%CI)	
DDI Scenario	**Obs**	**Pred**	**Obs**	**Pred**	**Obs**	**Pred**	**Obs**	**Pred**
Strong CYP3A inhibitor								
Mitapivat 20 mg SD	413	414	1942	1857	1.71	1.40	4.88	5.01
+ Itraconazole (200 mg QD)	704	579	9475	9307	(1.58-1.84)		(4.41-5.39)	
Strong CYP3A inducer								
Mitapivat 50 mg SD	1263	1029	5681	4470	0.23	0.25	0.087	0.09
+ Rifampin (600 mg QD)	292	261	496	304	(0.12-0.43)		(0.06-0.22)	

Source: Applicant's response to a Clinical Pharmacology Information Request.
Pharmacokinetic data are geometric means. C_{max} and AUC ratios are expressed as with/without modulator. Observed: Study AG-348-C-012.
Abbreviations: AUC_{inf}, area under the concentration-time curve to infinity; CI, confidence interval; C_{max}, maximum serum concentration; CYP3A, cytochrome P450 family 3 subfamily A gene; DDI, division of drug information; Obs, observed; Pred, predicted; SD, single dose; QD, once daily

Additional comments: When the default value of CYP3A4 Indmax value (=16) for the library model of rifampin (V17) was applied, DDI simulations underpredicted the induction effect of rifampin on both the AUC_{inf} (PE=116%) and C_{max} of mitapivat (PE=89%). A higher induction potency (CYP3A4 Indmax 30.6) in the rifampin model was needed to recover the reported induction effect on the pharmacokinetics of mitapivat (PE<10%). The possible reasons for the underprediction of rifampin DDI effect on mitapivat using the default, lower Indmax value were not addressed by the Applicant. In response to our information request, the Applicant argued that one possible reason for underprediction using the default Indmax value was not accounting for a possible induction of intestinal P-gp pathway by rifampin in the model (see the Methods section for rationale of intestinal P-gp contribution).

DDI simulations were conducted to predict the effect of strong and moderate CYP3A inhibitors or inducers on the pharmacokinetics of mitapivat following multiple doses of mitapivat 5, 20, and 50 mg BID. The predicted geometric mean ratios for AUC_{tau} and C_{max} values of mitapivat in the absence and presence of a CYP3A modulator are listed in Table 108.

Table 108. Predicted Pharmacokinetic Changes of Mitapivat After Twice Daily Dosing by Coadministration of CYP3A Modulators

	Mitapivat GMR C_{max}			Mitapivat GMR AUC_{tau}		
	Mitapivat Dosage			Mitapivat Dosage		
CYP3A Modulator	**5 mg BID**	**20 mg BID**	**50 mg BID**	**5 mg BID**	**20 mg BID**	**50 mg BID**
Itraconazole 200 mg **QD** Strong CYP3A inhibitor	2.5	2.4	2.2	5.0	4.3	3.6
Ketoconazole 400 mg **QD** Strong CYP3A inhibitor	2.0	2.3	2.4	3.6	3.9	3.9
Fluconazole 200 mg **QD** Moderate CYP3A inhibitor	1.6	1.6	1.6	2.6	2.6	2.6
*Erythromycin 500 mg **BID EC** Moderate CYP3A inhibitor	1.8	1.8	1.7	3.1	3.0	2.9
*Verapamil 80 mg **TID** Moderate CYP3A inhibitor	1.9	2.0	2.0	3.3	3.3	3.2
Rifampin 600 mg **QD** Strong CYP3A inducer	0.15	0.15	0.17	0.05	0.05	0.06
Efavirenz 600 mg **QD** Moderate CYP3A inducer	0.68	0.72	0.76	0.37	0.39	0.45

Source: Applicant's response to a Clinical Pharmacology Information Request and * Reviewer's analysis.
Data are geometric means ratios of C_{max} and AUC, calculated as with/without modulator. Mitapivat administered as 5, 20 or 50 mg BID doses for 14 days with the inhibitor or inducer being administered for 14 days.
Abbreviations: AUC_{tau}, area under the concentration-time curve for the dosing interval; BID, twice daily; C_{max}, maximum serum concentration; CYP3A, cytochrome 450 family 3 subfamily A gene; EC, enteric-coated; GMR, geometric mean ratio; QD, once daily; TID, three times daily

DDI simulations predicted a 3.6- to 5-fold increase in mitapivat AUC_{tau} with concomitant use of a strong CYP3A inhibitor such as itraconazole and ketoconazole. For the mitapivat BID dosing regimen, a dose-dependent interaction effect on the pharmacokinetics of mitapivat was predicted with itraconazole due to the induction effect of mitapivat on the plasma exposure of this CYP3A modulator, which is also a CYP3A substrate. The plasma exposures of itraconazole and its metabolite OH-itraconazole were predicted to decrease with time when coadministered with mitapivat dosages 20 mg BID and higher. The interaction effect of ketoconazole on the pharmacokinetics of mitapivat was dose-independent because ketoconazole's main elimination pathway was not susceptible to modification by mitapivat interaction via CYP3A induction.

Concomitant use of a moderate CYP3A inhibitor, such as fluconazole, erythromycin, and verapamil, increased mitapivat AUC_{tau} by approximately 3-fold (range 2.6- to 3.3-fold across mitapivat BID doses and moderate inhibitors).

DDI simulations predicted an approximately 95% reduction of mitapivat AUC_{tau} with concomitant use of a strong CYP3A inducer such as rifampin. Concomitant use of a moderate CYP3A inducer such as efavirenz reduced the mitapivat AUC_{tau} by 55% to 60%. When coadministered with efavirenz, an increase of mitapivat dosing to 110 mg BID resulted in mitapivat AUC_{tau} comparable to that of 50 mg BID at steady state. For the mitapivat BID dosing regimen, a slightly dose-dependent interaction effect on the pharmacokinetics of mitapivat was predicted with CYP3A inducers because the CYP3A4 induction effects are inversely related to baseline CYP3A levels (Gorski et al. 2003). The higher the mitapivat dose level/exposure, the higher the steady-state CYP3A4 level due to autoinduction. As a result, the CYP3A4 induction effect due to rifampin or efavirenz was slightly smaller.

Additional comments: Sensitivity analyses have been performed for DDI simulations of mitapivat with rifampin and efavirenz using the additive and multiplicative induction models to investigate

the net effect when multiple inducers are coadministered. Results suggested that these alternative induction models had minimal impact on predicted interaction effect of CYP3A inducers on the pharmacokinetics of mitapivat (C_{max} and AUC ratio) compared to the default induction model.

Q3. Can PBPK Analysis Predict the Interaction Effect of Mitapivat on the Pharmacokinetics of a CYP3A Substrate?

Yes, PBPK analysis was considered adequate to estimate the interaction effects of mitapivat on the pharmacokinetics of a sensitive CYP3A substrate, such as midazolam.

Mitapivat was determined to be both a time-dependent inhibitor and inducer of CYP3A and a sensitive substrate of CYP3A. The CYP3A interaction parameters were verified in vivo using clinical multiple ascending doses pharmacokinetics data of mitapivat (see Q2), considering the potential for autoinduction of CYP3A by multiple doses of mitapivat. DDI simulations were conducted considering the combined CYP3A inhibition and induction effect of mitapivat (5, 20, and 50 mg BID) on the pharmacokinetics of midazolam (single 5 mg oral dose on Day 14). Predicted geometric mean ratios of AUC_{inf} and C_{max} for midazolam in the absence and presence of multiple doses of mitapivat are listed in Table 109. A moderate induction effect on the pharmacokinetics of the sensitive CYP3A substrate, midazolam, was predicted at a mitapivat dosage of 50 mg BID.

Table 109. Predicted Interaction Effect of Mitapivat on the Pharmacokinetics of Midazolam

Mitapivat Dosage	*5 mg BID		20 mg BID		50 mg BID	
CYP3A Substrate	GMR C_{max}	GMR AUC_{inf}	GMR C_{max}	GMR AUC_{inf}	GMR C_{max}	GMR AUC_{inf}
Midazolam	0.82	0.80	0.61	0.57	0.48	0.43

Source: Applicant's response to a Clinical Pharmacology Information Request and * Reviewer's analysis.
Predicted geometric mean ratios of C_{max} and AUC_{inf} for midazolam calculated as C_{max} and AUC_{inf} in the presence vs. absence of multiple doses of mitapivat.
Abbreviations: AUC_{inf}, area under the concentration-time curve to infinity; BID, twice daily; C_{max}, maximum serum concentration; CYP3A, cytochrome P450 family 3 subfamily A gene; GMR, geometric mean ratio

Q4. Can PBPK Analyses Predict the Induction Effect of Mitapivat on the Pharmacokinetics of CYP2C Substrates?

No, the PBPK analysis was inadequate to predict the interaction effect of mitapivat on the substrates of CYP2C8 (repaglinide), CYP2C9 (S-warfarin), and CYP2C19 (omeprazole), for the following reasons:

(b) (6)

In summary, there is a low level of confidence to predict the CYP2C induction effect mediated by mitapivat using PBPK analysis. Considering the in vitro and in vivo CYP3A induction potential and in vitro induction data on CYP2C isoforms, the possibility of in vivo interactions of mitapivat with CYP2C substrates via induction cannot be excluded.

Q5. Can PBPK Analysis Predict the Interaction Effect of Mitapivat on the Pharmacokinetics of a CYP2B6 Substrate?

No, the PBPK analysis was considered inadequate to assess the induction effect of mitapivat on a CYP2B6 substrate for the following reasons:

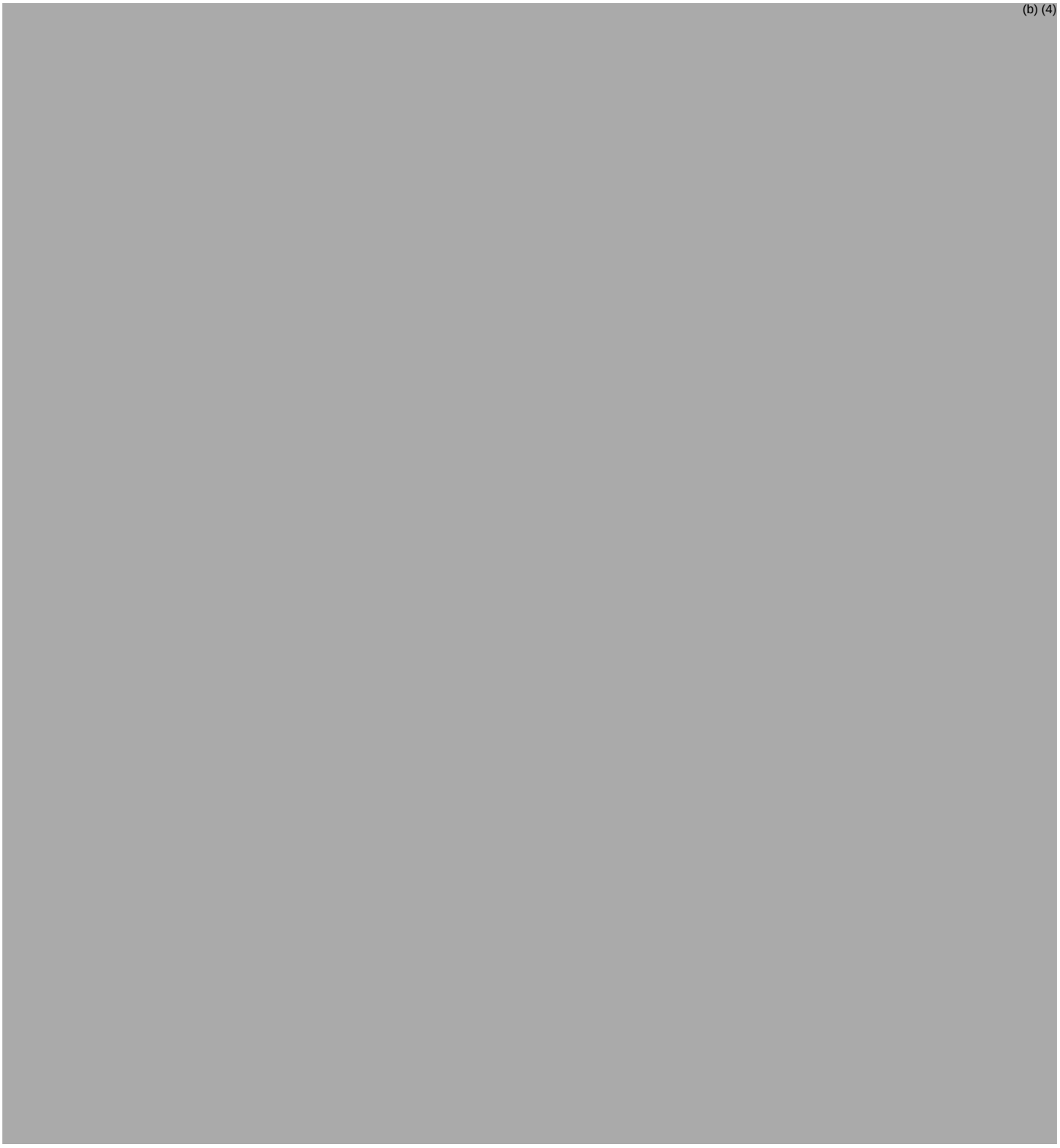
(b) (4)

Q6. Can PBPK Analysis Predict the Interaction Effect of Mitapivat on the Pharmacokinetics of P-gp Substrates?

No, the PBPK analysis could provide only supportive information on the potential for interaction of mitapivat with substrates of P-gp, such as digoxin and dabigatran etexilate.

(b) (4)

(b) (4)



Q7. Can PBPK Analyses Predict the Interaction Effect of Mitapivat on the Pharmacokinetics of Substrates of the Transporters OATP1B and MATE/OCT?

No, the PBPK analyses were considered inadequate to assess the interaction potential of mitapivat on substrates of the transporters OATP1B1 and MATE1/OCT2 for the following reasons:

Q8. Can PBPK Analyses Predict the Inhibitory Effects of Mitapivat on the Pharmacokinetics of Substrates of the Transporter OAT3?

Yes, the PBPK analysis was considered adequate to assess the DDI potential of mitapivat with methotrexate, a substrate of the renal uptake transporter OAT3.

DDI simulations assessed the inhibition potential of mitapivat with methotrexate (single 15 mg IV dose on Day 13), using the experimentally measured Ki inhibition parameter (Ki=IC_{50}=12.1μM) of mitapivat (Report AG-348-N-057). No interaction effect on methotrexate (AUC ratio=1) was predicted with coadministration of mitapivat.

The methotrexate model has been validated, by the software developer, for the interaction effect with probenecid, assuming the interaction occurred only by inhibition of renal OAT3. This verification was conducted using the lowest in vitro inhibition constant value reported in the literature for probenecid (Ki =0.76μM). In response to our request for information, the Applicant acknowledged significant variability in in vitro-determined OAT3 IC_{50} values for probenecid (reported in vitro OAT3 IC_{50} values range from 0.76 to 18.7μM (Shen et al. 2013; Chioukh et al. 2014)). Therefore, a PSA was conducted to assess the impact of the OAT3 Ki value on the predicted magnitude of interaction of mitapivat with methotrexate. A 10-fold reduction of the in vitro Ki (=IC_{50}) value indicated no interaction effect on methotrexate (AUC ratio <1.25) with mitapivat at 50 mg BID.

The Reviewer noted that the calculated R-value (=0.004) was several fold lower than the threshold of 0.1 for mitapivat exposure expected with 50 mg BID at steady state. Therefore, the static model and PBPK analysis indicated no DDI liability of mitapivat with an OAT3 substrate.

Q9. Can PBPK Analyses Predict the Effects of Changes in Gastric pH on the Pharmacokinetics of Mitapivat?

Yes, the mitapivat ADAM-PBPK model was adequate to evaluate the effect of elevated gastric pH on the exposure of mitapivat.

The ADAM-PBPK model of mitapivat was validated against the observed pharmacokinetics in healthy subjects following a single dose of 30, 120, 360, and 700 mg mitapivat (Study AG-348-C-001) and multiple-doses of 15, 60, and 120 mg BID mitapivat (Study AG-348-C-002). Overall, the ADAM-PBPK model was able to recover the observed pharmacokinetics of mitapivat within a 38% prediction error for mean C_{max} and AUC values, except for the 15 and 60 mg BID dosages (PE=59% and 47% for AUC_{last} on Day 1, respectively). The predicted median T_{max} values were delayed (≈2 hours) compared to the observed T_{max} values (≈1 hour). The model predicted fraction absorbed (fa) value was linear with a single dose up to 500 mg.

The ADAM-PBPK model was applied to assess the impact of simulating an increase in gastric pH on the pharmacokinetics and fraction absorbed of mitapivat. The pharmacokinetics of mitapivat following a single 100 mg dose with differing gastric pH values (3.5, 4.5, and 5) were generated and compared to the default gastric pH value of 1.5. Elevated gastric pH (up to 5) had no significant effect on mitapivat pharmacokinetics and fraction absorbed: the predicted decreases in C_{max} and AUC were ≤2%.

PSA was conducted for certain input parameters in the ADAM-PBPK model. The default value of 10 µm for particle radius was used in the model, whereas the drug product specification for particle radius ranged from (b) (4) µm. PSA showed that the particle size value had no effect on mitapivat pharmacokinetics predictions following a single 50 mg dose at a gastric pH value of 1.5. Similarly, a higher particle size value had no significant effect on the predicted absorption (fa value decreased 10%) and pharmacokinetics parameters (AUC and C_{max} values decreased ≤20%) of mitapivat at a gastric pH value of 5.5, for a mitapivat single 50 mg dose (Reviewer's analysis).

In the absence of experimental data for mitapivat critical supersaturation ratio (CSR), the default value of 1000 was used in the model. PSA was conducted to investigate the impact of the uncertainty in this parameter in mitapivat absorption. Results showed that predicted fa is sensitive to the CSR value. A fa value of around 1 was predicted using a CSR value in the range of 300 to 1000 at the dose level of 120 mg. A CSR value of 30 is approximately the lowest value recovering mitapivat pharmacokinetics profile and the reported high fa (>90%), based on human ADME data (120 mg dose, Study 009). Further, PSA of CSR values in the range of 30 to 1000 were conducted to investigate its impact on mitapivat pharmacokinetics at elevated gastric pH conditions. The results showed no effect of CSR value on the predicted absorption and pharmacokinetics parameters of mitapivat (decrease ≤5%) at a 50 mg dose for the gastric pH range of 1.5 to 5.5 (Reviewer's analysis).

The other key input parameter was the SF1. The input value (SF1=1255) was derived from the in vitro aqueous solubility data of mitapivat at differing pH values (1.2 to 6.8) (AG-348 074125-13-03). PSA showed that a two-fold reduction of the SF1 value had no effect on the predicted

absorption and pharmacokinetics parameters of mitapivat (decrease ≤5%) at the 50 mg dose for the gastric pH range of 1.5 to 5.5.

Simulations were conducted using a different set of parameter values for SF1 (=628), particle size (=22 µm), and CSR (=30) compared to the original values used in the model. The pharmacokinetics of mitapivat 50 mg dose at gastric pH of 1.5 was similar between this modified model and Applicant's model. This modeling effort predicted a decrease in C_{max} and AUC by 20% and 10%, respectively, at 50 mg dose at gastric pH value of 5.5 (Reviewer's analysis).

The Reviewer also considered the available in vitro and clinical pharmacokinetics data in the assessment of the elevated pH effect on mitapivat pharmacokinetics:

- Mitapivat sulfate showed high solubility (defined by the biopharmaceutical classification system) in aqueous solution at pH 5.5. The expected drug concentration in the GI tract calculated by 50 mg (mitapivat dose) ÷ 250 mL (stomach fluid volume) is 0.2 mg/mL, which is lower than the in vitro solubility measured at pH 5.5 (AG-348 074125-13-03).
- Based on the food effect study (AG-348-C-014), a high-fat meal affected the absorption rate of mitapivat following a 100 mg dose (2×50 mg tablets), but not the extent of absorption.
- Clinical Pharmacology data suggested no effect of solubility and dissolution on the absorption of higher dose levels of mitapivat
- A near complete oral absorption of mitapivat from the 120 mg dose (<1% eliminated unchanged in feces) was reported in the human ADME study (Study 009).
- A linear increase in C_{max} and AUC for dose levels up 200 mg was reported in Study 004.

In summary, the totality of evidence suggested that increasing gastric pH may have no clinically relevant impact on the absorption and pharmacokinetics of mitapivat. The lack of a pH effect may be attributed to the high solubility and nearly complete absorption of the drug at the therapeutic dose levels.

Conclusions

- PBPK analyses were adequate to predict the interaction effect of CYP3A inhibitors on mitapivat exposure at steady state. The model predicted that coadministration of a strong CYP3A inhibitor may increase mitapivat AUC_{tau} from 3.5- to 5-fold. Coadministration of a moderate CYP3A inhibitor may increase mitapivat AUC_{tau} from 2.6-to 3.3-fold.
- PBPK analyses were adequate to predict the interaction effect of CYP3A inducers on mitapivat exposure at steady state. The model predicted that coadministration of a strong CYP3A inducer may decrease mitapivat AUC_{tau} by approximately 95%; whereas a moderate CYP3A inducer may decrease AUC_{tau} by approximately 55 to 60%.
- The PBPK analysis indicated a moderate induction effect of mitapivat (50 mg BID) with a sensitive substrate of CYP3A.
- The PBPK analyses were inadequate to predict the induction DDI potential of mitapivat with substrates of CYP2C8, 2C9 and 2C19. However, considerations about in vitro induction data towards CYP3A and CYP2C isoforms and in vivo CYP3A auto-induction effect on mitapivat pharmacokinetics suggested that the DDI potential of mitapivat with substrates of CYP2C isoforms cannot be excluded.
- The PBPK analysis indicated no DDI liability of mitapivat with a substrate of OAT3.

- The PBPK analyses were inadequate to confirm no DDI potential of mitapivat with substrates of OATP1B and MATE1/OCT2 due to the lack of IVIVE for these transporter-mediated DDIs and the limitations identified in the substrate PBPK models.
- The PBPK analysis was inadequate to evaluate the DDI potential of mitapivat with a substrate of CYP2B6 due to the lack of IVIVE for CYP2B6 induction and uncertainty over the contribution of CYP2B6 to elimination of the substrate bupropion.
- The inhibition effect of mitapivat on a substrate of P-gp could not be accurately predicted by PBPK analysis. However, a DDI risk assessment, considering model uncertainties, indicated that a DDI potential of mitapivat with a substrate of P-gp cannot be excluded.
- The ADAM-PBPK model simulations, along with in vitro solubility and human absorption data, indicated a low potential for clinically relevant changes in mitapivat exposure with elevated gastric pH due to concomitant use of an acid-reducing agent.

14.5. Genomics Review

PKD is an autosomal recessive disorder caused by variants in the pyruvate kinase liver and red blood cell (PKLR) gene. The clinical variability observed in patients may be explained by the wide genetic heterogeneity of PK deficiency and the poor genotype-phenotype correlation. The most common variants are missense substitutions affecting residues critical for the structure and/or function of PK; less common are frameshift and splicing variants and premature stop codons, which has been categorized by the Applicant as nonmissense variants.

Study 003

Study 003 was a Phase 2, open-label, two-arm, multicenter, randomized, dose-ranging study in adult subjects with PKD; the study was divided into a Core Period (up to 24 weeks) and an Extension Period (up to 8 years).

Randomized patients were stratified by PKLR variant status. The PKLR variant stratification factor consisted of four levels (p.R510Q [c.1529G > A], p.R486W [c.1456C > T], p.R479H [c.1436G > A], and all other variants). Variant status was defined by the presence of at least one of the indicated variants.

The PKLR genotyping was performed by the designated central laboratory at screening.

The PKLR variants were reviewed by the Applicant and assigned the following descriptions and classifications listed in Table 111.

Table 111. PKLR Genotypes and Corresponding Variant Description and Classification, Study 003

Mutation	Mutation description	Mutation classification
1003 G>A	V335M	Missense
1008 A>AA	insertion (frameshift)	non-missense
1010 G>A	R337Q	Missense
1022 G>C	G341A	Missense
1072 G>A	G358R	Missense
1091 G>A	G364D	Missense
1151 C>T	T384M	Missense
1153 A>T	R385W	Missense
1178 A>G	N393S	Missense
1178 A>G, exon4IVS-IV+10G>T	N393S/splicing	Non-missense
1179 T>A	N393K	Missense
1223 C>T	T408I	Missense
1228 A>G	K410E	Missense
1318 G>T	truncation	Non-missense
1373 G>A	G458D	Missense
142_159del	in-frame deletion	Non-missense
1435 C>T	R479C	Missense
1436 G>A	R479H	Missense
1442 C>T	A481V	Missense
1456 C>T	R486W	Missense
1463 G>A	R488Q	Missense
1483 G>A	A495T	Missense
1484 C>T	A495V	Missense
1487 T>G	V496G	Missense
1493 G>A	R498H	Missense
1528 C>T	truncation (R510stop)	Non-missense
1529 G>A	R510Q	Missense
1574 G>A	truncation	Non-missense
1574 G>GG (1612 G>GG for site 102)*	insertion (frameshift)	Non-missense
1594 C>T (1633 CGG>TGG for site 102)*	R532W	Missense
284-2A>C	splicing	Non-missense
307delC	in-frame deletion	Non-missense
376-2A>C	splicing	Non-missense
389 C>A	S130Y	Missense
391_393delATC	in-frame deletion	Non-missense
401 T>A	V134D	Missense
494 G>T	G165V	Missense
507+1G>A	splicing	Non-missense
664 G>A	G222R	Missense
695-3C>G	splicing	Non-missense
-70 A>C	splicing	Non-missense
721 G>T (760 GAG>TAG or 760 G>T for site 102)*	truncation	Non-missense
721 G>T, 826delG	truncation/frameshift	Non-missense
92 C>T (131 GCT>GTT or 131 C>T for site 102)*	A31V	Missense
953_955delAAG	in-frame deletion	Non-missense
exon10del	deletion	Non-missense
exons3-9del	deletion	Non-missense

Source: Analysis Data Reviewer's Guide (Study 003).
Abbreviation: PKLR, pyruvate kinase liver and red blood cell

Table 112 lists the PKLR genotypes of the patients enrolled in Study 003.

Table 112. PKLR Genotype Baseline Characteristics, Full Analysis Set, Study 003

Allele	Mitapivat 300 mg BID N=25	Mitapivat 50 mg BID N=27	Total N=52
Allele 1 variant category, n (%)			
p.R486W	1 (4)	1 (4)	2 (4)
p.R510Q	1 (4)	3 (11)	4 (8)
p.R479H	3 (12)	5 (19)	8 (15)
Other	20 (80)	18 (67)	38 (73)

Allele	Mitapivat 300 mg BID N=25	Mitapivat 50 mg BID N=27	Total N=52
Allele 2 variant category, n (%)			
p.R486W	1 (4)	2 (7)	3 (6)
p.R510Q	6 (24)	5 (19)	11 (21)
p.R479H	3 (12)	3 (11)	6 (12)
Other	15 (60)	17 (63)	32 (62)
PKLR variant category, n (%)			
Missense/missense	17 (68)	15 (56)	32 (62)
Missense/nonmissense	4 (16)	6 (22)	10 (19)
Nonmissense/nonmissense	4 (16)	6 (22)	10 (19)

Source: Reviewer-generated table based on Table 8, Clinical Study Report (Study 003).
Abbreviations: BID, twice a day; PKLR, pyruvate kinase liver and red blood cell

An ad hoc subgroup analysis of hemoglobin (Hb) responders, which was not prespecified in the statistical analysis plan, by variant category was added to replicate the exploratory analyses that would help inform the Phase 3 studies (Studies 006 and 007).

Hemoglobin response by variant stratum group and PKLR variant category is summarized in Table 113.

Table 113. Hemoglobin Response by Variant Group, Full Analysis Set, Study 003, Core Period

	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)

Source: Applicant's analysis, Clinical Study Report (Study 003).
Hemoglobin response is defined as a change from baseline in hemoglobin ≥15 g/L (1.5 g/dL) at >50% of assessments in the Core Period, excluding those within 61 days after a transfusion.
N1 is the number of subjects in the full analysis set within each subgroup category and treatment group.
95% CI is based on the Clopper-Pearson method.
Abbreviations: BID, twice daily; CI, confidence interval; PKLR, pyruvate kinase liver and red blood cell

Reviewer's comment: The PKLR variant description and classification appear to be reasonable. No genotyping methods were provided. Based on the Applicant's analysis, carriers of nonmissense/nonmissense variants do not seem to respond to mitapivat therapy. In addition, limited Hb response was observed in carriers of p.R510Q and p.R479H variants. However, interpretation of these results is limited by the exploratory nature of the analysis and the small numbers of patients with these variants.

Study 006

Study 006 was a Phase 3, randomized, double-blind, placebo-controlled study to evaluate the efficacy and safety of mitapivat in not regularly transfused adult subjects with PKD.

Patients included in Study 006 had documented clinical laboratory confirmation of PKD, defined as documented presence of at least two variant alleles in the PKLR gene, of which at least one was a missense variant, as determined per the genotyping and genotype classification (i.e., missense or nonmissense) performed by the central genotyping laboratory. Missense variants were considered to be a single nucleotide change that might result in amino acid substitutions.

The effects of missense variants can include a loss of catalytic efficiency and/or a loss of protein stability of the enzyme.

Categorization of nonmissense variants included those that cause premature truncations of the enzyme, deletions or frameshifts, or variants that affect splicing of the enzyme. Many of these nonmissense variants are predicted to be null alleles of PKLR, resulting in a lack of functional protein expression.

Patients homozygous for the p.R479H variant or who had two nonmissense variants, without another missense variant, in the PKLR gene were not included in Study 006. According to the Applicant, this decision was based on the results of the Phase 2 Study 003, suggesting that patients with two nonmissense variants and patients with the p.R479H/p.R479H genotype might have a decreased response to mitapivat.

The randomization was stratified by the average screening Hb concentration (<85 g/L versus Hb ≥85 g/L) and PKLR gene variant category (missense/missense versus missense/nonmissense). Table 114 lists the PKLR genotypes of patients enrolled in Study 006.

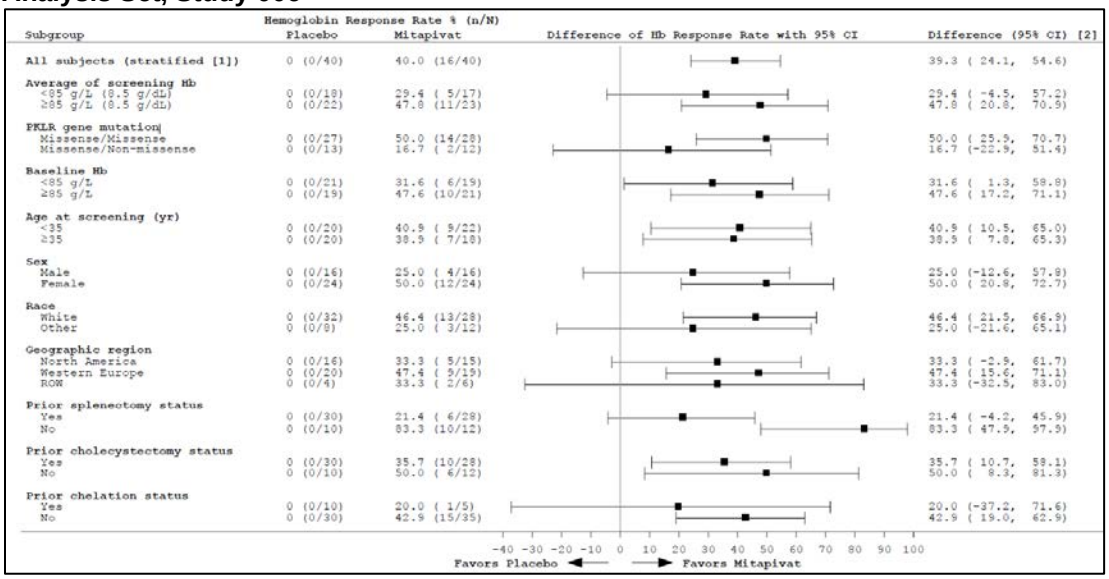
Table 114. PKLR Genotype Baseline Characteristics, Full Analysis Set, Study 006

PKLR Genotype	Placebo, N=40	Mitapivat, N=40	Total, N=80
PKLR variant category, n (%)			
Missense/missense	27 (68)	28 (70)	55 (69)
Missense/nonmissense	13 (32)	12 (30)	25 (31)
Average of screening Hb concentrations and PKLR gene variant category, n (%)			
Hb <85 g/L and missense/missense	12 (30)	12 (30)	24 (30)
Hb <85 g/L and missense/nonmissense	6 (15)	5 (13)	11 (14)
Hb ≥85 g/L and missense/missense	15 (38)	16 (40)	31 (39)
Hb ≥85 g/L and missense/nonmissense	7 (18)	7 (18)	14 (18)

Source: Reviewer-generated table based on Table 3, Clinical Study Report (Study 006).
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 screening Hb concentration (<85 g/L versus ≥85 g/L) and PKLR gene variant category (missense/missense vs. missense/nonmissense).
Abbreviations: Hb, hemoglobin; PKLR, pyruvate kinase liver and red blood cell

Subgroup analyses were prespecified for the primary endpoint (Hb response). Hemoglobin response rates (Figure 48) and the average mean changes from baseline in hemoglobin at Weeks 16, 20, and 24 were higher in the mitapivat arm than the placebo arm across all prespecified subgroups.

Figure 48. Forest Plot for Difference in Hemoglobin Response Rates, Subgroup Analyses, Full Analysis Set, Study 006



Source: Applicant's analysis, Clinical Study Report (Study 006).
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 screening Hb concentration (<85 g/L vs. ≥85 g/L) and PKLR gene variant 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.
Abbreviations: CI, confidence interval; Hb, hemoglobin; IXRS, interactive response technology; PKLR, pyruvate kinase liver and red blood cell; ROW, rest of world

Based on the Applicant's subgroup analysis, the Hb response was observed in both the missense/missense and missense/nonmissense patient subgroups. However, a larger effect on the Hb response was observed in the missense/missense group receiving mitapivat when compared to placebo. A nonsignificant Hb response was observed in the missense/nonmissense group when compared to placebo.

Reviewer's comment: The PKLR variant description and classification appear to be reasonable. No genotyping methods were provided. Of note, interpretation of the Hb response stratified by PKLR genotype is limited by a small number of patients and overlapping confidence intervals.

Study 007

Study 007 was an open-label study to evaluate the efficacy and safety of mitapivat in regularly transfused adult subjects with PKD.

Patients included in Study 007 had documented clinical laboratory confirmation of PKD, defined as documented presence of at least two variant alleles in the PKLR gene, of which at least one was a missense variant, as determined per the genotyping and genotype classification (i.e., missense or nonmissense) performed by the central genotyping laboratory (Table 115).

Missense variants were considered to be a single nucleotide change that might result in an amino acid substitution. The effects of missense mutations can include a loss of catalytic efficiency and/or a loss of protein stability of the enzyme. Categorization of nonmissense variants included those that cause premature truncations of the enzyme, deletions or frameshifts, or variants that

affect splicing of the enzyme. Many of these nonmissense variants are predicted to be null alleles of PKLR, resulting in a lack of functional protein expression.

Patients homozygous for the p.R479H variant or had two nonmissense variants, without another missense variant, in the PKLR gene as determined per the genotyping performed by the study central genotyping laboratory were excluded.

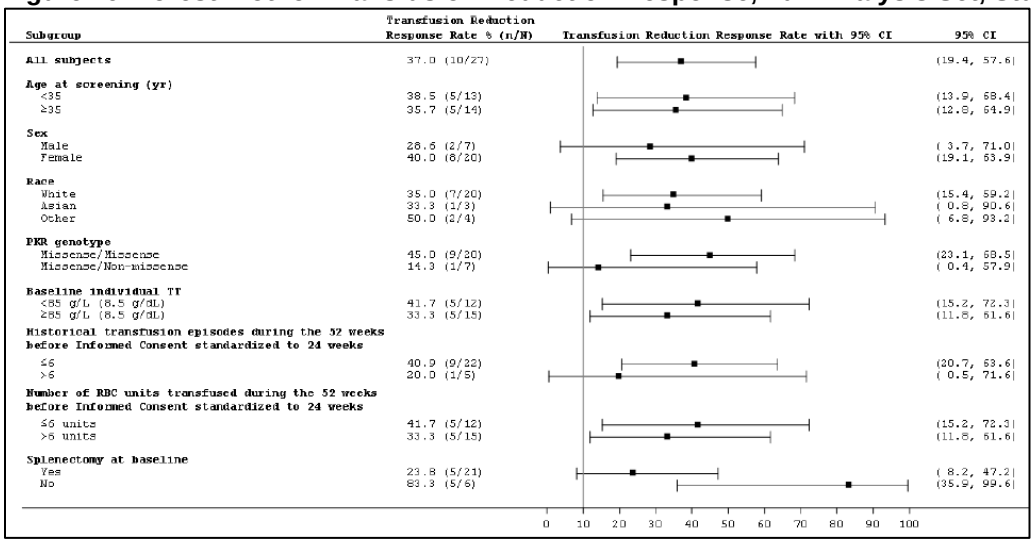
Table 115. PKLR Genotype Baseline Characteristics, Full Analysis Set, Study 007

PKLR Variant Category n (%)	Mitapivat N=27
Missense/missense	20 (74)
Missense/nonmissense	7 (26)

Source: Reviewer-generated table based on Table 6, Clinical Study Report (Study 007).
Abbreviation: PKLR, pyruvate kinase liver and red blood cell

Transfusion reduction response (TRR) was assessed in prespecified subgroups, including those defined by PKLR genotype (Figure 49). TRR rate was summarized for each category within each subgroup with the 95% exact CI using the Clopper-Pearson method. Efficacy analyses in subgroups were exploratory and were intended to evaluate the consistency of the treatment effect.

Figure 49. Forest Plot for Transfusion Reduction Response, Full Analysis Set, Study 007



Source: Applicant's analysis, Clinical Study Report (Study 007), Figure 14.2-3.1.
Notes: N is the number of subjects in each subgroup. Transfusion reduction responders: subjects who had a ≥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.
Abbreviations: PK-R, red blood cell isoform of pyruvate kinase; RBC, red blood cell; TT, transfusion trigger

Based on the Applicant's subgroup analysis, transfusion reduction responders were observed in all prespecified subgroups. The transfusion reduction was observed in both the missense/missense and missense/nonmissense patient subgroups. However, a larger effect was observed in the missense/missense group receiving mitapivat. A nonsignificant effect was observed in the missense/nonmissense group.

Reviewer's comment: The PKLR variant description and classification appear to be reasonable. No genotyping methods were provided. Of note, interpretation of the TRR analysis stratified by the PKLR genotype was limited by its exploratory nature, small number of patients, and overlapping confidence intervals.

Summary and Conclusions

PKD is an autosomal recessive disorder caused by variants in the PKLR gene. The clinical variability observed in patients may be explained by the wide genetic heterogeneity of PKD and the poor genotype-phenotype correlation. The most common variants are missense substitutions affecting residues critical for the structure and/or function of PK; less common are frameshift and splicing variants and premature stop codons, which are categorized by the Applicant as nonmissense variants.

Results from Study 003 demonstrated that carriers of nonmissense/nonmissense variants do not respond to mitapivat therapy. In addition, a limited hemoglobin response was observed in carriers of p.R479H variants. Based on the results of Study 003, patients homozygous for the p.R479H variant or with two nonmissense variants, without another missense variant, in the PKLR gene were not included in Studies 006 and 007.

For additional details please refer to the Section II.6.2 and Section III.16.2.

14.6. Summary of Bioanalytical Method Validation and Performance

Bioanalytical methods were reviewed for both mitapivat and its N-dealkylated metabolite (AGI-8702) in plasma and urine (Table 116 to Table 122). The bioanalytical methods used in the clinical studies are listed in Table 116. Several analytical methods were used to quantify mitapivat and its metabolite in human plasma during the drug development period. The primary method used was Method AG-348-N-077 (Table 117 and Table 118) in all clinical studies that included mitapivat pharmacokinetic measurements, with the exception of Studies 004 (ethnic bridging study) and 009 (human ADME study), which used the assays listed in Table 119 to Table 121, respectively.

Table 116. Bioanalytical Methods Overview

	Method AG348-N-077	Method AG348-Q-004	Method AG348-Q-009	Method AG348-N-069
Analyte	Mitapivat and its N-dealkylated metabolite, AGI-8702	Mitapivat and its N-dealkylated metabolite, AGI-8702	Mitapivat and [$^{13}C_6$]-mitapivat	Mitapivat and its N-dealkylated metabolite, AGI-8702
Validation Type	Full validation	Full validation	Full validation	Full validation
Method ID	AG348-N-077 (Agios method ID) 422-1401 (CRO Method ID)	AG348-Q-004 (Agios Method ID) 422-1706 (CRO Method ID)	AG348-Q-009 (Agios Method ID) 422-1801 (CRO Method ID)	AG348-N-069 (Agios Method ID) 422-1312 (CRO Method ID)
Duration of time method is in use	04/2014 – 02/2021	08/2017 – 10/2017	06/2018	07/2014, 06/2018
Bioanalytical site	(b) (4)			
Matrix	Human plasma (EDTA)			Human urine
Platform	Liquid chromatography/tandem mass spectrometry (LC-MS/MS)			
Format	Reversed-phase ultra-performance liquid chromatography (UPLC), electrospray positive ionization (ESI+), multiple reaction monitoring detection			
Stock reference, lot number, and expiration date used during validation	AGI-0001480 (b) (4) (b) (4) (AG-348), Lot 804002-61-C, expiration date 06/14/2015 AG-348 Sulfate Hydrate, Lot PT-C14060349-D14002M, expiration date 11/29/2018 AGI-0008702, Lot NB541-066, expiration date 06/14/2015 AGI-0008702, Lot NB571-035, expiration date 12/26/2015 AGI-0008702, Lot b5, expiration date 03/31/2019	AG-348 Sulfate Hydrate, Lot PT-C14060349-D14002M, expiration date 11/29/2018 AG-348 Sulfate, Lot C14060349-D17001M, expiration date 06/01/2021 AGI-0008702, Lot b5, expiration date 03/31/2020	AG-348 Sulfate Hydrate, Lot PT-C14060349-D14002M, expiration date 11/29/2018 [$^{13}C_6$]AG-348, Lot 50060MAR18-01, expiration date 09/21/2018	AGI-0001480 (b) (4) (b) (4) (AG-348), Lot 804002-61-C, expiration date 06/14/2015 AGI-0008702, Lot NB541-066, expiration date 06/14/2015 AGI-0008702, Lot NB571-035, expiration date 12/18/2016
Calibration range from the lower limit of quantitation (LLOQ) to the upper limit of quantitation (ULOQ)	Mitapivat: 0.500–1,500 ng/mL AGI-8702: 0.500–1,500 ng/mL	Mitapivat: 0.100–200 ng/mL AGI-8702: 0.200–400 ng/mL	Mitapivat: 0.500–500 ng/mL [$^{13}C_6$]-mitapivat: 5.00–5,000 pg/mL	Mitapivat: 5.00–15,000 ng/mL AGI-8702: 5.00–15,000 ng/mL
Synopsis of amendment history	Amendment 1: Extended long-term storage stability (96 days) Amendment 2: Extended long-term storage stability (187 days) Amendment 3: Extended long-term storage stability (374 days) Amendment 4: Extended long-term storage stability (1726 days for AG-348 only) Amendment 5: Updated stock solution stability (Reference to AG348-Q-004 Amendment 2)	Amendment 1: Extended long-term storage stability (370 days, LQC) Amendment 2: Extended long-term storage stability (679 days); extended stock solution storage stability (370 and 407 days for mitapivat and AGI-8702, respectively)	Not applicable	Amendment 1: Extended long-term stability (97 days) Amendment 2: Extended long-term stability (164 days) Amendment 3: Extended long-term stability (416 and 358 days at -20°C and -70°C, respectively)

Source: Applicant's report.
Abbreviations: CRO, contract research organization; EDTA, ethylenediaminetetraacetic acid

Table 117. Summary Method Validation for Mitapivat in Human Plasma, Method AG-348-N-077

Bioanalytical method validation report name, amendments, and hyperlinks	Report AG348-N-077	
Method description	CRO Method Number 422-1401 Simultaneous measurement of mitapivat and its *N*-dealkylated metabolite, AGI-8702, by LC-MS/MS detection	
Materials used for standard calibration curve and concentration	Human (EDTA) plasma 0.500; 1.00; 10.0; 50.0; 250; 1,000; 1,350; and 1,500 ng/mL	
Validated assay range	0.500–1,500 ng/mL	
Material used for quality controls (QCs) and concentration	Human (EDTA) plasma 0.500, 1.50, 30.0, 750, and 1,200 ng/mL	
Minimum required dilutions (MRDs)	Not applicable	
Source and lot of reagents	Not applicable	
Regression model and weighting	Linear, $1/x^2$	
Validation parameters	**Method validation summary**	
Standard calibration curve performance	Number of standard calibrators from LLOQ to ULOQ	8
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-6.0% to 7.0%
	Cumulative precision (%CV) from LLOQ to ULOQ	≤6.4%
Performance of QCs during accuracy and precision runs	**Cumulative accuracy (%bias) in 5 QCs**	-2.5% to 8.7%
	Interbatch %CV	≤5.0%
	Total Error (TE)	Not applicable
Selectivity & matrix effect	Results from all 6 lots of human plasma tested met selectivity acceptance criteria. Accuracy (%RE) of the LLOQ spike-in selectivity from the 6 lots was within ±12.8%. The precision (%CV) of IS-normalized matrix factor across 6 lots of human plasma was ≤2.2% at the Low and High QC levels; therefore, no matrix effect was observed which would significantly impact study data.	
Interference & specificity	Results from all 6 lots of human plasma tested met acceptance criteria. No interference was observed for mitapivat or its stable isotope-labeled internal standard.	
Hemolysis effect	Results from Low and High QC levels tested in a single lot of 2% hemolyzed plasma met acceptance criteria. Accuracy (%RE) was ±6.0% (Low QC) and ±-2.5% (High QC).	
Lipemic effect	Not applicable	
Dilution linearity	Dilution of 40-fold tested at 3,000 ng/mL. Precision (%CV) and accuracy (%RE) were 4.9% and 1.3% respectively.	
Bench-top/process stability	Mitapivat was determined to be stable in human plasma at ambient temperature for up to 25 hours. Based on the Low and High QC, precision (%CV) and accuracy (%RE) were ≤2.6% and ±6.7% respectively. Mitapivat was determined to be stable in human whole blood at ambient temperature for up to 2 hours. Based on the Low and High QC, precision (%CV) and accuracy (% difference from time 0) were ≤4.4% and ±4.3% respectively. Mitapivat was determined to be stable in processed samples stored at 4°C for up to 142 hours. Based on the Low and High QC, precision (%CV) and accuracy (%RE) were 2.1% and ±7.3% respectively.	
Freeze-Thaw stability	Mitapivat was determined to be stable in human plasma stored at -20°C through 5 freeze-thaw cycles. Based on the Low and High QC, precision (%CV) and accuracy (%RE) were ≤2.9% and ±6.0% respectively. Mitapivat was determined to be stable in human plasma stored at -70°C through 5 freeze-thaw cycles. Based on the Low and High QC, precision (%CV) and accuracy (%RE) were ≤4.1% and ±5.3% respectively.	
Long-term storage	Mitapivat was determined to be stable in human plasma stored at -20°C for up to 1,726 days. Based on the Low and High QC, precision (%CV) and accuracy (% difference from Day 0) were ≤2.2% and ±10.9% respectively. Mitapivat was determined to be stable in human plasma stored at -70°C for up to 1,726 days. Based on the Low and High QC, precision (%CV) and accuracy (% difference from Day 0) were ≤4.8% and ±7.9% respectively.	
Parallelism	Not applicable	
Carry over	Mitapivat carryover (>20% of LLOQ) was observed in 2 validation runs, and is monitored during the production use of this method for sample-to-sample injection carryover impact.	

Source: Applicant's report.
Abbreviations: CRO, contract research organization; CV, coefficient of variation; EDTA, ethylenediaminetetraacetic acid; LC-MS/MS, liquid chromatography – mass spectrometry; LLOQ, lower limit of quantitation; QC, quality control; RE, relative error; ULOQ, upper limit of quantitation

Table 118. Summary Method Performance for Mitapivat in Human Plasma, Method AG-348-N-077

Method performance in Study AG348-C-001 (Report 422-1406A)	
Assay passing rate	100% (8/8) analytical runs passed acceptance criteria.
Standard curve performance	Cumulative accuracy (%bias) from LLOQ to ULOQ: -7.4% to 8.0% Cumulative precision (%CV) from LLOQ to ULOQ: ≤6.8%
QC performance	Cumulative accuracy (%bias) from 4 levels of QCs: -5.8% to 7.7% Cumulative precision (%CV) from 4 levels of QCs: ≤6.8%
Method reproducibility	Of the 815 study samples, 88 were reanalyzed for incurred sample reproducibility (ISR). Two samples were not included in the final ISR calculation because their IS response did not meet acceptance criteria in the ISR run. Of the remaining 86 samples reanalyzed, 100% (86/86) of the samples met the ISR acceptance criteria for mitapivat.
Study sample analysis/stability	Sample storage duration was a maximum of 98 days (date of first sample collection – date of last injection). All samples were analyzed within the established long-term stability at -70°C.
Method performance in Study AG348-C-002 (Report 422-1408A)	
Assay passing rate	82% (14/17) analytical runs passed acceptance criteria. Two runs were rejected due to QCs not meeting acceptance criteria, and one run was rejected due to an error in the standard curve preparation.
Standard curve performance	Cumulative accuracy (%bias) from LLOQ to ULOQ: -5.3% to 5.0% Cumulative precision (%CV) from LLOQ to ULOQ: ≤6.1%
QC performance	Cumulative accuracy (%bias) from 4 levels of QCs: -5.0% to 6.7% Cumulative precision (%CV) from 4 levels of QCs: ≤6.6%
Method reproducibility	Of the 1,390 analyzed study samples, 137 were reanalyzed for incurred sample reproducibility (ISR). Two samples were not included in the final ISR calculation because reanalysis values were above the quantifiable limit in the ISR run. Of the remaining 135 samples reanalyzed, 99.3% (134/135) of the samples met the ISR acceptance criteria for mitapivat.
Study sample analysis/stability	Sample storage duration was a maximum of 158 days (date of first sample collection – date of last injection). All samples were analyzed within the established long-term stability at -70°C.
Method performance in Study AG348-C-003 (Report AG348-C-003-BA)	
Assay passing rate	85% (18/21) analytical runs passed acceptance criteria. One run was rejected due to suspected contamination, one run was rejected due to QCs not meeting acceptance criteria, and one run was rejected due to standards not meeting acceptance criteria.
Standard curve performance	Cumulative accuracy (%bias) from LLOQ to ULOQ: -4.0% to 6.0% Cumulative precision (%CV) from LLOQ to ULOQ: ≤6.8%
QC performance	Cumulative accuracy (%bias) from 4 levels of QCs: -1.7% to 8.0% Cumulative precision (%CV) from 4 levels of QCs: ≤13.4%
Method reproducibility	Of the 720 analyzed study samples, 74 were reanalyzed for incurred sample reproducibility (ISR). 79.7% (59/74) of the samples met the ISR acceptance criteria for mitapivat.
Study sample analysis/stability	Sample storage duration was a maximum of 1,303 days (date of first sample collection – date of last injection). All samples were analyzed within the established long-term stability at -70°C.
Method performance in Study AG348-C-004 (Report AG348-C-004-BA)	
Assay passing rate	100% (10/10) analytical runs passed acceptance criteria.
Standard curve performance	Cumulative accuracy (%bias) from LLOQ to ULOQ: -3.3% to 2.4% Cumulative precision (%CV) from LLOQ to ULOQ: ≤7.7%

QC performance	QC performance is reported with and without 3 outliers (2 replicates of LQC and 1 replicate of MQC over 2 analytical runs): *QC outliers included in statistics:* Cumulative accuracy (%bias) from 4 levels of QCs: 0.0% to 18.0% Cumulative precision (%CV) from 4 levels of QCs: ≤41.3% *QC outliers excluded from statistics:* Cumulative accuracy (%bias) from 4 levels of QCs: 0.0% to 4.5% Cumulative precision (%CV) from 4 levels of QCs: ≤4.8%
Method reproducibility	Of the 960 study samples, 114 were reanalyzed for incurred sample reproducibility (ISR). Two assays were used to support this study; the same assay used to generate a sample's original result was used for ISR reanalysis. Six samples were not included in the final ISR calculation due to failed Dilution QCs or carryover impact in the ISR run(s). Of the remaining 108 samples reanalyzed, 99.1% (107/108) of the samples met the ISR acceptance criteria for mitapivat.
Study sample analysis/stability	Sample storage duration was a maximum of 77 days (date of first sample collection – date of last injection). All samples were analyzed within the established long-term stability at -70°C.
Method performance in Study AG348-C-005 **(Report AG348-C-005-BA)**	
Assay passing rate	70% (7/10) analytical runs passed acceptance criteria. Three runs were rejected due to QCs not meeting the acceptance criteria.
Standard curve performance	Cumulative accuracy (%bias) from LLOQ to ULOQ: -4.7% to 5.0% Cumulative precision (%CV) from LLOQ to ULOQ: ≤7.9%
QC performance	Cumulative accuracy (%bias) from 4 levels of QCs: -1.7% to 7.3% Cumulative precision (%CV) from 4 levels of QCs: ≤14.1%
Method reproducibility	Of the 764 analyzed study samples, 77 were reanalyzed for incurred sample reproducibility (ISR). 96.1% (74/77) of the samples met the ISR acceptance criteria for mitapivat.
Study sample analysis/stability	Sample storage duration was a maximum of 24 days (date of first sample collection – date of last injection). All samples were analyzed within the established long-term stability at -70°C.
Method performance in Study AG348-C-006 **(Report AG348-C-006-BA)**	
Assay passing rate	100% (6/6) analytical runs passed acceptance criteria.
Standard curve performance	Cumulative accuracy (%bias) from LLOQ to ULOQ: -4.4% to 6.0% Cumulative precision (%CV) from LLOQ to ULOQ: ≤5.6%
QC performance	Cumulative accuracy (%bias) from 4 levels of QCs: -3.3% to 4.7% Cumulative precision (%CV) from 4 levels of QCs: ≤5.5%
Method reproducibility	Of the 400 analyzed study samples, 42 were reanalyzed for incurred sample reproducibility (ISR). 100% (42/42) of the samples met the ISR acceptance criteria for mitapivat.
Study sample analysis/stability	Sample storage duration was a maximum of 750 days (date of first sample collection – date of last injection). All samples were analyzed within the established long-term stability at -70°C.
Method performance in Study AG348-C-007 **(Report AG348-C-007-BA)**	
Assay passing rate	100% (5/5) analytical runs passed acceptance criteria.
Standard curve performance	Cumulative accuracy (%bias) from LLOQ to ULOQ: -4.4% to 5.2% Cumulative precision (%CV) from LLOQ to ULOQ: ≤7.6%
QC performance	Cumulative accuracy (%bias) from 4 levels of QCs: -1.7% to 2.7% Cumulative precision (%CV) from 4 levels of QCs: ≤5.6%
Method reproducibility	Of the 107 study samples, 20 were reanalyzed for incurred sample reproducibility (ISR). 100% (20/20) of the samples met the ISR acceptance criteria for mitapivat.
Study sample analysis/stability	Sample storage duration was a maximum of 574 days (date of first sample collection – date of last injection). All samples were analyzed within the established long-term stability at -70°C.

	Method performance in Study AG348-C-011 (Report AG348-C-011-BA)
Assay passing rate	100% (5/5) analytical runs passed acceptance criteria.
Standard curve performance	Cumulative accuracy (%bias) from LLOQ to ULOQ: -4.0% to 4.4% Cumulative precision (%CV) from LLOQ to ULOQ: ≤9.2%
QC performance	Cumulative accuracy (%bias) from 4 levels of QCs: -0.8% to 4.0% Cumulative precision (%CV) from 4 levels of QCs: ≤4.4%
Method reproducibility	Incurred Sample Reproducibility was not performed for this study.
Study sample analysis/stability	Sample storage duration was a maximum of 624 days (date of first sample collection – date of last injection). All samples were analyzed within the established long-term stability at -70°C.
	Method performance in Study AG348-C-012 (Report AG348-C-012-BA)
Assay passing rate	100% (8/8) analytical runs passed acceptance criteria.
Standard curve performance	Cumulative accuracy (%bias) from LLOQ to ULOQ: -3.3% to 5.2% Cumulative precision (%CV) from LLOQ to ULOQ: ≤3.8%
QC performance	Cumulative accuracy (%bias) from 4 levels of QCs: -1.9% to 0.3% Cumulative precision (%CV) from 4 levels of QCs: ≤6.0%
Method reproducibility	Of the 952 study samples, 103 were reanalyzed for incurred sample reproducibility (ISR). 99.0% (102/103) of the samples met the ISR acceptance criteria for mitapivat.
Study sample analysis/stability	Sample storage duration was a maximum of 52 days (date of first sample collection – date of last injection). All samples were analyzed within the established long-term stability at -70°C.
	Method performance in Study AG348-C-014 (Report AG348-C-014-BA)
Assay passing rate	100% (25/25) analytical runs passed acceptance criteria.
Standard curve performance	Cumulative accuracy (%bias) from LLOQ to ULOQ: -4.7% to 5.6% Cumulative precision (%CV) from LLOQ to ULOQ: ≤7.7%
QC performance	Cumulative accuracy (%bias) from 4 levels of QCs: -1.7% to 2.7% Cumulative precision (%CV) from 4 levels of QCs: ≤5.7%
Method reproducibility	Of the 2,228 study samples, 135 were reanalyzed for incurred sample reproducibility (ISR). Five samples were not included in the final ISR calculation because their IS response did not meet acceptance criteria in the ISR run. Of the remaining 130 samples reanalyzed, 100% (130/130) of the samples met the ISR acceptance criteria for mitapivat.
Study sample analysis/stability	Sample storage duration was a maximum of 216 days (date of first sample collection – date of last injection). All samples were analyzed within the established long-term stability at -70°C.

Source: Applicant's report.
Abbreviations: CRO, contract research organization; CV, coefficient of variation; LLOQ, lower limit of quantitation; LQC, lower quality control; MQC, middle quality control; QC, quality control; ULOQ, upper limit of quantitation

Table 119. Summary Method Validation and Performance for Mitapivat in Human Plasma, Method AG-348-Q-004

Bioanalytical method validation report name, amendments, and hyperlinks	Report AG348-Q-004
Method description	CRO Method Number 422-1706 Simultaneous measurement of mitapivat and its N-dealkylated metabolite, AGI-8702, by LC-MS/MS detection
Materials used for standard calibration curve and concentration	Human (EDTA) plasma 0.100, 0.200, 1.00, 2.00, 20.0, 100, 180, and 200 ng/mL
Validated assay range	0.100–200 ng/mL
Material used for quality controls (QCs) and concentration	Human (EDTA) plasma 0.100, 0.300, 10.0, 40.0, 80.0, and 160 ng/mL
Regression model and weighting	Linear, $1/x^2$

Validation parameters	Method validation summary	
Standard calibration curve performance	Number of standard calibrators from LLOQ to ULOQ	8
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-5.6% to 5.0%
	Cumulative precision (%CV) from LLOQ to ULOQ	≤6.6%
Performance of QCs during accuracy and precision runs	Cumulative accuracy (%bias) in 6 QCs	-6.3% to 2.7%
	Interbatch %CV	≤7.1%
	Total Error (TE)	Not applicable
Selectivity & matrix effect	Results from all 6 lots of human plasma tested met selectivity acceptance criteria. Accuracy (%RE) of the LLOQ spike-in selectivity from the 6 lots was within ±16.2%. The precision (%CV) of IS-normalized matrix factor across 6 lots of human plasma was ≤2.3% at the Low and High QC levels; therefore, no matrix effect was observed which would significantly impact study data.	
Interference & specificity	Results from all 6 lots of human plasma tested met acceptance criteria. No interference was observed for mitapivat or its stable isotope-labeled internal standard.	
Hemolysis effect	Results from Low and High QC levels tested in a single lot of 2% hemolyzed plasma met acceptance criteria. Accuracy (%RE) was 3.7% (Low QC) and -8.8% (High QC).	
Lipemic effect	Results from Low and High QC levels tested in a single lot of lipemic plasma met acceptance criteria. Accuracy (%RE) was 5.3% (Low QC) and -4.4% (High QC).	
Dilution linearity	Dilution of 20-fold tested at 400 ng/mL. Precision (%CV) and accuracy (%RE) were 4.4% and 4.0% respectively.	
Bench-top/process stability	Mitapivat was determined to be stable in human plasma at ambient temperature for up to 25 hours. Based on the Low QC, precision (%CV) and accuracy (%RE) were 4.5% and 0.7% respectively. Mitapivat was determined to be stable in human whole blood at ambient temperature for up to 2 hours. Based on the Low QC, precision (%CV) and accuracy (% difference from Time 0) were 5.5% and -10.5% respectively. Mitapivat was determined to be stable in processed samples stored at 4°C for up to 99 hours. Based on the Low and High QC, precision (%CV) and accuracy (%RE) were ≤3.7% and ±5.0% respectively.	
Freeze-Thaw stability	Mitapivat was determined to be stable in human plasma stored at -20°C through 5 freeze-thaw cycles. Based on the Low QC, precision (%CV) and accuracy (%RE) were 6.4% and -1.0% respectively. Mitapivat was determined to be stable in human plasma stored at -70°C through 5 freeze-thaw cycles. Based on the Low QC, precision (%CV) and accuracy (%RE) were 1.7% and 6.0% respectively.	
Long-term storage	Mitapivat was determined to be stable in human plasma stored at -20°C for up to 370 days. Based on the Low QC, precision (%CV) and accuracy (%RE) were 3.9% and 0.0% respectively. Mitapivat was determined to be stable in human plasma stored at -70°C for up to 679 days. Based on the Low and High QC, precision (%CV) and accuracy (%RE) were ≤4.2% and ±10.0% respectively.	
Parallelism	Not applicable	
Carry over	Carryover met acceptance criteria for mitapivat and its stable isotope-labeled internal standard in all validation runs.	

	Method performance in Study AG348-C-004 (Report AG348-C-004-BA)
Assay passing rate	100% (5/5) analytical runs met acceptance criteria.
Standard curve performance	Cumulative accuracy (%bias) from LLOQ to ULOQ: -5.0% to 4.0% Cumulative precision (%CV) from LLOQ to ULOQ: ≤6.6%
QC performance	Cumulative accuracy (%bias) from 4 levels of QCs: -2.5% to 8.3% Cumulative precision (%CV) from 4 levels of QCs: ≤5.6%
Method reproducibility	Of the 960 study samples, 114 were reanalyzed for incurred sample reproducibility (ISR). Two assays were used to support this study; the same assay used to generate a sample's original result was used for ISR reanalysis. Six samples were not included in the final ISR calculation due to failed Dilution QCs or carryover impact in the ISR run. Of the remaining 108 samples reanalyzed, 99.1% (107/108) of the samples met the ISR acceptance criteria for mitapivat.
Study sample analysis/ stability	Sample storage duration was a maximum of 77 days (date of first sample collection – date of last injection). All samples were analyzed within the established long-term stability at -70°C.

Source: Applicant's report.
Abbreviations: CRO, contract research organization; CV, coefficient of variation; EDTA, ethylenediaminetetraacetic acid; LC-MS/MS, liquid chromatography – mass spectrometry; LLOQ, lower limit of quantitation; QC, quality control; RE, relative error; ULOQ, upper limit of quantitation

Table 120. Summary Method Validation and Performance for Mitapivat in Human Plasma, Method AG-348-Q-009

Bioanalytical method validation report name, amendments, and hyperlinks	Report AG348-Q-009	
Method description	CRO Method Number 422-1801 Simultaneous measurement of mitapivat and [$^{13}C_6$]mitapivat by LC-MS/MS detection	
Materials used for standard calibration curve and concentration	Human (EDTA) plasma 0.500, 1.00, 5.00, 10.0, 50.0, 100, 450, and 500 ng/mL	
Validated assay range	0.500–500 ng/mL	
Material used for quality controls (QCs) and concentration	Human (EDTA) plasma 0.500, 1.50, 20.0, 200, and 400 ng/mL	
Minimum required dilutions (MRDs)	Not applicable	
Source and lot of reagents	Not applicable	
Regression model and weighting	Linear, $1/x^2$	
Validation parameters	**Method validation summary**	
Standard calibration curve performance	Number of standard calibrators from LLOQ to ULOQ	8
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-1.3% to 1.6%
	Cumulative precision (%CV) from LLOQ to ULOQ	≤7.6%
Performance of QCs during accuracy and precision runs	**Cumulative accuracy (%bias) in 5 QCs**	-7.5% to -0.6%
	Interbatch %CV	≤9.1%
	Total Error (TE)	Not applicable
Selectivity & matrix effect	Results from 5 of 6 lots of human plasma tested met selectivity acceptance criteria. The maximum accuracy (%RE) of the LLOQ spike-in selectivity from the 6 lots was 21.2%; but for the other 5 lots accuracy was within ±6.8%. The precision (%CV) of IS-normalized matrix factor across 6 lots of human plasma was ≤2.9% at the Low and High QC levels; therefore, no matrix effect was observed which would significantly impact study data.	
Interference & specificity	Results from all 6 lots of human plasma tested met acceptance criteria. No interference was observed for mitapivat or its stable isotope-labeled internal standard. An interference test was performed to evaluate the potential interference to [$^{13}C_6$]mitapivat (Low QC 15.0 pg/mL) when mitapivat was present at concentrations 1500-fold higher. The interference test met acceptance criteria.	
Hemolysis effect	Results from Low and High QC levels tested in a single lot of 2% hemolyzed plasma met acceptance criteria. Accuracy (%RE) was -2.0% (Low QC) and -6.5% (High QC).	
Lipemic effect	Results from Low and High QC levels tested in a single lot of lipemic plasma met acceptance criteria. Accuracy (%RE) was -5.3% (Low QC) and -6.5% (High QC).	
Dilution linearity	Dilution of 20-fold tested at 1000 ng/mL. Precision (%CV) and accuracy (%RE) were 2.9% and -10.1% respectively.	
Bench-top/process stability	Mitapivat was determined to be stable in human plasma at ambient temperature for up to 15 hours. Based on the Low and High QC, precision (%CV) and accuracy (%RE) were ≤5.4% and ±9.3% respectively. Mitapivat was determined to be stable in human whole blood at ambient temperature for up to 2 hours. Based on the Low and High QC, precision (%CV) and accuracy (% difference from Time 0) were ≤4.5% and ±3.5% respectively. Mitapivat was determined to be stable in processed samples stored at 4°C for up to 148 hours. Based on the Low and High QC, precision (%CV) and accuracy (%RE) were ≤4.5% and ±7.8% respectively.	
Freeze-Thaw stability	Mitapivat was determined to be stable in human plasma stored at -20°C through 5 freeze-thaw cycles. Based on the Low and High QC, precision (%CV) and accuracy (%RE) were ≤2.6% and ±8.0% respectively. Mitapivat was determined to be stable in human plasma stored at -70°C through 5 freeze-thaw cycles. Based on the Low and High QC, precision (%CV) and accuracy (%RE) were ≤3.7% and ±8.3% respectively.	
Long-term storage	Mitapivat was determined to be stable in human plasma stored at -20°C for up to 45 days. Based on the Low and High QC, precision (%CV) and accuracy (%RE) were ≤5.3% and ±8.8% respectively. Mitapivat was determined to be stable in human plasma stored at -70°C for up to 45 days. Based on the Low and High QC, precision (%CV) and accuracy (%RE) were ≤1.9% and ±8.8% respectively.	
Parallelism	Not applicable	
Carry over	Carryover met acceptance criteria for mitapivat and its stable isotope-labeled internal standard in all validation runs.	

	Method performance in Study AG348-C-009 (Report AG348-C-009-BA-Plasma)
Assay passing rate	80% (4/5) analytical runs met acceptance criteria. One run was rejected due to QCs not meeting acceptance criteria.
Standard curve performance	Cumulative accuracy (%bias) from LLOQ to ULOQ: -5.0% to 7.1% Cumulative precision (%CV) from LLOQ to ULOQ: ≤9.2%
QC performance	Cumulative accuracy (%bias) from 4 levels of QCs: -5.3% to 8.0% Cumulative precision (%CV) from 4 levels of QCs: ≤11.6%
Method reproducibility	Of the 154 analyzed study samples, 22 were reanalyzed for incurred sample reproducibility (ISR). 100% (22/22) of the samples met the ISR acceptance criteria for mitapivat.
Study sample analysis/ stability	Sample storage duration was a maximum of 32 days (date of first sample collection – date of last injection). All samples were analyzed within the established long-term stability at -70°C.

Source: Applicant's report.
Abbreviations: CRO, contract research organization; CV, coefficient of variation; EDTA, ethylenediaminetetraacetic acid; LC-MS/MS, liquid chromatography – mass spectrometry; LLOQ, lower limit of quantitation; QC, quality control; RE, relative error; ULOQ, upper limit of quantitation

Table 121. Summary Method Validation and Performance for [$^{13}C_6$] Mitapivat in Human Plasma, Method AG-348-Q-009

Bioanalytical method validation report name, amendments, and hyperlinks	Report AG348-Q-009	
Method description	CRO Method Number 422-1801 Simultaneous measurement of mitapivat and [$^{13}C_6$]mitapivat by LC-MS/MS detection	
Materials used for standard calibration curve and concentration	Human (EDTA) plasma 5.00; 10.0; 50.0; 100; 500; 1,000; 4,500; and 5,000 pg/mL	
Validated assay range	5.00–5,000 pg/mL	
Material used for quality controls (QCs) and concentration	Human (EDTA) plasma 5.00; 15.0; 200; 2,000; and 4,000 pg/mL	
Minimum required dilutions (MRDs)	Not applicable	
Source and lot of reagents	Not applicable	
Regression model and weighting	Linear, $1/x^2$	
Validation parameters	**Method validation summary**	
Standard calibration curve performance	Number of standard calibrators from LLOQ to ULOQ	8
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-2.2% to 1.2%
	Cumulative precision (%CV) from LLOQ to ULOQ	≤5.7%
Performance of QCs during accuracy and precision runs	**Cumulative accuracy (%bias) in 5 QCs**	-7.8% to 2.4%
	Interbatch %CV	≤12.9%
	Total Error (TE)	Not applicable
Selectivity & matrix effect	Results from all 6 lots of human plasma tested met selectivity acceptance criteria. Accuracy (%RE) of the LLOQ spike-in selectivity from the 6 lots was within ±19.4%. The precision (%CV) of IS-normalized matrix factor across 6 lots of human plasma was ≤2.9% at the Low and High QC levels; therefore, no matrix effect was observed which would significantly impact study data.	
Interference & specificity	Results from all 6 lots of human plasma tested met acceptance criteria. No interference was observed for [$^{13}C_6$]mitapivat or its stable isotope-labeled internal standard. An interference test was performed to evaluate the potential interference to [$^{13}C_6$]mitapivat (Low QC 15.0 pg/mL, n=5) when mitapivat was present at concentrations 1500-fold higher. Precision (%CV) and accuracy (%RE) were 4.6% and 10.7% respectively.	

Hemolysis effect	Results from Low and High QC levels tested in a single lot of 2% hemolyzed plasma met acceptance criteria. Accuracy (%RE) was -0.7% (Low QC) and -6.3% (High QC).
Lipemic effect	Results from Low and High QC levels tested in a single lot of lipemic plasma met acceptance criteria. Accuracy (%RE) was -1.3% (Low QC) and -7.0% (High QC).
Dilution linearity	Dilution of 20-fold tested at 10,000 pg/mL. Precision (%CV) and accuracy (%RE) were 2.9% and -8.7% respectively.
Bench-top/process stability	[$^{13}C_6$]mitapivat was determined to be stable in human plasma at ambient temperature for up to 15 hours. Based on the Low and High QC, precision (%CV) and accuracy (%RE) were ≤3.8% and ±7.8% respectively. [$^{13}C_6$]mitapivat was determined to be stable in human whole blood at ambient temperature for up to 2 hours. Based on the Low and High QC, precision (%CV) and accuracy (% difference from Time 0) were ≤3.9% and ±2.0% respectively. [$^{13}C_6$]mitapivat was determined to be stable in processed samples stored at 4°C for up to 148 hours. Based on the Low and High QC, precision (%CV) and accuracy (%RE) were ≤3.3% and ±8.3% respectively.
Freeze-Thaw stability	[$^{13}C_6$]mitapivat was determined to be stable in human plasma stored at -20°C through 5 freeze-thaw cycles. Based on the Low and High QC, precision (%CV) and accuracy (%RE) were ≤2.6% and ±8.8% respectively. [$^{13}C_6$]mitapivat was determined to be stable in human plasma stored at -70°C through 5 freeze-thaw cycles. Based on the Low and High QC, precision (%CV) and accuracy (%RE) were ≤5.3% and ±8.3% respectively.
Long-term storage	[$^{13}C_6$]mitapivat was determined to be stable in human plasma stored at -20°C for up to 45 days. Based on the Low and High QC, precision (%CV) and accuracy (%RE) were ≤2.9% and ±8.8% respectively. [$^{13}C_6$]mitapivat was determined to be stable in human plasma stored at -70°C for up to 45 days. Based on the Low and High QC, precision (%CV) and accuracy (%RE) were ≤2.5% and ±9.8% respectively.
Parallelism	Not applicable
Carry over	Carryover met acceptance criteria for [$^{13}C_6$]mitapivat and its stable isotope-labeled internal standard in all validation runs.

Method performance in Study AG348-C-009 (Report AG348-C-009-BA-Plasma)	
Assay passing rate	60% (3/5) analytical runs met acceptance criteria. One run was rejected due to QCs not meeting acceptance criteria, and one run was rejected due to suspected contamination.
Standard curve performance	Cumulative accuracy (%bias) from LLOQ to ULOQ: -3.7% to 6.4% Cumulative precision (%CV) from LLOQ to ULOQ: ≤7.9%
QC performance	Cumulative accuracy (%bias) from 4 levels of QCs: -5.3% to 5.5% Cumulative precision (%CV) from 4 levels of QCs: ≤5.7%
Method reproducibility	Of the 154 analyzed study samples, 22 were reanalyzed for incurred sample reproducibility (ISR). 100% (22/22) of the samples met the ISR acceptance criteria for [$^{13}C_6$]mitapivat.
Study sample analysis/stability	Sample storage duration was a maximum of 32 days (date of first sample collection – date of last injection). All samples were analyzed within the established long-term stability at -70°C.

Source: Applicant's report.
Abbreviations: CRO, contract research organization; CV, coefficient of variation; EDTA, ethylenediaminetetraacetic acid; LC-MS/MS, liquid chromatography – mass spectrometry; LLOQ, lower limit of quantitation; QC, quality control; RE, relative error; ULOQ, upper limit of quantitation

Table 122. Summary Method Validation and Performance for Mitapivat in Human Urine, Method AG-348-N-069

Bioanalytical method validation report name, amendments, and hyperlinks	Report AG348-N-069
Method description	CRO Method Number 422-1312 Simultaneous measurement of mitapivat and its N-dealkylated metabolite, AGI-8702, by LC-MS/MS detection
Materials used for standard calibration curve and concentration	Human urine 5.00; 10.0; 100; 500; 2,500; 10,000; 13,500; and 15,000 ng/mL
Validated assay range	5.00–15,000 ng/mL

Material used for quality controls (QCs) and concentration	Human urine 5.00; 15.0; 300; 7,500; and 12,000 ng/mL	
Minimum required dilutions (MRDs)	Not applicable	
Source and lot of reagents	Not applicable	
Regression model and weighting	Linear, $1/x^2$	
Validation parameters	**Method validation summary**	
Standard calibration curve performance	Number of standard calibrators from LLOQ to ULOQ	8
	Cumulative accuracy (%bias) from LLOQ to ULOQ	-4.7% to 5.0%
	Cumulative precision (%CV) from LLOQ to ULOQ	≤3.9%
Performance of QCs during accuracy and precision runs	**Cumulative accuracy (%bias) in 5 QCs**	-4.4% to 4.0%
	Interbatch %CV	≤3.7%
	Total Error (TE)	Not applicable
Selectivity & matrix effect	Results from all 6 lots of human urine tested met selectivity acceptance criteria. Accuracy (%RE) of the LLOQ spike-in selectivity from the 6 lots was within ±20.0%. The precision (%CV) of IS-normalized matrix factor across 6 lots of human plasma was ≤2.3% at the Low and High QC levels; therefore, no matrix effect was observed which would significantly impact study data.	
Interference & specificity	Results from all 6 lots of human urine tested met acceptance criteria for mitapivat and its stable isotope-labeled internal standard. No interference was observed for mitapivat or its stable isotope-labeled internal standard.	
Hemolysis effect	Not applicable	
Lipemic effect	Not applicable	
Dilution linearity	Dilution of 40-fold tested at 30,000 ng/mL. Precision (%CV) and accuracy (%RE) were 5.6% and -1.3% respectively.	
Bench-top/process stability	Mitapivat was determined to be stable in human urine at ambient temperature for up to 15.5 hours. Based on the Low and High QC, precision (%CV) and accuracy (%RE) were ≤1.9% and ±5.3% respectively. Mitapivat was determined to be stable in processed samples stored at 4°C for up to 183 hours. Based on the Low and High QC, precision (%CV) and accuracy (%RE) were ≤1.8% and ±6.7% respectively.	
Freeze-Thaw stability	Mitapivat was determined to be stable in human urine stored at -20°C through 5 freeze-thaw cycles. Based on the Low and High QC, precision (%CV) and accuracy (%RE) were ≤4.2% and ±8.0% respectively. Mitapivat was determined to be stable in human urine stored at -70°C through 5 freeze-thaw cycles. Based on the Low and High QC, precision (%CV) and accuracy (%RE) were ≤3.5% and ±4.0% respectively.	
Long-term storage	Mitapivat was determined to be stable in human urine stored at -20°C for up to 416 days. Based on the Low and High QC, precision (%CV) and accuracy (% difference from Day 0) were ≤5.5% and ±3.4% respectively. Mitapivat was determined to be stable in human urine stored at -70°C for up to 358 days. Based on the Low and High QC, precision (%CV) and accuracy (% difference from Day 0) were ≤4.0% and ±8.3% respectively.	
Parallelism	Not applicable	
Carry over	Mitapivat carryover (>20% of LLOQ) was observed in 2 validation runs, and is monitored during the production use of this method for any sample-to-sample injection carryover impact.	

	Method performance in Study AG348-C-001 (Report 422-1406B)
Assay passing rate	100% (3/3) analytical runs met acceptance criteria.
Standard curve performance	Cumulative accuracy (%bias) from LLOQ to ULOQ: -9.6% to 12.0% Cumulative precision (%CV) from LLOQ to ULOQ: ≤6.7%
QC performance	Cumulative accuracy (%bias) from 4 levels of QCs: -7.5% to 9.3% Cumulative precision (%CV) from 4 levels of QCs: ≤6.3%
Method reproducibility	Of the 192 study samples, 22 were reanalyzed for incurred sample reproducibility (ISR). 100% (22/22) of the samples met the ISR acceptance criteria for mitapivat.
Study sample analysis/ stability	Sample storage duration was a maximum of 92 days (date of first sample collection – date of last injection). All samples were analyzed within the established long-term stability at -20°C.

	Method performance in Study AG348-C-009 (Report AG348-C-009-BA-Urine)
Assay passing rate	66.7% (2/3) analytical runs met acceptance criteria. One run was rejected due to QCs not meeting acceptance criteria.
Standard curve performance	Cumulative accuracy (%bias) from LLOQ to ULOQ: -2.6% to 3.0% Cumulative precision (%CV) from LLOQ to ULOQ: ≤9.5%
QC performance	Cumulative accuracy (%bias) from 5 levels of QCs: 3.3% to 8.0% Cumulative precision (%CV) from 5 levels of QCs: ≤6.7%
Method reproducibility	Of the 76 analyzed study samples, 21 were reanalyzed for incurred sample reproducibility (ISR). 100% (21/21) of the samples met the ISR acceptance criteria for mitapivat.
Study sample analysis/ stability	Sample storage duration was a maximum of 27 days (date of first sample collection – date of last injection). All samples were analyzed within the established long-term stability at -70°C.

Source: Applicant's report.
Abbreviations: CRO, contract research organization; CV, coefficient of variation; LC-MS/MS, liquid chromatography – mass spectrometry; LLOQ, lower limit of quantitation; QC, quality control; RE, relative error; ULOQ, upper limit of quantitation

All methods were well described by the Applicant and reviewed, which satisfied the method validation criteria in accordance with the FDA guidance. The performance of the assay is considered acceptable for sample analysis.

15. Trial Design: Additional Information and Assessment

15.1. Protocol Amendments

The clinical trial landmarks and protocol amendments for Studies 006 and 007 are summarized in Table 123 and Table 124, respectively.

Table 123. Key Landmarks and Key Protocol Amendments, Study 006

Date	Landmarks
November 30, 2017	Version 1
February 26, 2018	Version 2 Removed dose escalation restrictions after the Week 8 Visit. Added guidance on reintroducing or escalating study treatment after resolution of a Grade 3 AE that caused study treatment to be stopped or reduced. Removed the requirement that subjects must be receiving study treatment at Week 24 to be eligible for an extension study. Added an exclusion criterion to exclude subjects who have not stopped using hematopoietic stimulating agents at least 28 days before the first dose of study treatment. Added clarity to the dose modification guidance for Grade 3 and Grade 4 AEs that are deemed to be related to study treatment.

Date	Landmarks
August 15, 2018	Version 3
	Consolidated iron-related secondary and exploratory endpoints into one exploratory endpoint for markers of iron metabolism.
	Revised the instructions for dose optimization.
	Clarified that unblinding before database lock will occur only in the subjects who enter the planned mitapivat extension study and that subjects undergoing a dose taper should remain blinded through the taper.
	Amended the inclusion criterion for renal function (adding eGFR requirement of ≥60 mL/min/1.73 m^2).
	Amended the absolute neutrophil count and platelet count inclusion criteria to be assessed via two measurements.
	Added an exception for subjects who have concurrent disorders that in isolation are predicted to be insufficient to explain the observed clinical phenotype to the exclusion criterion for congenital or genetic disorders.
	Corrected the exclusion criterion for splenectomy to require subjects to wait at least 12 months after splenectomy before starting screening.
	Redefined the definition of hemoglobin overshoot, and subsequent study treatment dose decrease, to higher than 20 g/L (2 g/dL) below the ULN.
	Added details for assessments after a transaminase increase.
October 1, 2018	First subject enrollment
August 14, 2019	Version 4
	Revised the dose optimization language to allow dosing decisions to be based on results from local laboratories at the Week 4 and Week 8 Visits.
	Revised the inclusion criterion for platelet count.
	Removed the option for a rapid dose taper and simplified the recommended gradual dose taper.
	Added language to provide previously ineligible subjects the opportunity to rescreen for enrollment into the study should they become eligible based on an amended protocol.
	Revised the requirements for clinical laboratory results, allowing the flexibility to use local laboratory results when results from central laboratories are not available.
	Added further details for assessments after a transaminase increase that meet the criteria for an AESI.
October 9, 2020	Last subject completed

Source: Study 006 Clinical Study Report.
Abbreviations: AE, adverse event; AESI, adverse event of special interest; eGFR, estimated glomerular filtration rate; ULN, upper limit of normal

Table 124. Key Landmarks and Key Protocol Amendments, Study 007

Date	Landmark
September 28, 2017	Version 1
June 26, 2018	First subject enrollment
October 15, 2018	Version 2
	Revised the dose-escalation restrictions during the Fixed-Dose Period.
	Revised the instructions for dose optimization during the Dose Optimization Period.
	Revised the language on hemoglobin monitoring in relation to the subject's transfusion trigger during the Fixed-Dose Period to make the collection of additional hematology assessments more flexible.
	Amended the inclusion criterion for renal function.

Date	Landmark
	Amended the inclusion criterion for platelet count. Added an exception for subjects who have genetic findings that, in isolation, are predicted to be insufficient to explain the observed clinical phenotype to the exclusion criterion for congenital or genetic disorders. Corrected the exclusion criterion for splenectomy to require subjects to wait at least 12 months after splenectomy before starting screening. Added an exclusion criterion to exclude subjects who did not stop using hematopoietic stimulating agents at least 28 days before the first dose of study treatment. Redefined hemoglobin overshoot, and subsequent study treatment dose decrease, to more than 2 g/dL below the upper limit of normal. Added guidance on reintroducing or escalating study treatment after resolution of a Grade 3 AE that caused study treatment to be stopped or the dose to be reduced. Added clarity to the dose-modification guidance for Grade 3 and Grade 4 AEs that are deemed to be related to study treatment. Added language to provide previously ineligible subjects the opportunity to rescreen for enrollment into the study should they become eligible based on an amended protocol. Added further details for assessments after a transaminase increase that meets the criteria for an AE of special interest. Removed the use of an Independent Data Monitoring Committee.
March 19, 2019	Version 3 Increased the sample size from “approximately 15-20” to “a minimum of 20, with up to 40 adult subjects.” Revised the central laboratory requirements for additional blood sampling necessary for subjects whose individual transfusion trigger has not been reached.
November 12, 2020	Last subject completed.

Source: Study 007 Clinical Study Report.
Abbreviations: AE, adverse event

15.2. Eligibility Criteria

Study 006

The abbreviated eligibility criteria are provided in Section 6.2.1.3. Other key inclusion and exclusion criteria are provided below.

Key Inclusion Criteria

1. Age ≥ 18 years.
2. Documented clinical laboratory confirmation of PK deficiency, defined as documented presence of at least 2 variant alleles in the PKLR gene, of which at least 1 is a missense variant, as determined per the genotyping performed by the central genotyping laboratory.
3. Hemoglobin concentration ≤ 10.0 g/dL (6.21 mmol/L) regardless of gender (average of at least 2 Hb measurements [separated by a minimum of 7 days] during the Screening Period).
4. 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 prior to the first day of study treatment.

5. Received at least 0.8 mg oral folic acid daily for at least 21 days prior to the first dose of study treatment, to be continued daily during study participation.
6. Adequate organ function, as defined by:
    a. AST ≤2.5 × ULN (unless the increased AST was assessed as due to hemolysis and/or hepatic iron deposition) and ALT ≤2.5 × ULN (unless the increased ALT was assessed as due to hepatic iron deposition).
    b. Normal or elevated levels of serum bilirubin. In subjects with serum bilirubin >ULN, the elevation must not be associated with choledocholithiasis, cholecystitis, biliary obstruction, or hepatocellular disease. Elevated bilirubin attributed to hemolysis with or without Gilbert's syndrome is not exclusionary.
    c. GFR ≥60 mL/min/1.73 m^2, measured GFR≥60 mL/min, or CrCL ≥60 mL/min.
    d. ANC ≥1.0 × 10^9/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 × 10^9/L in the absence of a spleen, or platelet count ≥50 × 10^9/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 (aPTT) and international normalized ratio (INR) ≤1.25 × ULN, unless the subject is receiving therapeutic anticoagulants.
7. For women of reproductive potential as well as men with partners who are women of reproductive potential, be abstinent as part of their usual lifestyle, or agree to use 2 forms of contraception, 1 of which must be considered highly effective, from the time of giving informed consent, during the study, and for 28 days following the last dose of study treatment for women and 90 days following the last dose of study treatment for men.

Key Exclusion Criteria

1. Homozygous for the R479H variant or have 2 non-missense variants, without the presence of another missense variant, in the PKLR gene as determined per the genotyping performed by the central genotyping laboratory.
2. Significant medical condition that confers an unacceptable risk to participating in the study that includes following:
    a. Poorly controlled hypertension (defined as systolic blood pressure [BP] >150 mmHg or diastolic BP >90 mmHg) refractory to medical management.
    b. History of recent (within 6 months prior to signing informed consent) congestive heart failure; myocardial infarction or unstable angina pectoris; hemorrhagic, embolic, or thrombotic stroke; deep venous thrombosis; or pulmonary or arterial embolism.
    c. Cardiac dysrhythmias judged as clinically significant.
    d. Heart-rate corrected QT interval-Fridericia's method (QTcF) >450 msec (average of triplicate ECGs) with the exception of subjects with right or left bundle branch block.
    e. Clinically symptomatic cholelithiasis or cholecystitis. Prior cholecystectomy is not exclusionary. Subjects with symptomatic cholelithiasis or cholecystitis may be

rescreened once the disorder has been treated and clinical symptoms have resolved.

f. History of drug-induced cholestatic hepatitis.
g. Iron overload sufficiently severe to result in a clinical diagnosis of cardiac (e.g., clinically significant impaired left ventricular ejection fraction), hepatic (e.g., fibrosis, cirrhosis), or pancreatic (eg, diabetes) dysfunction.
h. Diagnosis of any other congenital or acquired blood disorder or any other hemolytic process, except mild allo-immunization, as a consequence of transfusion therapy. Genetic findings that in isolation are predicted to be insufficient to explain the observed clinical phenotype may be allowed (e.g., heterozygous status for certain recessive red blood cell disorders).
i. Positive test for HBsAg or HCVAb with signs of active hepatitis B or C virus infection.
j. Positive test for HIV-1 or -2 Ab.
k. Active infection requiring the use of parenteral antimicrobial agents or Grade ≥3 in severity within 2 months prior to the first dose of study treatment.
l. Diabetes mellitus judged to be under poor control or requiring >3 antidiabetic agents, including insulin (all insulins are considered 1 agent); use of insulin per se is not exclusionary.
m. History of any primary malignancy, with the exception of: curatively treated nonmelanomatous skin cancer; curatively treated cervical or breast carcinoma in situ; or other primary tumor treated with curative intent, no known active disease present, and no treatment administered during the last 3 years.
n. Unstable extramedullary hematopoiesis that could pose a risk of imminent neurologic compromise.

3. Splenectomy scheduled during the study treatment period or have undergone splenectomy within 12 months.
4. Prior treatment with a pyruvate kinase activator.
5. Prior bone marrow or stem cell transplant.
6. History of major surgery within 6 months.
7. Are currently receiving medications that are strong inhibitors of CYP3A4, strong inducers of CYP3A4, strong inhibitors of P-glycoprotein (P-gp), or digoxin (a P-gp sensitive substrate medication) that have not been stopped for a duration of at least 5 days or a timeframe equivalent to 5 half-lives (whichever is longer) prior to the first dose of study treatment.
8. Currently receiving hematopoietic stimulating agents (eg, erythropoietin, granulocyte colony stimulating factors, thrombopoietin) that have not been stopped for a duration of at least 28 days prior to the first dose of study treatment.
9. History of allergy to sulfonamides if characterized by acute hemolytic anemia, drug-induced liver injury, anaphylaxis, rash of erythema multiforme type or Stevens-Johnson syndrome, cholestatic hepatitis, or other serious clinical manifestations.
10. Currently receiving anabolic steroids, including testosterone preparations, within 28 days prior to the first dose of study treatment.

Patients were allowed to continue iron chelation therapy with deferoxamine, deferasirox, or deferiprone. Patients were to continue to take at least 0.8 mg oral folic acid daily for the duration of the study.

Study 007

The abbreviated eligibility criteria are provided in Section 6.2.3.3. Other key inclusion and exclusion criteria are listed below.

Key Inclusion Criteria

1. Aged ≥ 18 years.
2. Documented clinical laboratory confirmation of PK deficiency, defined as documented presence of at least 2 variant alleles in the PKLR gene, of which at least 1 is a missense variant, as determined per the genotyping performed by the study central genotyping laboratory.
3. History of a minimum of 6 transfusion episodes in the 52-week period prior to date of informed consent as documented in the transfusion history of the subject, which reflects the subject's typical transfusion burden.
4. Complete records of transfusion history, defined as having the following available for the 52 weeks prior to the date of informed consent: (1) all the transfusion dates, (2) the number of blood units transfused for all the transfusions, and (3) hemoglobin concentrations within 1 week prior to transfusion for at least 80% of the transfusions.
5. Received at least 0.8 mg oral folic acid daily for at least 21 days prior to the first dose of study drug, to be continued daily during study participation.
6. Have adequate organ function, as defined by in Study AG-348-C-006.
7. Use of adequate contraceptive methods for women of reproductive potential as well as men with partners who are women of reproductive potential, as defined in Study AG-348-C-006.

Key Exclusion Criteria

1. Homozygous for the R479H variant or have 2 non-missense variants, without the presence of another missense variant, in the PKLR gene as determined per the genotyping performed by the central genotyping laboratory.
2. Significant medical condition that confers an unacceptable risk to participating in the study consistent with those in Study AG-348-C-006.
3. Current or recent history of psychiatric disorder that could compromise the ability of the subject to cooperate with study visits and procedures.
4. History of transfusions occurring on average more frequently than once every 3 weeks during the 52 weeks prior to signing informed consent.
5. Splenectomy scheduled during the study drug period or have undergone splenectomy within 12 months prior to signing informed consent.
6. Prior bone marrow or stem cell transplant.
7. History of major surgery within 6 months.
8. Currently receiving medications that are strong inhibitors of CYP3A4, strong inducers of CYP3A4, strong inhibitors of P-glycoprotein (P-gp), or digoxin (a P-gp sensitive substrate medication) that have not been stopped for a duration of at least 5 days or a timeframe equivalent to 5 half-lives (whichever is longer) prior to the first dose of study drug.
9. Currently receiving hematopoietic stimulating agents (e.g., erythropoietins [EPOs],

granulocyte colony stimulating factors, thrombopoietins) that have not been stopped for a duration of at least 28 days prior to the first dose of study drug.
10. History of allergy to sulfonamides if characterized by acute hemolytic anemia, drug-induced liver injury, anaphylaxis, rash of erythema multiforme type or Stevens-Johnson syndrome, cholestatic hepatitis, or other serious clinical manifestations.
11. History of allergy to AG-348 or its excipients (microcrystalline cellulose, croscarmellose sodium, sodium stearyl fumarate, and mannitol).
12. Currently receiving anabolic steroids, including testosterone preparations, within 28 days prior to the first dose of study drug.

As in Study 006, patients were allowed to continue iron chelation therapy and were to continue taking at least 0.8 mg oral folic acid daily during the study.

15.3. Treatment Modification Plan

Study 006

In general, it was recommended that a patient not abruptly discontinue or interrupt study treatment due to the risk of withdrawal hemolysis. A patient's study treatment dose could be reduced (to 5 mg BID or 20 mg BID), interrupted, and/or discontinued in the following cases:

- Excessive hemoglobin response, with the patient's hemoglobin >2 g/dL (1.24 mmol/L) below the ULN (i.e., higher than 15.0 g/dL [9.31 mmol/L] in men and 13.5 g/dL [8.38 mmol/L] in women) up to the ULN (i.e., 17.0 g/dL [10.55 mmol/L] in men and 15.5 g/dL [9.62 mmol/L] in women), a dose decrease to the next lower dose level was considered, without a need for a dose taper (i.e., if the patient experienced an excessive Hb response at 50 mg BID, the dose was decreased to 20 mg BID). The same rule applied to the decrease from 20 mg BID to 5 mg BID.
- Occurrence of study treatment-related AEs (except for excessive hemoglobin response).
- Planned study treatment discontinuation with the recommended dose taper.

Table 125. Dose Modification for Adverse Reactions (Except for Excessive Hb Response), Study 006

Related AEs	Dose Modification
Grade 1	None required.
Grade 2	None required. Specific cases could be managed as Grade 3 events (see below).
Grade 3	Based on the relative risk on study treatment versus risk of withdrawal hemolysis when stopping treatment abruptly or reducing the dose, the following options were available: Maintaining the current dose: At least once weekly monitoring was performed until the event resolves to baseline or Grade 1 (whichever is lower). If the event persisted, the recommended dose taper or stopping the study treatment abruptly was considered. Performing the recommended dose taper or stopping the study treatment abruptly: If the recommended dose taper was conducted or study treatment was stopped, the following instructions were followed for reintroduction or re-escalation of the study treatment, respectively. In all cases, reintroduction and re-escalation of study treatment was performed only after discussion with the Independent Medical Monitor, or designee. Restarting study treatment after dosing was stopped: Once the event resolved to baseline or Grade 1 (whichever is lower) and the decision was made to restart treatment, the study treatment was reintroduced at the 5 mg BID dose level. If the event did not re-occur after at least 4 weeks on 5 mg BID (with at

Related AEs	Dose Modification
	least once weekly monitoring), the dose could be increased from 5 mg BID to 20 mg BID. If the event did not re-occur after at least 4 weeks on 20 mg BID (with at least once weekly monitoring), the dose could be increased from 20 mg BID to 50 mg BID. Events that were resolving during the recommended dose taper (i.e., the subject is still on study treatment): If during the recommended dose taper, the event resolved to baseline or Grade 1 (whichever is lower), the study treatment was maintained at the dose at which the event resolved for at least 4 weeks (with at least once weekly monitoring). If the event did not re-occur after at least 4 weeks, then the dose could be increased to the next highest BID dose (5 mg BID, 20 mg BID, or 50 mg BID) with at least once weekly monitoring. If the event did not recur after at least 4 weeks (with at least once weekly monitoring), and the patient was not already receiving 50 mg BID, then an increase to the next BID dose level was considered. Re-occurrence of the AE: If the AE recurred at any point during the above scenarios, the patient underwent the recommended dose taper or stopped study treatment abruptly if necessitated by the risk of the AE. If the patient underwent the recommended dose taper and the AE resolved during the taper, study treatment was maintained at the next lowest BID dose below the dose at which the AE resolved. If the subject could not tolerate BID dosing, another regimen was allowed. If the AE did not resolve to baseline or Grade 1 (whichever is lower) after the dose was decreased, a further decrease in the dose was considered. If the AE still did not resolve, study treatment was permanently discontinued.
Grade 4	After careful consideration of the relative risk of withdrawal hemolysis when stopping study treatment abruptly versus reducing the dose, the following options were available: Performing the recommended dose taper, or Stopping the study treatment abruptly. If the event resolved, and it was believed that reintroducing study treatment was justified, the Independent Medical Monitor, or designee, was consulted before any further study treatment was administered.

Source: Study 006 protocol.
Abbreviations: AE, adverse event; BID, twice daily; Hb, hemoglobin

Patients who discontinued or interrupted study treatment during the study were to undergo the recommended dose taper regimen shown in Table 126. This regimen was based on the study treatment dose administered at the start of the taper and occurred in one or two sequential steps. Patients who were unblinded and allocated to placebo did not need to undergo the recommended dose taper when discontinuing or interrupting study treatment. Patients undergoing the recommended dose taper were to be monitored as clinically indicated for signs of withdrawal hemolysis and worsening of anemia. If the recommended dose taper was performed in order to permanently discontinue study treatment, patients were to stop taking the study treatment after the taper was completed.

Table 126. Recommended Dose Taper Regimen, Study 006

Starting Dose (at the time of the dose taper)	First Step ×7 days	Second Step ×7 days
5 mg BID	5 mg QD	---
20 mg BID	20 mg QD	5 mg QD
50 mg BID	50 mg QD	20 mg QD

Source: Study 006 protocol.
Abbreviations: BID, twice daily; QD, once daily

Study 007

As in Study 006, it was recommended that a patient not abruptly discontinue or interrupt study treatment due to the risk of withdrawal hemolysis. A patient's study treatment dose could be reduced (to 5 mg BID or 20 mg BID), interrupted, and/or discontinued as in Study 006 (see Table 125). After consideration of the risk of maintaining the patient on the study drug versus withdrawal hemolysis when stopping study drug abruptly versus reducing the dose rapidly, a gradual dose taper (GDT and rapid dose taper were evaluated.

In patients undergoing a rapid dose taper, the regimen as detailed in Table 127 was to be followed and patients were to be monitored for signs of hemolysis and worsening of anemia.

Table 127. Rapid Dose Taper Regimen, Study 007

Starting Dose	First Step ×3 days	Second Step ×3 days	Third Step ×3 days
5 mg BID	5 mg QD	5 mg QOD	n/a
20 mg BID	20 mg QD	20 mg QOD	n/a
50 mg BID	50 mg QD	20 mg QD	20 mg QOD

Source: Study 007 protocol.
Abbreviations: BID, twice daily; n/a, not applicable; QD, once daily; QOD, every other day

In patients undergoing a GDT, the regimen detailed below and in Table 128 was to be followed. For patients on either 5 mg BID or 20 mg BID of study drug, the GDT started with reducing the frequency of dosing from their usual regimen of 1 tablet BID to 1 tablet QD for 7 days, followed by 1 tablet every other day (QOD) for 7 days prior to stopping study drug. For patients on 50 mg BID, the GDT started with reducing the frequency of dosing from their usual regimen of one tablet BID to one tablet QD for 7 days; then switched to one tablet of 20 mg QD for 7 days, followed by one tablet 20 mg QOD for 7 days prior to stopping study drug. Subjects going through a GDT were to be monitored for signs of hemolysis and worsening of anemia. If a GDT was performed to discontinue dosing permanently, subjects stopped taking the study drug after the taper was completed.

Table 128. Gradual Dose Taper Regimen, Study 007

Starting Dose	First Step ×7 days	Second Step ×7 days	Third Step ×7 days
5 mg BID	5 mg QD	5 mg QOD	n/a
20 mg BID	20 mg QD	20 mg QOD	n/a
50 mg BID	50 mg QD	20 mg QD	20 mg QOD

Source: Study 007 protocol.
Abbreviations: BID, twice daily; n/a, not applicable; QD, once daily; QOD, every other day

16. Efficacy: Additional Information and Assessment

16.1. Study 011

Study Design

Study 011 (Study 011) is an ongoing Phase 3, open-label, multicenter, extension study to evaluate the long-term safety and efficacy of mitapivat therapy in patients who were previously enrolled in Study 006 or 007. Patients were required to have completed Study 006 or 007 through Part 2 (Fixed-Dose Period), Week 24 before enrolling in Study 011. Patients who received placebo in Study 006 started treatment with mitapivat (Cohort 1); and patients who received mitapivat in Study 006 (Cohort 2) and Study 007 (Cohort 3) continued to receive treatment with mitapivat. For Cohorts 2 and 3, patients were required to have demonstrated clinical benefit from mitapivat in the antecedent study. The primary endpoint was to evaluate the long-term safety. The secondary endpoints included efficacy assessment of mitapivat as follows:

Cohort 1 Only

- 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 two or more scheduled assessments at Weeks 16, 20, and 24
- Average change from baseline in Hb concentration at Weeks 16, 20, and 24

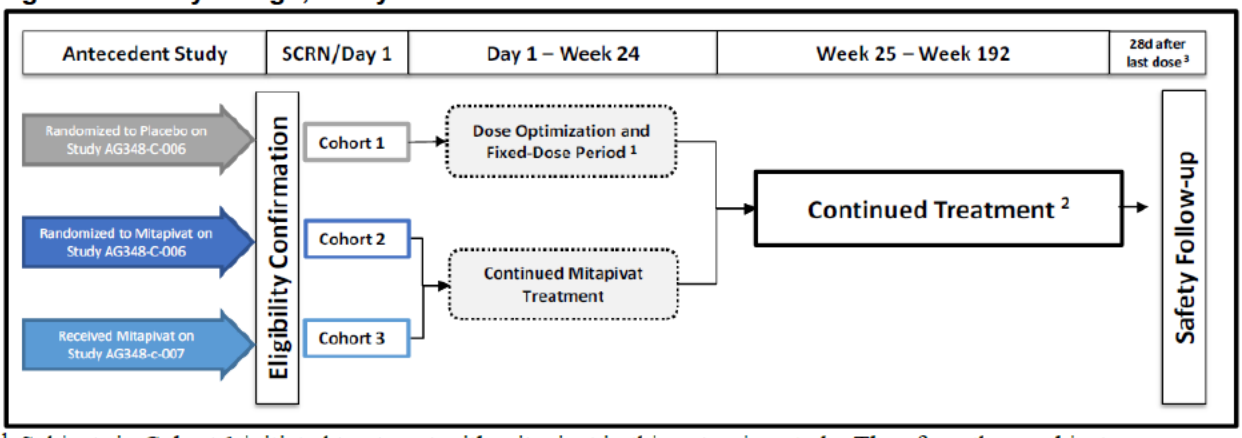
All Cohorts

- Change from baseline in Hb concentration
- Change from baseline in markers of hemolysis: 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
- Change from baseline in the number of RBC units transfused
- Change from baseline in patient-reported outcomes (PRO) scores: Pyruvate Kinase Deficiency Diary (PKDD) and Pyruvate Kinase Deficiency Impact Assessment (PKDIA)

The duration of mitapivat treatment was scheduled for up to a maximum of 192 weeks (approximately 3.7 years). For patients in Cohort 1, individual dose titration was conducted,

consistent with Study 006 to escalate to a dose of mitapivat that maximized increase in hemoglobin while maintaining an acceptable safety profile. Patients in Cohort 2 and 3 remained on the same dose that they were receiving at the end of Study 006 or 007.

Figure 50. Study Design, Study 011



[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.
Source: Study 011 Clinical Study Report.
Abbreviations: d, days; SCRN, screening

Study Results

Patient Disposition

Most of the patients who completed the antecedent Studies (i.e., 006 and 007) through Week 24 or Part 2, Week 24 enrolled in Study 011 [a total of 88 patients (Cohort 1: 36 patients, Cohort 2: 35 patients, Cohort 3: 17 patients)]. At the time of the clinical cut-off date of November 12, 2020, none of the patients completed the study. A total of 10 patients (11.4%) (Cohort 1: five patients, Cohort 2: two patients, Cohort 3: three patients) discontinued study treatment and eight patients (9.1%) (Cohort 1: four patients, Cohort 2: one patient, Cohort 3: three patients) discontinued the study prematurely, mostly due to lack of efficacy or withdrawal by the subject.

Protocol Deviations

A total of 16 patients (18.2%) had major protocol deviations: 11 patients (14.8%) were related to informed consent, 2 patients (2.3%) did not meet the selection criteria (i.e., demonstration of clinical benefit from mitapivat treatment in the antecedent study; and completion of the antecedent mitapivat study) and 2 patients (2.3%) had study treatment deviations.

Patient Demographics and Disease Characteristics

In Study 011, the median age was 36 years (range: 18, 78 years), 53 patients (60.2%) were female and 65 patients (73.9%) were white. The baseline disease characteristics were generally consistent with those in Studies 006 and 007, as shown in Table 129.

Table 129. Baseline Disease Characteristics, Full Analysis Set, Study 011

Disease Characteristic, n (%)	Cohort 1 (N=36)	Cohort 2 (N=35)	Cohort 3 (N=17)	Total (N=88)
Hemoglobin (g/dL)				
Median	8.4	8.8	8.9	8.7
Range	6.2, 10.3	6.4, 10.2	7.4, 10.8	6.2, 10.8
Hemoglobin category (g/dL)				
<8.5	19 (52.8%)	15 (42.9%)	6 (35.3%)	40 (45.5%)
PKLR gene variant				
Missense/missense	23 (63.9%)	26 (74.3%)	13 (76.5%)	62 (70.5%)
Missense/nonmissense	13 (36.1%)	9 (25.7%)	4 (23.5%)	26 (29.5%)
Ferritin (µg/L)				
Median	497	381	613	494
Range	59, 2286	21, 5890	163, 2320	21, 5890
Prior splenectomy				
Yes	26 (72.2%)	23 (65.7%)	12 (70.6%)	61 (69.3%)
Prior cholecystectomy				
Yes	27 (75.0%)	24 (68.6%)	15 (88.2%)	66 (75.0%)
Prior chelation*				
Yes	11 (30.6%)	4 (11.4%)	14 (82.4%)	29 (33.0%)

Source: ADSLSUB.xpt.
* Within 52 weeks (364 days) before the first dose of study treatment.
Abbreviations: N, total number of subjects; n, number of subjects in each category; PKLR, pyruvate kinase liver and red blood cell

Efficacy Results

Duration of Hb Response in Subjects Not Regularly Transfused

All data for subjects who achieved Hb response in Study 006 are included in the analysis below (Table 130). Hemoglobin response is defined as a ≥15 g/L increase in Hb concentration from baseline that is sustained at two or more scheduled assessments at Weeks 16, 20, and 24 during the Fixed-Dose Period in Study 006, excluding assessments within 61 days after a transfusion. These results highlight the durability of Hb response with long-term treatment with mitapivat. Hb responses ongoing at the last available Hb assessment are denoted by a "+." Duration of Hb response was calculated as follows:

- Loss of Hb response was defined to have occurred if, after the last assessment in the Fixed-Dose Period with increase in Hb ≥15 g/L from baseline, the change from baseline at the next scheduled Hb assessment was <15 g/L.
- Duration of Hb response was defined as the time from the date a subject first achieved an increase in Hb ≥15 g/L from baseline to the date of the last Hb assessment before loss of Hb response.

We can observe from Table 130:

- Hb response is ongoing for 13 of the 16 Hb responders at the last available Hb assessment.
- The shortest duration of response is 3.3 months.
- The longest duration of response is >18.4 months

Table 130. Duration of Hb Response (Hb Responders in the Full Analysis Set, Not Regularly Transfused)

Hb Response	Total (N=16)
Duration of Hb response (months)	
n (%)	13 (81.25)
Mean (SD)	8.7 (4.5)
Median (Q1, Q3)	6.9 (6.2, 11.3)
Minimum, maximum	3.3, 18.4+

Source: Applicant's response to an information request; Statistical Reviewer's analysis.
Abbreviations: Hb, hemoglobin; N, total number of subjects; n, number of subjects who maintained response; Q1, the first quartile; Q3, the third quartile; SD, standard deviation; +, ongoing Hb response

Duration of Transfusion-Free Response in Subjects Regularly Transfused

All data for subjects who were regularly transfused and achieved a transfusion-free response in Study 007 are included in the analysis (Table 131). Transfusion-free responders are subjects who did not receive RBC transfusions for a period of ≥24 weeks any time between start of treatment in Study 007 through the end of the on-treatment period (last dose date +28 days for subjects who discontinued treatment, or cutoff date for subjects ongoing treatment). These results highlight the durability of transfusion-free response with long-term treatment with mitapivat. Transfusion-free responses ongoing at the time of treatment discontinuation or, for subjects who have not discontinued, as of the cutoff date for Study 011, are denoted by a "+."

We can observe from Table 131:

- Transfusion-free response is ongoing for the six transfusion-free responders at the time of treatment discontinuation or, for subjects who have not discontinued, as of the cutoff date for Study 011.
- The shortest duration of response is >11.5 months.
- The longest duration of response is >21.8 months.

Table 131. Duration of Transfusion-Free Response (Transfusion-Free Responders in the Full Analysis Set, Regularly Transfused)

Hb Response	Total (N=6)
Duration of Hb response (months)	
n (%)	6 (100)
Mean (SD)	16.7 (4.4)
Median (Q1, Q3)	17.0 (12.5, 20.2)
Minimum, maximum	11.5+, 21.8+

Source: Applicant's response to an information request; Statistical Reviewer's analysis.
Abbreviations: Hb, hemoglobin; N, total number of subjects; n, number of subjects who maintained response; Q1, first quartile; Q3, third quartile; SD, standard deviation; +, ongoing Hb response

16.2. Supplemental Efficacy, Study AG-3480-C-003

Study 003

Study 003 was a Phase 2, open-label, two-arm, multicenter, randomized, dose-ranging study in adult subjects with PKD. The study consisted 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 were based on the highest safely tolerated dose (Arm 1: 300 mg twice daily) and the lowest dose with

potentially relevant PD activity (Arm 2: 50 mg twice daily) from the AG-348-C-002 multiple ascending dose study in healthy adult subjects.

Inclusion criteria (summarized) included patients aged 18 years and older who had a documented clinical laboratory confirmation of PKD, Hgb ≤12 g/dL if male and ≤11 g/dL if female and transfusion independent (less than or equal to three units of RBC transfusions in the 12-month period up to the first day of study drug dosing and no transfusions within 4 months before the first day of study dosing). Patients either still had their spleen or had undergone splenectomy and had an Eastern Cooperative Oncology group Performance Status of ≤2, and adequate organ function as defined in protocol.

The primary objective was to evaluate the safety and tolerability of up to 24 weeks of mitapivat administration in subjects with PKD. The endpoints include a safety evaluation and clinical activity as measured by changes in Hb, HCT, reticulocyte count, LDH, total and indirect bilirubin, erythropoietins, hepcidin, ferritin, and transferrin saturation.

In this study, hemoglobin response was defined as change from baseline in hemoglobin of ≥1.5 g/dL at >50% of the assessments in the Core Period (excluding those within 61 days after a transfusion). There were 27 patients enrolled in the mitapivat 50 mg BID arm and 25 patients in the mitapivat 300 mg BID arm, for a total of 52 patients. Of the 52 patients, 43 (83%) completed treatment in the core period and 9 discontinued treatment before completing the core period of 24 weeks. Thirty-six (69%) continued into the extension period, including 18 subjects still receiving treatment.

The median age was 34 (range 22, 43) years, with 32 males and 20 females enrolled. The median Hb level was 8.9 g/dL at baseline (range 7.7 to 9.8 g/dL) and baseline median ferritin was 764 mcg/L (range 506to 1077 mcg/L), 82% had a prior splenectomy and 25% had prior chelation therapy.

Among the 27 patients assigned to the 50 mg BID dose group, 9 (33.3%) (95% CI, 16.5, 54) achieved a hemoglobin response. In the mitapivat 300 mg BID arm, 10 of the 25 patients (40%) achieved a hemoglobin response with 95% CI (21.1, 61.3). Among the 9 patients who achieved the primary endpoint in the 50 mg BID dose group, the response rate was higher in patients who had the missense/nonmissense PK-R genotype (66.7%, 4 of 6 patients) compared to patients who had missense/missense variants (33.3%, 5 of 15 patients), which is not consistent with the results of Study 006 or 007. Study 003 did not exclude patients homozygous for the missense variant, R479H, whereas Studies 006 and 007 excluded these patients. In Study 003, all five patients with the R479H/R479H missense variant were nonresponders. However, given the small numbers in Study 003 and small sizes of Studies 006 and 007, it is difficult to reach a conclusion on this difference. There were six patients who had the nonmissense/nonmissense variants in the 50 mg BID dose group; no patients achieved the primary endpoint with this genotype. In addition, there were no responders among patients with the nonmissense/nonmissense variants (n=4) in the 300 mg BID dose group (n=25). Further discussion regarding dose and dose responsiveness can be found in Section II.6.1.

16.3. Sensitivity Analyses for Secondary Endpoints, Study 006

This section supplements the analyses and interpretation presented in Section II.6.2.2.2 regarding the sensitivity analyses by analysis of covariance (ANCOVA) for the key secondary endpoint and six other secondary endpoints that were included in the Applicant's statistical testing hierarchy for Study 006 shown in Figure 3.

The Applicant's primary analyses for the seven secondary endpoints were conducted using the mixed-effect model repeat measurement (MMRM) method, which assumes missing at random when handling missing data. To evaluate the robustness of the results, sensitivity analyses by ANCOVA were requested by the statistical review team for the seven secondary endpoints.

The Applicant's submitted ANCOVAs on the seven secondary endpoints based on the observed values (i.e., subjects with missing data were excluded). The analyses results are summarized in Table 132 and were verified by the statistical review team.

According to the Applicant's statistical testing hierarchy (Figure 3), the Hochberg testing procedure was used for the lactate dehydrogenase, haptoglobin, PKDD, and PKDIA endpoints with a two-sided α=0.05. Four two-sided p-values for lactate dehydrogenase, haptoglobin, PKDD and PKDIA endpoints were ordered from largest to smallest and compared to a set of critical values. The comparisons were conducted sequentially by comparing the largest p-value to α=0.05, then the second-largest p-value to $\alpha/2$=0.025, then the third largest p-value to $\alpha/3\approx$0.0167, and the fourth largest p-value to $\alpha/4$=0.0125, until a p-value for an endpoint is smaller than the corresponding critical value, whereupon the Hochberg procedure provides a conclusion on the statistical significance of the effect of mitapivat compared to placebo for that endpoint and all endpoints with smaller p-values. The two-sided p-value for the lactate dehydrogenase, haptoglobin, PKDD, and PKDIA endpoints by ANCOVA based on the observed values were 0.013, 0.011, 0.079, and 0.034, respectively. Thus, the two-sided p-values were no longer nominally significant for the PKDD and PKDIA endpoints by ANCOVA based on the observed values.

Table 132. Sensitivity Analysis of Secondary Endpoints by ANCOVA With Observed Values, Full Analysis Set, Study 006

Visit	Mitapivat N=40	Placebo N=40
Hemoglobin (g/dL)		
Average of Weeks 16, 20, and 24		
Change from baseline		
n	39	37
LS Mean (SE)	1.6 (0.2)	-0.1 (0.2)
95% CI	(1.2, 2.1)	(-0.6, 0.3)
Difference in LS mean (SE) (mitapivat-placebo)		1.8 (0.3)
95% CI		(1.2, 2.4)
Two-sided p-value		<0.0001

Visit	Mitapivat N=40	Placebo N=40
Indirect bilirubin (mg/dL)		
Average of Weeks 16, 20, and 24		
Change from baseline		
n	34	36
LS Mean (SE)	-1.1 (0.3)	0.2 (0.2)
95% CI	(-1.6, -0.6)	(-0.2, 0.7)
Difference in LS mean (SE) (mitapivat-placebo)		-1.3 (0.3)
95% CI		(-2.0, -0.7)
Two-sided p-value		0.0002
Reticulocyte percentage (Fraction of 1)		
Average of Weeks 16, 20, and 24		
Change from baseline		
n	39	37
LS Mean (SE)	-0.09 (0.014)	0.01 (0.01)
95% CI	(-0.12, -0.06)	(-0.02, 0.04)
Difference in LS mean (SE) (mitapivat-placebo)		-0.10 (0.02)
95% CI		(-0.14, -0.06)
Two-sided p-value		<0.0001
Lactate dehydrogenase (U/L)		
Average of Weeks 16, 20, and 24		
Change from baseline		
n	38	37
LS Mean (SE)	-80 (15)	-29 (15)
95% CI	(-109, -51)	(-58, 0)
Difference in LS mean (SE) (mitapivat-placebo)		-51 (20)
95% CI		(-91, -11)
Two-sided p-value		0.013
Haptoglobin (g/dL)		
Average of Weeks 16, 20, and 24		
Change from baseline		
n	39	37
LS Mean (SE)	0.0148 (0.00435)	-0.0005 (0.00431)
95% CI	(0.0061, 0.0234)	(-0.0091, 0.0081)
Difference in LS mean (SE) (mitapivat-placebo)		0.0152 (0.00587)
95% CI		(0.0035, 0.0269)
Two-sided p-value		0.011
PKDD		
Week 24		
Change from baseline		
n	36	31
LS Mean (SE)	-4.6 (1.0)	-2.2 (1.0)
95% CI	(-6.6, -2.7)	(-4.2, -0.1)
Difference in LS mean (SE) (mitapivat-placebo)		-2.5 (1.4)
95% CI		(-5.3, 0.3)
Two-sided p-value		0.079

Visit	Mitapivat N=40	Placebo N=40
PKDIA		
Week 24		
Change from baseline		
n	39	34
LS Mean (SE)	-4.5 (1.2)	-1 (1.2)
95% CI	(-6.8, -2.2)	(-3.4, 1.5)
Difference in LS mean (SE) (mitapivat-placebo)		-3.5 (1.6)
95% CI		(-6.8, -0.3)
Two-sided p-value		0.034

Source: Applicant's response to an Information Request; Statistical Reviewer's analysis.
For hemoglobin, indirect bilirubin, reticulocyte percentage, lactate dehydrogenase and haptoglobin, the estimates, 95% CIs, and p-value were based on ANCOVA, which included change from baseline at Weeks 16, 20, and 24 as the dependent variable, baseline as a covariate, and treatment group and the randomization stratification factors as fixed factors.
For PKDD and PKDIA, the estimates, 95% CIs, and p-values were based on ANCOVA, which included change from baseline at Week 24 as the dependent variable, baseline as a covariate, and treatment group and the randomization stratification factors as fixed factors.
Abbreviations: ANCOVA, analysis of covariance; CI, confidence interval; LS, least square; N, total number of subjects in each treatment arm; n, number of subjects who attended the visit; PKDIA, Pyruvate Kinase Deficiency Impact Assessment; PKDD, Pyruvate Kinase Deficiency Diary; SE, standard error

However, ANCOVA with only observed values can be biased due to the relatively small sample size of the study and missing data. To further evaluate the robustness of the results, sensitivity analyses by ANCOVA with multiple imputation via a control-based pattern-mixture model were performed by the Applicant and the Statistical Reviewer for the PKDD and PKDIA endpoints (Table 133). Results for PKDD (nominal p=0.015) and PKDIA (nominal p=0.044) analyzed by ANCOVA-multiple imputation (MI) using T-scores were nominally statistically significant, which is consistent with the primary analysis results by MMRM. Note that although the results of PKDIA reached nominal statistical significance, the review team has concern over the clinical meaningfulness of PKDIA. See Section II.6.3.1 for a detailed explanation.

Table 133. Analysis of Change From Baseline in PKDD Weekly Mean Score and PKDIA Score at Week 24 by ANCOVA With Multiple Imputation, Full Analysis Set, Study 006

Endpoint	LS Mean (SE) (Mitapivat)	LS Mean (SE) (Placebo)	LS Mean Difference (SE) (Mitapivat-Placebo)	2-Sided P-value
PKDD	-4.9 (1. 1)	-1.3 (1.1)	-3.6 (1.5)	0.015
PKDIA	-4.4 (1.2)	-1.2 (1.2)	-3.3 (1.6)	0.044

Source: Applicant's response to an Information Request; Statistical Reviewer's analysis.
Notes: The estimates and p-values are based on ANCOVA, which includes the change from baseline at Week 24 as the dependent variable, baseline as a covariate, and treatment group and the randomization stratification factors as fixed factors.
Control-based pattern-mixture model was used for multiple imputation.
Abbreviations: ANCOVA, analysis of covariance; LS, least squares; PKDIA, pyruvate kinase deficiency impact assessment; PKDD, pyruvate kinase deficiency diary; SE, standard error

The T-score analyses were conducted for average scores of several items, for each instrument and were not comparable to the individual raw scores. To further assess the PRO endpoints as discussed in Sections II.6.3.1 and 16.4, the Applicant was requested to provide the changes at Week 24 from baseline of the individual items of the PKDD instrument (based on weekly average raw scores) analyzed by both MMRM and ANCOVA with multiple imputation (ANCOVA-MI) via a control-based pattern-mixture model. The weekly raw score analyses results based on MMRM are listed in Table 32, and the analyses results based on ANCOVA-MI are listed in Table 134.

Table 134. Analysis of Covariance With Multiple Imputation Using a Control-Based Pattern-Mixture Model for Change From Baseline in PKDD Weekly Raw Score at Week 24 Based on Average Daily Raw Total Scores for Each Individual Item, Full Analysis Set, Study 006

PKDD Parameter	Placebo LS Mean (SE)	Mitapivat LS Mean (SE)	Difference in LS Mean (SE) (Mitapivat-Placebo)
Tired worst (0-10)	-0.2 (0.3)	-1.3 (0.3)	-1.0 (0.4)
Daily activities (0-10)	-0.5 (0.3)	-1.2 (0.3)	-0.8 (0.4)
Jaundice (0-4)	-0.1 (0.1)	-0.4 (0.1)	-0.3 (0.1)
Bone pain worst (0-10)			
Multiple imputation [1]	-0.1 (0.2)	-0.1 (0.2)	0.0 (0.3)
Multiple imputation [2]	-0.1 (0.2)	-0.1 (0.2)	0.0 (0.3)
Breathe activities (0-10)	-0.5 (0.2)	-0.6 (0.2)	-0.1 (0.3)
Energy beginning (0-10)	-0.5 (0.3)	-0.3 (0.3)	0.3 (0.4)
Energy end (0-10)	-0.4 (0.3)	-0.7 (0.3)	-0.2 (0.4)

Source: Applicant's response to information request dated December 15, 2021; Statistical Reviewer's analysis.
Results are based on an ANCOVA that includes the change from baseline in the individual PKDD item at Week 24 as the dependent variable, baseline PKDD item value as a covariate, and treatment group and the randomization stratification factors as fixed factors. Control-based pattern-mixture model was used for multiple imputation.
Baseline for each individual PKDD item 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.
[1] Due to singular covariance matrix, imputation performed including scores at baseline and scheduled visits on or after Week 10.
[2] Due to singular covariance matrix, imputation performed including scores at baseline, Week 2, and alternative weeks thereafter (i.e., Week 4, Week 6, etc.).
Abbreviations: ANCOVA, analysis of covariance; LS, least squares; PKDD, pyruvate kinase deficiency diary; PMM, pattern-mixture model; SE, standard error

For the individual PKDD item analyses based on raw score, although the differences in least squares mean (mitapivat − placebo) were small (by MMRM analysis), there was a general tendency of more improvement of the PKDD items in the mitapivat arm compared to those in the placebo arm. The ANCOVA-MI results were generally consistent with the MMRM results.

16.4. DCOA Review Summary of the PROs

Study 006 used the following PRO assessments as secondary endpoints to assess the signs and symptoms of PKD and their associated impacts:

- PKDD
- PKDIA

The PKDD was collected daily during Screening (up to 6 weeks) and daily throughout Part 1 and Part 2 (24 weeks). During Part 1 and Part 2 of Study 006, the PKDIA was collected at Weeks 4, 8, 12, and 24. In addition, the PKDIA was collected at Weeks 16 and 20.

The PKDD is a seven-item PRO instrument designed to assess the signs and symptoms of pyruvate kinase deficiency. Items are rated on multiple response scales. Six items are rated using an 11-point numeric rating scale ranging from 0 ("No or not at all sign/symptom") to 10 ("Extremely or worst possible or high sign/symptom). One item (Item 3, jaundice) is rated on a five-point verbal rating scale that ranges from 0 ("No yellow eyes and/or skin") to 4 ("Very severe yellow eyes and/or skin"). Item 4 (bone pain) also contains a "Not experience" response option for participants who have never experienced this symptom. Item 5 (shortness of breath) has a "Not applicable" response option, as well as an option for those participants who avoided the activity due to the inability to perform moderate physical activity. The recall period is "today (from the time you woke up this morning to the time you are completing this questionnaire)." A copy of the instrument is provided in Appendix A. The PKDD generates a weekly average T-

score based on a raw score to T-score transformation table. The T-score ranges from 25 to 76, with higher scores indicating greater symptom severity. For the endpoint, a weekly average score was computed. The weekly average scoring includes Items 1, 2, 3, 4, 5, 6, and 7. Additional details regarding the scoring algorithm can be found in Appendix C.

The PKDIA is an eight-item PRO instrument designed to assess the impacts of pyruvate kinase deficiency. Seven items are rated on an 11-point numeric rating scale ranging from 0 ("None of the time" or "Not at all difficult") to 10 ("All of the time" or "Extremely difficult"). One item (Item 8, need for additional rest or sleep) uses a five-point verbal rating scale that ranges from 0 ("No additional rest or sleep") to 4 ("A lot of additional rest or sleep"). The recall period is the previous 7 days. A copy of the instrument is provided in Appendix B. The PKDIA generates a T-score based on a raw sum score to T-score transformation table. The T-score ranges from 30 to 76, with higher scores indicating greater negative impact. For the endpoint, a weekly average score was computed. PKDIA scoring includes Items 2, 5, 6, 7, 8, 10, 11b, and 12 (item numbering from original version of PKDIA).

The results of Study 006 demonstrated that mitapivat had statistically significant improvement in the selected secondary efficacy PRO endpoints compared with placebo. Despite achievement of statistical significance in both instruments, there are concerns regarding the T-scores used to support the PKDD and PKDIA endpoint definitions. Specifically, the Division of Clinical Outcome Assessment (DCOA) review team does not agree with the Applicant's methodology used to derive both the T-scores and the raw score to T-score transformation tables for PKDD and PKDIA, which resulted in challenges with interpreting the T-scores and corresponding PKDD and PKDIA endpoint results. Further, there were minimal observed score changes, if any, in the item-level data at the raw score scale (e.g., some item scores reflect less than one category change on the raw score scale).

(1) The PKDD and PKDIA were reviewed for content validity and other measurement properties as discussd below.

Issue 1: Content Validity

Both instruments measure important aspects of PKD symptoms and associated functional impacts. However, the relevancy of some of the items is questionable based on the qualitative and quantitative data. For example:

- Based on item-level distributional data from Study 006, the PKDD had skewed responses towards the lower ends of the response options, where 3.2 to 53.1% of participants responded with the least severe category at baseline across the items in the PKDD (i.e., participants reporting "not at all" to the experience of the symptom). This observed skewness was most extreme for Items 3 (jaundice) and 4 (bone pain). Qualitative data also showed that bone pain was less relevant and experienced by fewer participants than the other signs and symptoms.
- Similarly, PKDIA had skewed responses towards the lower ends of the response options, where 10.3% to 34.6% of participants selected the least severe category responses at baseline across the items in the PKDIA (i.e., participants reporting "none of the time/not at all" regarding the experience or difficulty with functioning).
- The PKDIA includes concepts that might be influenced by factors beyond the treatment (i.e., interference with leisure activities, negative impact on relationships, additional rest,

or sleep) and may not accurately describe clinical benefit. The regulatory utility of these concepts is unclear.

Issue 2: Other Measurement Properties

- It is difficult to fully evaluate the psychometric properties of the PKDD and PKDIA because of the scoring approach used. Based on the skewness observed in the PKDD and PKDIA item-response distributions, the Applicant collapsed response options for select items rated 0 to 10 to result in five response options and summed the items into a daily sum score. The daily sum score was subsequently converted to a T-score. The Applicant has not provided adequate justification for their scoring approach. The psychometric analyses were subsequently performed using this scoring approach. As such, the reliability and validity of the T-scores created from PKDD and PKDIA are unable to be verified because of the limitations of the scoring approach.

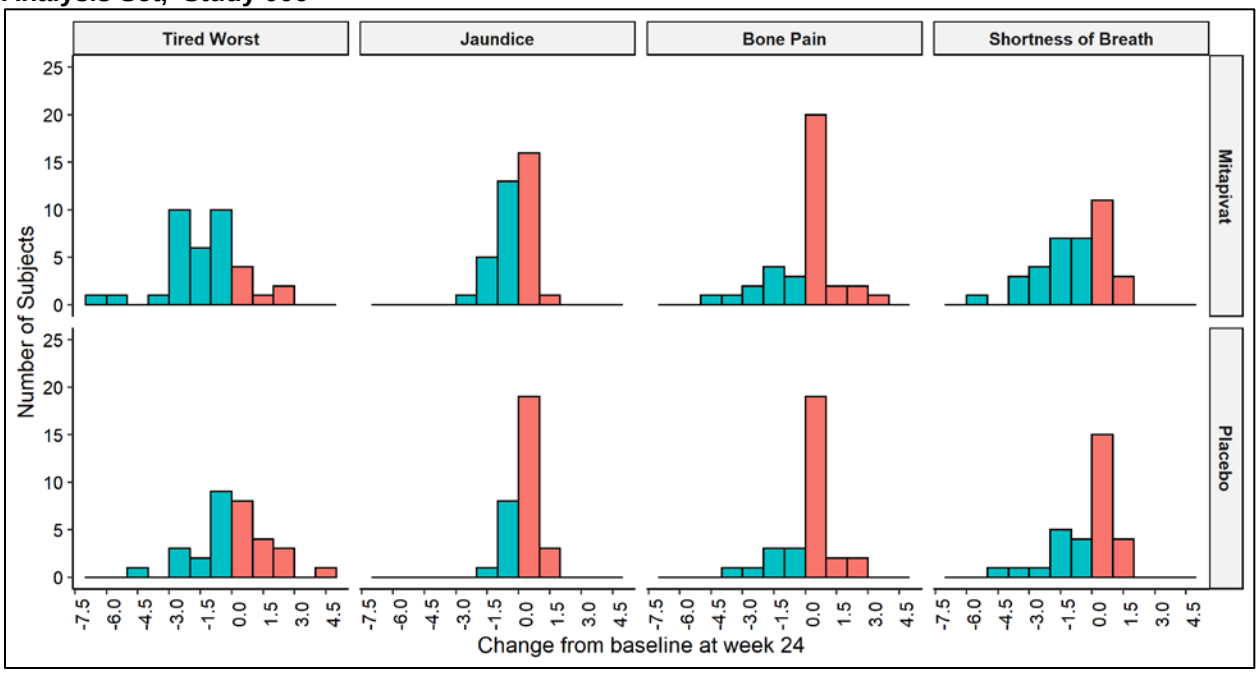
Issue 3: Data Interpretability

- The scoring algorithms adopt multiple score transformations, making it difficult to interpret the final score. Further, the small sample size (n=80) can result in large measurement error in item response theory item calibration, contributing to pervasive uncertainties in item response theory scoring.
-
- Based on the response distributions, minimal change is observed at the item-level in the raw score scale of the PKDD and PKDIA. Because there was minimal change, it is difficult to assess its clinical meaningfulness. Further, the external anchor used for anchor-based analyses has limitations (i.e., the measurement concept of the external anchor is the global impact of PKD ["PK deficiency affected me"] and not individually related to PKD symptoms or functional impacts, specifically).

If PRO data are labeled from the PKDD, DCOA recommends a description of the items driving the total score to avoid inclusion of misleading claims and highlight important limitations regarding the interpretability of the data. Refer to the DCOA review by Dr. Mira Patel, dated December 23, 2021.

The clinical and statistical team conducted sensitivity analyses evaluating the raw scores for the PKDD and PKDIA using the ANCOVA and MMRM. Study 006 enrolled patients with PKD who were not transfusion-dependent (required less frequent transfusions), however, they still had symptoms and signs of the disease at baseline that affected how they function and feel. As shown from the following histograms (Figures 50, there was an improvement in tiredness, shortness of breath, and jaundice in the treatment group compared to the control group, but not bone pain. The histograms are based on the observed data without any imputation. These symptoms are common symptoms of anemia and ineffective erythropoiesis and frequently reported in patients with PKD. The improvement in these symptoms reflects the treatment effect of mitapivat and impacts and improves how the patients function and feel and is a clinically meaningful benefit for these patients. For additional discussion, see Sections II.6.2, II.6.3, and 16.3.

Figure 51. Change From Baseline to Week 24 for Core Sign and Symptom Items of PKDD, Full Analysis Set, Study 006



Source: FDA analyses
Note that blue bars are for improvements and red bars are for worsening.

Figure 52. Appendix A: PKDD

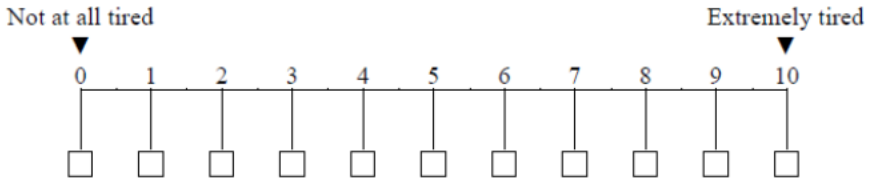
Instructions: Please answer the following questions based on your experience with Pyruvate Kinase (PK) Deficiency **today (from the time you woke up this morning to the time you are completing this questionnaire)**. Please select the box corresponding to the number that best describes you.

1. Describe how **tired** you were at its worst today.

Not at all tired — Extremely tired

0 1 2 3 4 5 6 7 8 9 10

2. Describe how **tired you felt after finishing your daily activities (e.g., work, social, leisure, physical or household activities)** today.



3. Describe your **jaundice** (how **yellow your eyes and/or skin** appeared) when you looked in the mirror today.

☐0 No yellow eyes and/or skin

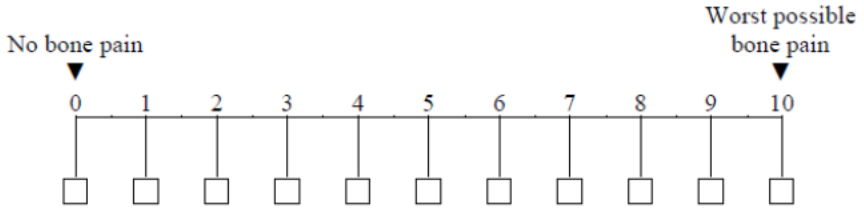
☐1 Mild yellow eyes and/or skin

☐2 Moderate yellow eyes and/or skin

☐3 Severe yellow eyes and/or skin

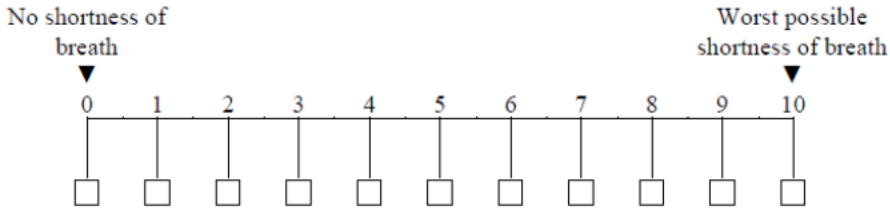
☐4 Very severe yellow eyes and/or skin

4. Describe your **bone pain** at its worst today.



☐ I have never experienced bone pain

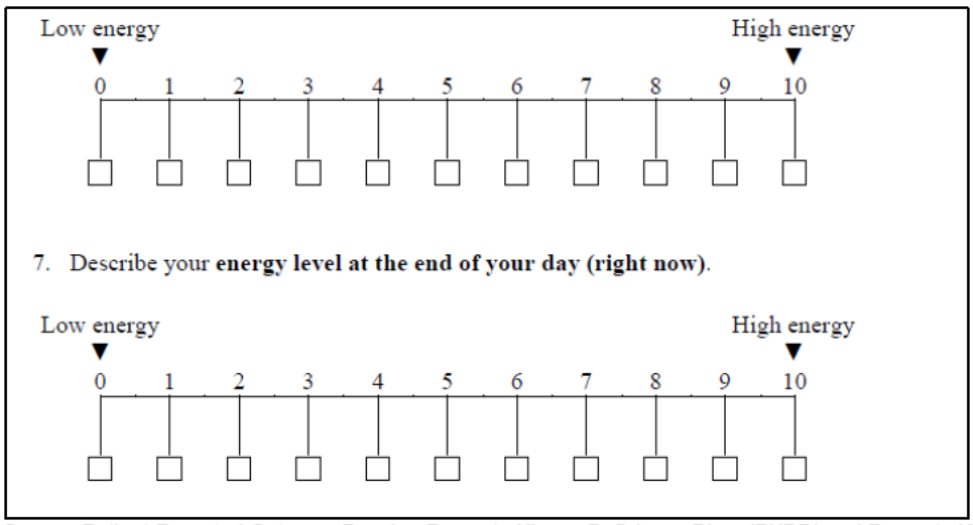
5. Describe your **shortness of breath** during moderate (e.g., walking on an incline or upstairs) physical activity you did today.



☐ I avoided this activity because it was too difficult for me to do moderate physical activity

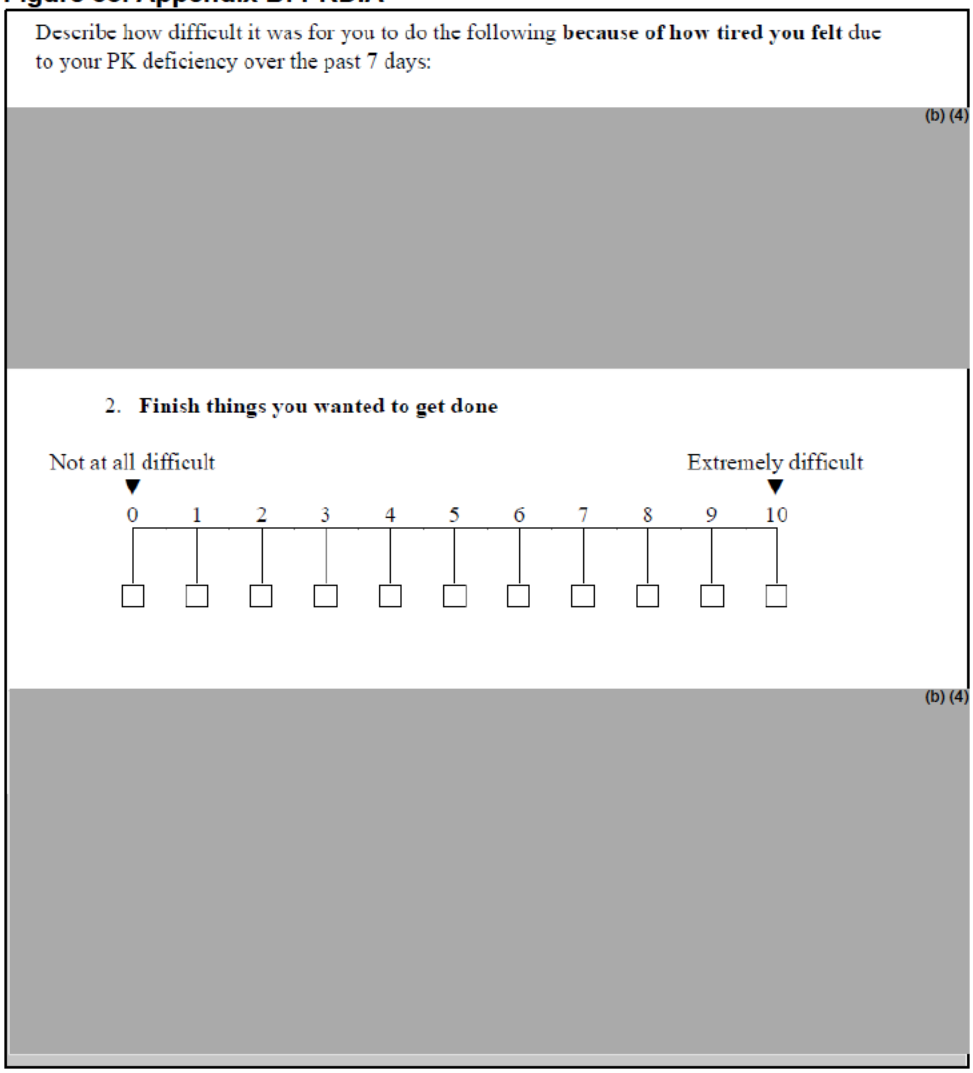
☐ Not applicable, because I did not have the opportunity to do moderate physical activity

6. Describe your **energy level at the beginning of your day (after being awake for one hour)**.



Source: Patient-Reported Outcome Dossier: Pyruvate Kinase Deficiency Diary (PKDD) and Pyruvate Kinase Impact Assessment (PKDIA).

Figure 53. Appendix B: PKDIA



(b) (4)

5. How often did your PK deficiency interfere with your ability to do **household activities (e.g., chores, cleaning, laundry)** over the past 7 days?

None of the time ▼ All of the time ▼

0 1 2 3 4 5 6 7 8 9 10

6. How often did a lack of energy due to your PK deficiency interfere with participating in **social activities (e.g., doing something together with friends)** over the past 7 days?

None of the time ▼ All of the time ▼

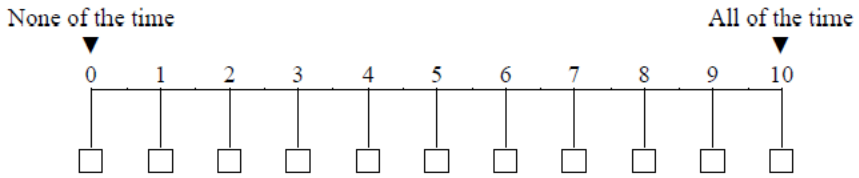
0 1 2 3 4 5 6 7 8 9 10

7. How often did your PK deficiency interfere with **leisure activities (i.e., hobbies or things you do for fun in your free time) over the past 7 days**?

None of the time ▼ All of the time ▼

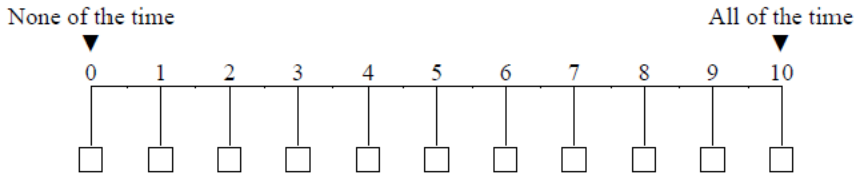
0 1 2 3 4 5 6 7 8 9 10

8. How often did you feel **your relationships with friends or family were negatively affected** because of your PK deficiency over the past 7 days?



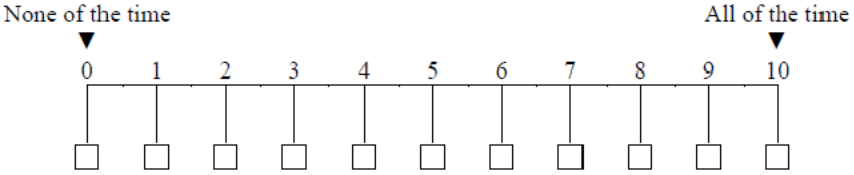
(b) (4)

10. How often did you have **difficulty concentrating** because of your PK deficiency over the past 7 days?



(b) (4)

11b [redacted] (b) (4) How often did you have difficulty performing **moderate (e.g., walking on an incline or up stairs) physical activity** because of your PK deficiency over the past 7 days?



12. How much **additional rest or sleep did you feel you needed** because of your PK deficiency over the past 7 days?

- ☐0 No additional rest or sleep
- ☐1 A little additional rest or sleep
- ☐2 Some additional rest or sleep
- ☐3 Quite a bit additional rest or sleep
- ☐4 A lot of additional rest or sleep

Source: Patient-Reported Outcome Dossier: Pyruvate Kinase Deficiency Diary (PKDD) and Pyruvate Kinase Impact Assessment (PKDIA)
Items not included in scoring are presented in gray.
Abbreviation: PK, pyruvate kinase

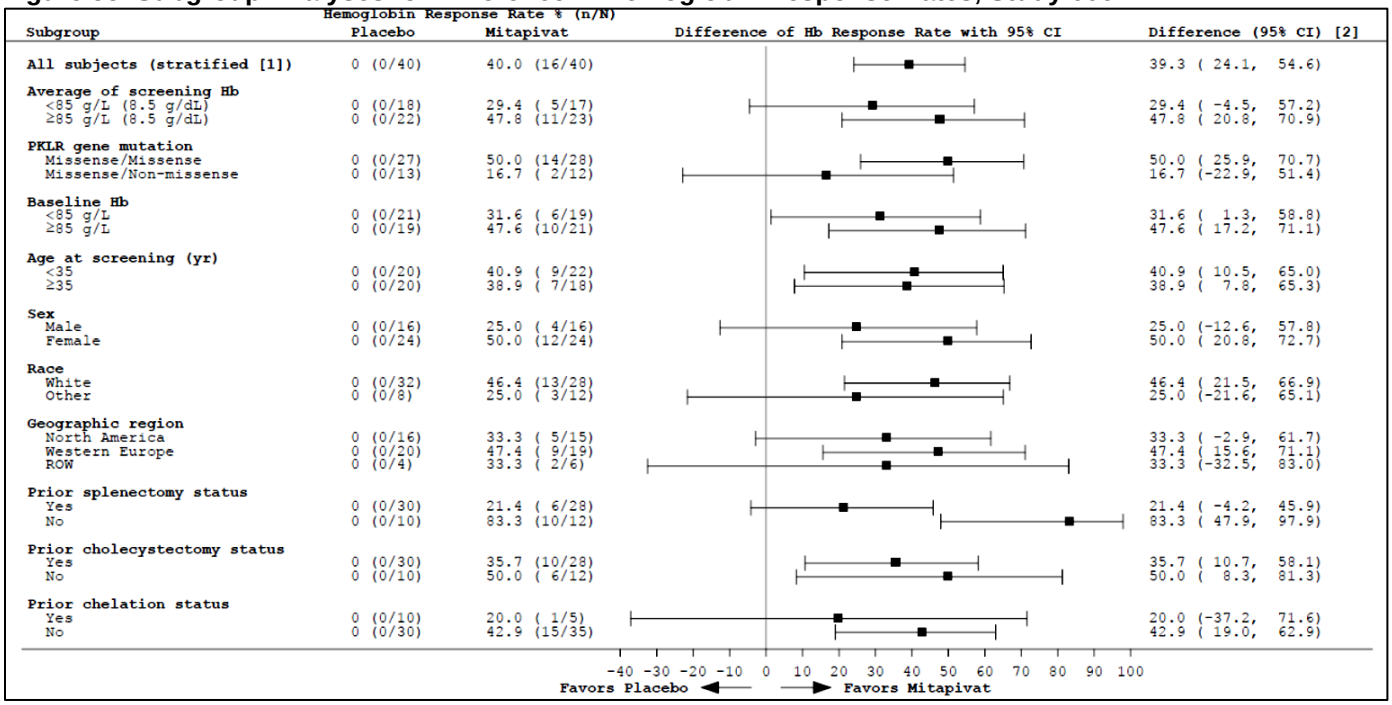
3 Pages have been Withheld in Full as b4 (CCI/TS) immediately following this page

(b) (4)

16.5. Subgroup Analyses for the Primary Endpoint, Study 006

The section supplements the analyses and interpretation presented in Section II.6.2.2.3. Several subgroup analyses were performed to determine whether there are baseline demographic or clinical characteristics that affect the primary endpoint (Hb response). The subgroup analyses results are summarized in Figure 55. Overall, the treatment effect of mitapivat compared to placebo was consistent across baseline demographics and clinical characteristics subgroups of average of screening Hb, PKLR gene mutation, baseline Hb, age at screening (years), sex, race, geographic region, prior splenectomy status, prior cholecystectomy status, and prior chelation status. Of note, these subgroup analyses are limited by the small numbers of patients, and so the results should be interpreted with caution.

Figure 55. Subgroup Analyses for Difference in Hemoglobin Response Rates, Study 006

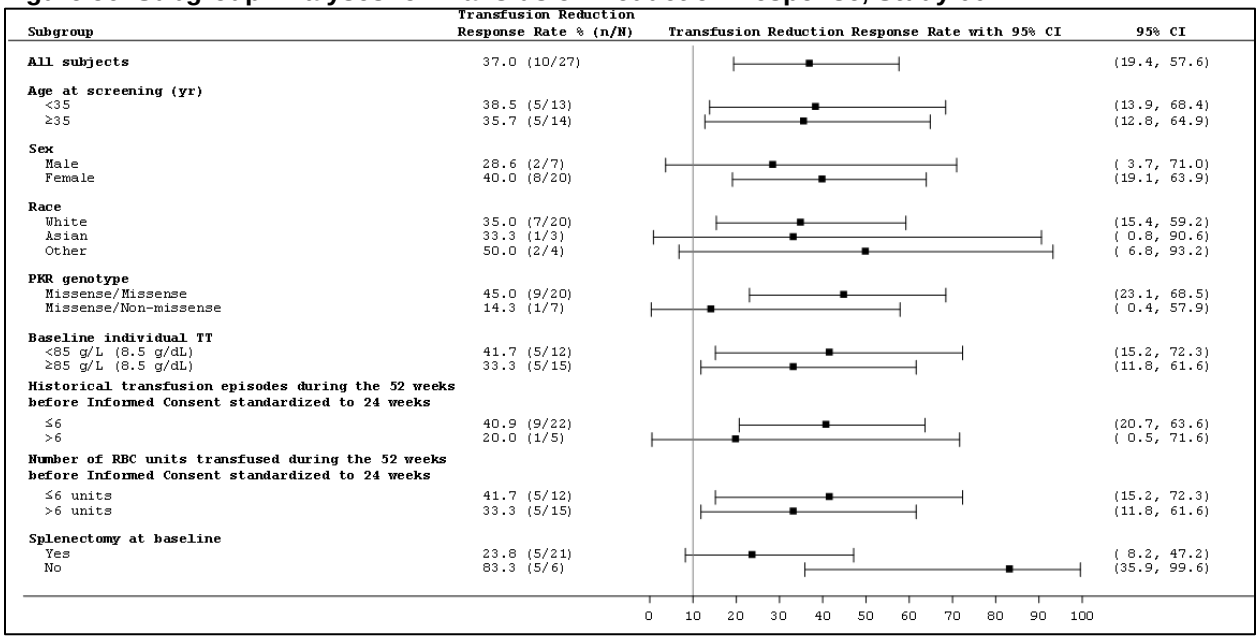


Source: Study 006 Clinical Study Report, Figure 7 (p. 63); Statistical Reviewer's analysis.
N is the number of subjects in the Full Analysis Set within each subgroup category and treatment arm.
Prespecified subgroups with ≤10% of the subjects in the FAS were pooled (race: Asian and Other were pooled).
[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.
Abbreviations: CI, confidence interval; Hb, hemoglobin; IXRS, interactive response technology; PKLR, pyruvate kinase liver and red blood cell

16.6. Subgroup Analyses for the Primary Endpoint, Study 007

The section supplements the analyses and interpretation presented in Section II.6.2.4.3. Several subgroup analyses were performed to identify whether there were any baseline demographic or clinical characteristics impacting the primary endpoint (TRR). The subgroup analyses results are shown in Figure 56. TRRs were identified in all prespecified subgroups across baseline demographics and disease characteristics subgroups of age at screening (years), sex, race, PKR genotype, baseline individual transfusion trigger, historical transfusion episodes during the 52 weeks before informed consent standardized to 24 weeks, number of RBC units transfused during the 52 weeks before informed consent standardized to 24 weeks, and splenectomy at baseline. Of note, these subgroup analyses are limited by the small numbers of patients, and so the results should be interpreted with caution.

Figure 56. Subgroup Analyses for Transfusion Reduction Response, Study 007



Source: Study 007 Clinical Study Report, Figure 2 (p. 50); Statistical Reviewer's analysis.
N is the number of subjects in each subgroup.
Transfusion reduction responders: Subjects with a ≥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.
Abbreviations: CI, confidence interval; PK-R, red blood cell isoform of pyruvate kinase; RBC, red blood cell; TT, transfusion trigger

16.7. Analyses of Other Secondary Endpoints, Study 007

The section summarizes the Applicant's efficacy results for the secondary endpoints in Study 007 that were not included in the statistical testing hierarchy or labeling.

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

Table 135. Annualized RBC Units Transfused, Full Analysis Set, Study 007

Annualized RBC Units Transfused	Total N=27
Annualized historical RBC units transfused[1]	
n	27
Mean (SD)	16.6 (8.6)
Median (Q1, Q3)	15.0 (11.0, 21.0)
Min, max	6.0, 44.0
Annualized total number of RBC units transfused (up to EOS)[2]	
n	27
Mean (SD)	11.4 (10.8)
Median (Q1, Q3)	8.7 (2.5, 18.5)
Min, max	0.0, 45.0

Annualized RBC Units Transfused	Total N=27
Reduction from annualized historical RBC units transfused (up to EOS)[2]	
n	27
Mean (SD)	5.3 (5.7)
Median (Q1, Q3)	3.4 (1.6, 9.7)
Min, max	-2.5, 18.1
Percentage reduction from annualized historical RBC units transfused (up to EOS)[2]	
n	27
Mean (SD)	38.6 (38.8)
Median (Q1, Q3)	26.5 (10.5, 78.5)
Min, max	-16.1, 100.0
Percentage 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)
Annualized total number of RBC units transfused (up to end of Fixed-Dose Period)[3]	
n	27
Mean (SD)	11.5 (10.5)
Median (Q1, Q3)	10.4 (2.6, 19.0)
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)	5.1 (5.8)
Median (Q1, Q3)	3.4 (1.34, 9.7)
Min, max	-3.2, 18.1
Percentage reduction from annualized historical RBC units transfused (up to end of Fixed-Dose Period)[3]	
n	27
Mean (SD)	36.6 (39.2)
Median (Q1, Q3)	20.6 (5.5, 78.5)
Min, max	-19.7, 100.0
Percentage 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: Study 007 Clinical Study Report, Table 11 (p. 45).
[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].
Abbreviations: EOS, end of study; max, maximum; min, minimum; N, total number of subjects; n, number of subjects in each category; Q1, first quartile; Q3, third quartile; RBC, red blood cells; SD, standard deviation

Transfusion Episodes During the Fixed-Dose Period Compared With the Standardized Control Period

Table 136. Number of Transfusion Episodes Standardized to 24 Weeks, Full Analysis Set, Study 007

Transfusion Episodes	Total N=27
Number of historical transfusion episodes	
n	27
Mean (SD)	4.5 (1.7)
Median (Q1, Q3)	4.2 (2.8, 5.1)
Min, max	2.8, 7.8
Number of transfusion episodes during the Fixed-Dose Period	
n	26
Mean (SD)	2.9 (2.7)
Median (Q1, Q3)	2.9 (0.00, 3.9)
Min, max	0.0, 9.0
Reduction from number of historical transfusion episodes	
n	26
Mean (SD)	1.6 (1.9)
Median (Q1, Q3)	1.2 (-0.1, 2.8)
Min, max	-1.6, 5.4
Percentage reduction from number of historical transfusion episodes	
n	26
Mean (SD)	39.6 (44.4)
Median (Q1, Q3)	21.3 (-0.8, 100.0)
Min, max	-22.3, 100.0

Source: Study 007 Clinical Study Report, Table 12 (p. 47).
Notes: The number of historical transfusion episodes and the number of transfusion episodes during the Fixed-Dose Period are standardized to 24 weeks.
Transfusions received over up to 3 consecutive days are counted as one episode.
Abbreviations: max, maximum; min, minimum; N, total number of subjects; n, number of subjects in each category; Q1, first quartile; Q3, third quartile; SD, standard deviation

Achievement of a Normal Hemoglobin Concentration

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 (Table 137).

Table 137. Summary of Subjects Who Achieved Normal Hb Concentrations, Full Analysis Set, Study 007

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

Source: Study 007 Clinical Study Report, Table 13 (p. 48).
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 the Clopper-Pearson method.
Abbreviations: CI, confidence interval; Hb, hemoglobin; N, total number of subjects; n, number of subjects in each category

17. Clinical Safety: Additional Information and Assessment

17.1. Safety Analyses by Demographic Subgroups

Safety Analyses by Age

In Studies 006 and 007, the number of patients who were ≥65 years of age (Study 006: four patients [mitapivat: two patients, placebo: two patients], Study 007: one patient) was too small to conduct a meaningful safety analysis by age. However, in Study 006 in patients younger than 65 years, the incidences of TEAEs and serious TEAEs were similar in the two arms, and largely consistent with those in Study 007.

Table 138. Safety Analysis by Age, Safety Analysis Set, Studies 006 and 007

	Study 006				Study 007	
	Mitapivat (N=40)		Placebo (N=39)		Mitapivat (N=27)	
Safety Analysis	<65 Yrs (N=38) n (%)	≥65 Yrs (N=2) n (%)	<65 Yrs (N=37) n (%)	≥65 Yrs (N=2) n (%)	<65 Yrs (N=26) n (%)	≥65 Yrs (N=1) n (%)
All TEAEs	33 (86.8)	2 (100)	34 (91.9)	1 (50)	26 (100)	1 (100)
Serious TEAEs	3 (7.9)	1 (50)	2 (5.4)	0	3 (11.5)	0
TEAEs leading to withdrawal	0	0	0	0	0	0
TEAEs leading to death	0	0	0	0	0	0

Source: ADAE.xpt.
Abbreviations: TEAE, treatment-emergent adverse event; N, total number of subjects in each study arm and age group; n, number of subjects with adverse event

Safety Analyses by Sex

In Study 006, the incidences of TEAEs (female: 87.5%, male: 87.5%) and serious TEAEs (female: 12.5%, male: 8.3%) were similar between females and males in the mitapivat arm, and similar to those reported in Study 007, as shown in Table 139.

Table 139. Safety Analysis by Sex, Safety Analysis Set, Studies 006 and 007

	Study 006				Study 007	
	Mitapivat (N=40)		Placebo (N=39)		Mitapivat (N=27)	
Safety Analysis	Male (N=16) n (%)	Female (N=24) n (%)	Male (N=15) n (%)	Female (N=24) n (%)	Male (N=7) n (%)	Female (N=20) n (%)
All TEAEs	14 (87.5)	21 (87.5)	14 (93.3)	21 (87.5)	7 (100)	20 (100)
Serious TEAEs	2 (12.5)	2 (8.3)	1 (6.7)	1 (4.2)	1 (14.3)	2 (10.0)
TEAEs leading to withdrawal	0	0	0	0	0	0
TEAEs leading to death	0	0	0	0	0	0

Source: ADAE.xpt.
Abbreviations: TEAE, treatment-emergent adverse event; N, total number of subjects in each study arm; n, number of subjects with adverse event

In Study 006, among patients who received mitapivat, the incidences of TEAEs that occurred more frequently (≥10% difference) in males compared to females included leukopenia (12.5% versus 0%), arrhythmia (12.5% versus 0) and nasopharyngitis (25% versus 12.5%); whereas diarrhea (0% versus 16.7%), back pain (6.2% versus 20.8%), dizziness (6.2% versus 16.7%) and headache (6.2% versus 20.8%) occurred more frequently (≥10% difference) in females. Because

the numbers of males and females are small, these results, however, should be interpreted with caution.

Table 140. TEAEs by Gender With ≥10% Difference Between Males and Females in the Mitapivat Arm, FDA Medical Query (Narrow), Safety Analysis Set, Study 006

	006-Mitapivat			006-Placebo		
System Organ Class FMQ (Narrow)	Male N=16 n (%)	Female N=24 n (%)	Risk Difference (%) (95% CI)	Male N=15 n (%)	Female N=24 n (%)	Risk Difference (%) (95% CI)
Blood and lymphatic system disorders						
Leukopenia	2 (12.5)	0	12.5 (-3.7, 28.7)	0	0	0 (0, 0)
Cardiac disorders						
Arrhythmia	2 (12.5)	0	12.5 (-3.7, 28.7)	0	0	0 (0, 0)
Gastrointestinal disorders						
Diarrhea	0	4 (16.7)	-16.7 (-31.6, -1.8)	3 (20.0)	4 (16.7)	3.3 (-21.8, 28.5)
Infections and infestations						
Nasopharyngitis	4 (25.0)	3 (12.5)	12.5 (-12.5, 37.5)	1 (6.7)	9 (37.5)	-30.8 (-54.0, -7.7)
Musculoskeletal and connective tissue disorders						
Back pain	1 (6.2)	5 (20.8)	-14.6 (-34.7, 5.5)	2 (13.3)	1 (4.2)	9.2 (-9.8, 28.1)
Nervous system disorders						
Dizziness	1 (6.2)	4 (16.7)	-10.4 (-29.5, 8.6)	3 (20.0)	2 (8.3)	11.7 (-11.4, 34.7)
Headache	1 (6.2)	5 (20.8)	-14.6 (-34.7, 5.5)	3 (20.0)	10 (41.7)	-21.7 (-49.9, 6.6)

Source: adae.xpt; software, R.
Treatment-emergent adverse events defined as adverse events with onset on or after initiation of any study therapy through 28 days after the last dose of any study therapy.
Duration is 26 weeks for Study 006.
Risk difference column shows difference (with 95% confidence interval) between total treatment and comparator.
Abbreviations: CI, confidence interval; FMQ, FDA Medical Query; N, number of subjects in treatment arm; n, number of subjects with adverse event

In Study 007, diarrhea, nausea, fatigue, nasopharyngitis, back pain, dyspnea, and rash occurred more frequently (≥15% difference) in females than in males. Headache was reported more often in males (females: 35%, males: 57.1%). Three female patients (15%) reported abnormal uterine bleeding. As in Study 006, the comparison of TEAEs in females and males should be interpreted with caution because of the small number of patients.

Table 141. TEAEs by Gender With ≥15% Difference Between Males and Females, FDA Medical Query (Narrow), Safety Analysis Set, Study 007

System Organ Class FMQ (Narrow)	Mitapivat Male N=7 n (%)	Mitapivat Female N=20 n (%)	Risk Difference (%) (95% CI)
Gastrointestinal disorders			
Diarrhea	0	3 (15.0)	-15.0 (-30.6, 0.6)
Nausea	0	5 (25.0)	-25.0 (-44.0, -6.0)
General disorders and administration site conditions			
Fatigue	0	6 (30.0)	-30.0 (-50.1, -9.9)
Infections and infestations			
Nasopharyngitis	1 (14.3)	6 (30.0)	-15.7 (-48.5, 17.1)
Musculoskeletal and connective tissue disorders			
Back pain	0	5 (25.0)	-25.0 (-44.0, -6.0)
Nervous system disorders			
Headache	4 (57.1)	7 (35.0)	22.1 (-20.1, 64.3)
Reproductive system and breast disorders			
Abnormal uterine bleeding	0	3 (15.0)	-15.0 (-30.6, 0.6)

System Organ Class FMQ (Narrow)	Mitapivat Male N=7 n (%)	Mitapivat Female N=20 n (%)	Risk Difference (%) (95% CI)
Respiratory, thoracic, and mediastinal disorders			
Dyspnea	0	5 (25.0)	-25.0 (-44.0, -6.0)
Skin and subcutaneous tissue disorders			
Rash	0	3 (15.0)	-15.0 (-30.6, 0.6)

Source: adae.xpt; software, R.
Treatment-emergent adverse events defined as adverse events with onset on or after initiation of any study therapy through 28 days after the last dose of any study therapy.
Duration is 42 weeks for Study 007.
Risk difference column shows difference (with 95% confidence interval) between total treatment and comparator.
Abbreviations: CI, confidence interval; FMQ, FDA Medical Query; N, number of subjects in treatment arm; n, number of subjects with adverse event

17.2. Additional Safety Explorations

Vital Signs

In Study 006, based on vital signs, at baseline the incidence of systolic blood pressure (BP) ≥ 140 mm Hg was 10.0% for mitapivat and 5.2% for placebo. Post-baseline, the incidence was higher in the mitapivat arm compared to placebo arm (mitapivat: 27.5%, placebo: 12.8%). The small numbers of patients limit conclusions. One patient in the mitapivat arm (166 mm Hg) and two patients in the placebo arm (161 mm Hg and 162 mm Hg, respectively) had post-baseline systolic BP > 160 mm Hg. No patients developed systolic BP > 180 mm Hg in Study 006.

There were no significant differences in the incidences of diastolic BP ≥ 90 mm Hg or heart rate ≥ 100 beats/min (or < 60 beats/min) post-baseline between the two arms. No patients developed diastolic BP > 100 mg Hg in Study 006.

In Study 007, the incidences of abnormal vital signs post-baseline were similar to those of the mitapivat arm in Study 006 except for heart rate ≥ 100 beats/min which was 33.3%.

Table 142. Vital Signs Abnormalities Postbaseline, Safety Analysis Set, Studies 006 and 007

	Study 006				Study 007	
	Mitapivat N=40 n (%)		Placebo N=39 n (%)		Mitapivat N=27 n (%)	
Maximum Postdose Value	Baseline	Postbaseline	Baseline	Postbaseline	Baseline	Postbaseline
Blood pressure (mm Hg)						
Systolic BP < 120	25 (62.5)	11 (27.5)	23 (59.0)	15 (38.5)	15 (55.6)	8 (29.6)
Systolic BP ≥ 140	4 (10.0)	11 (27.5)	2 (5.2)	5 (12.8)	4 (14.8)	8 (29.6)
Diastolic BP <80	37 (92.5)	21 (52.5)	36 (92.3)	31 (79.5)	23 (85.2)	14 (51.9)
Diastolic BP ≥ 90	1 (2.5)	2 (5.0)	1 (2.6)	4 (10.3)	1 (3.7)	3 (11.1)
Heart rate (beats/min)						
Heart Rate ≥ 100	0	4 (10.0)	0	4 (10.3)	1 (3.7)	9 (33.3)
Heart Rate < 60	3 (7.5)	0	1 (2.6)	0	1 (3.7)	0

Source: advs.xpt
Abbreviations: BP, blood pressure

Electrocardiograms and QT

The effect of mitapivat on QTc was assessed in Study 004, a Phase 1 study in healthy subjects of Japanese and non-Asian origin, and Study 014, a Phase 1 study in healthy subjects evaluating the

effect of mitapivat administered under fasting conditions on the QTcF. No clinically significant shift from baseline in QTcF level at mitapivat doses of 5, 50, or 200 mg was reported in Study 004. A concentration-QTc analysis using the results from Study 004 showed that the effects of mitapivat and its weakly active metabolite AGI-8702 on QTcF change from baseline fall below the 10 ms threshold. Mitapivat at doses of 100 mg and 300 mg had no clinically relevant effect on ECG parameters in Study 004. A concentration-QTc analysis showed that the effects of mitapivat and its metabolite AGI-8702 on placebo-corrected QTcF change from baseline were below the 10 ms threshold.

No significant QTc prolongation effect of mitapivat was detected in the QT-Interdisciplinary Review Team assessment. For details, refer to the QT-Interdisciplinary Review Team review dated September 29, 2021.

Human Carcinogenicity or Tumor Development

In the mitapivat clinical studies that enrolled patients with PKD (i.e., Studies 003, 006, 007, and 011), among patients who received mitapivat, four developed TEAEs in the Neoplasms benign, malignant, and unspecified System Organ Class (one case each of lipoma, renal cell carcinoma, seborrheic keratosis, and superficial spreading melanoma stage unspecified). There isn't a signal for carcinogenicity, but one would not expect to be able to detect such a signal if one existed given the limited number of patients and treatment duration. Of note, the carcinogenicity studies were negative and mitapivat is not genotoxic.

Pediatrics and Assessment of Effects on Growth

In the mitapivat clinical studies, patients younger than 18 years of age were excluded. The safety and efficacy of mitapivat have not been established in pediatric patients.

Overdose, Drug Abuse Potential, Withdrawal, and Rebound

No events of overdose were reported in subjects who received mitapivat in clinical studies. No potential for drug dependence or drug abuse has been reported for mitapivat during any of the studies with mitapivat. Subjects who abruptly interrupt or discontinue mitapivat may be at risk of developing acute hemolysis; a gradual reduction in dosing (dose taper) is recommended.

17.3. Patient Narratives

Transaminase Increase

Patient (b) (6) was a 63-year-old white male who participated in Study 006 and was randomized to the mitapivat arm. The patient narrative was not provided, however, from the datasets, the baseline ALT was 81 U/L (Grade 1), AST 112 U/L (Grade 2), and bilirubin 84 µmol/L (Grade 3). During treatment with the study drug, the ALT level fluctuated between normal and 120 U/L (Grade 1), the AST level fluctuated between normal and 152 U/L (Grade 2), and the bilirubin level between 42 µmol/L (Grade 2) and 103 µmol/L (Grade 3). Adverse events reported for this patient included cough, dehydration, dyspnea, erectile dysfunction, and fatigue.

Patient (b) (6) was a 23-year-old white male who received mitapivat in Study 007. The baseline ALT was 39 U/L (normal) and AST 38 U/L (Grade 1), and bilirubin 113 µmol/L (Grade 3). During treatment with the study drug, the ALT level fluctuated between normal and 145 U/L

(Grade 1), the AST level fluctuated between normal and 106 U/L (Grade 2), and the bilirubin level from 68 µmol/L (Grade 3) to 132 µmol/L (Grade 3). The AE of ALT increased was not resolved at EOS. The repeated AEs of ALT and/or AST increased were assessed as nonserious and not related to mitapivat. Confounding factors included medical history of ongoing hemolysis, iron overload, concomitant use of deferoxamine and deferiprone, and increased liver iron concentrations on study. Of note, the subject experienced concurrent AST >3× ULN and total bilirubin >2× ULN on Study Day 99. The subject had an elevated bilirubin (Grade 3) at baseline that decreased at the time of the event, and the subject consumed alcohol prior to sample collection for chemistry tests, likely resulting in the elevated transaminases. Therefore, this was not assessed as a potential drug-induced liver injury.

Patient (b) (6) was a 19-year-old white male who received mitapivat in Study 007. The baseline ALT was 19 U/L (normal), AST was 23 U/L (normal), and bilirubin was 96 mol/L (Grade 3). During treatment with study drug, the ALT level fluctuated between normal and 89 U/L (Grade 1), the AST level fluctuated between normal and 119 U/L (Grade 2), and the bilirubin level from 62 µmol/L (Grade 3) and 96 µmol/L (Grade 3). The subject experienced concurrent AST >3× ULN and total bilirubin >2× ULN on Study Day 169. The subject had elevated bilirubin (Grade 3) at baseline that decreased at the time of the event. The case was not assessed as a potential drug-induced liver injury. The investigator considered the events of ALT and AST increased (two times) to be not related to study treatment. Confounding factors included ongoing medical history of hemolysis, iron overload and concomitant use of deferasirox. On Study Day 282, the subject completed study treatment at a fixed dose of 50 mg BID mitapivat and enrolled in the extension study.

Patient (b) (6) was a 20-year-old white female who received mitapivat in Study 007. The baseline ALT was 9 U/L (normal), AST 13 U/L (normal), and bilirubin 35 µmol/L (Grade 2). During treatment with study drug, the bilirubin level was 41 µmol/L (Grade 2) to 59 µmol/L (Grade 2). On Study Day 229, the patient experienced ALT increased (75 U/L, Grade 1; ALT >2.5×baseline) and AST increased (195 U/L, Grade 3; AST >2.5×baseline). The bilirubin was 56 µmol/L (normal range: 6.8 to 20.5 µmol/L). Additional laboratory results included LDH 291 U/L. During the course of the events, the subject was feeling “fine.” The most recent dose prior to the events was received on Study Day 229. Ongoing AEs at the time of the events included osteoporosis (Grade 2) and asthenia (Grade 1). No action was taken with study treatment due to the events; however, the same day, study treatment with mitapivat 20 mg QOD was discontinued due to withdrawal by the subject. No treatment was reported for the events. On Study Day 256, the ALT increased and AST increased were considered resolved. ALT was 16 U/L (normal range: 0 to 55 U/L) and AST 17 U/L (normal range: 5 to 34 U/L). The investigator considered the events of ALT increased and AST increased to be related to study treatment. The Applicant assessed the events of ALT increased AST increased as nonserious AEs and not related to mitapivat. The subject experienced concurrent AST >3× ULN and total bilirubin >2× ULN on Study Day 229. The subject had an elevated bilirubin (Grade 3) at screening that increased slightly at the time of the event. The event was not considered a potential drug-induced liver injury because the subject had elevated bilirubin throughout the study, and the transaminase elevations are more likely attributable to the subject experiencing hemolysis as a result of an underlying PK deficiency anemia. This is evidenced by the decreased hemoglobin and increased LDH, bilirubin, and reticulocytes during the time of the event (Study Day 229; final day of mitapivat treatment) while on 20 mg QOD as part of the taper. The subject was dosed with mitapivat for over 200 days without any elevations in transaminases and only

experienced this elevation in liver test values after the dose of mitapivat was reduced due to a taper. Other confounding factors included medical history of ongoing hemolysis, iron overload and concomitant use of deferasirox.

Fractures

Patient (b) (6) was a 42-year-old female who received mitapivat in Study 006. Relevant medical conditions included osteoporosis (diagnosed 2017, Study Day -588). A screening DXA scan showed a total femoral T-score -2.73, Z-score -2.49, and BMD 0.608 g/cm^2 and adjusted spine T-score -3.62, Z-score -3.28, and BMD 0.649 g/cm^2. On Study Day 46, the patient experienced a serious adverse event (SAE) of rib fracture (Grade 3) and was hospitalized. At the time of the rib fracture, the patient was receiving 20 mg BID mitapivat in the Dose Optimization Period. No action was taken with study treatment due to the event. No DXA scan or bone densitometry scans were performed. Corset placement was recommended. On Study Day 49, the ECOG scale score was 1 and pain was controlled. A gamma scan was pending to assess possible vertebral fracture in the context of osteopenia and severe chronic hemolytic anemia as an underlying cause. Splenosis and worsening of the underlying disease were ruled out due to no increase in hemolysis, no worsening of hepatic iron overload, and no increase in spleen or liver size. On Study Day 52, the subject was discharged. The subject reported improvement in pain, but with some limitations in day-to-day life and sleeping. Treatment with analgesics continued. It was reported that the fracture occurred spontaneously and in the context of osteoporosis and was likely related to severe chronic hemolytic anemia (PKD). The dose was escalated to 50 mg BID mitapivat and the patient continued into the Fixed-Dose Period at the same dose. On Study Day 150, the subject had improved further, but continued to have residual pain. On Study Day 168, DXA scan results were a total femoral T-score -2.75, Z-score -2.49, and BMD 0.606 g/cm^2 and adjusted spine T-score -3.95, Z-score -3.58, and BMD 0.613 g/cm^2. The SAE of rib fracture was considered resolving at the end of the study and not related to study treatment but expected in the targeted disease/population. On Study Day 170, the subject completed study treatment at a fixed dose of 50 mg BID mitapivat and enrolled in the extension study.

Patient (b) (6) was a 46-year-old female who received mitapivat in Study 007. On Study Day 1 (November 25, 2019), the patient received 5 mg BID mitapivat. Relevant medical conditions included osteonecrosis (diagnosed in 2010) and osteoporosis (diagnosed in 2014). Relevant concomitant medications taken within 7 days prior to the start of the event of thoracic vertebral fracture included alendronic acid. Screening DXA scan results were a total femoral T-score -3.39, Z-score -3.04, and BMD 0.528 g/cm^2 and adjusted spine T-score -4.88, Z-score -4.34, and BMD 0.510 g/cm^2. No further DXA scan was conducted. On Study Day 61, the patient experienced nonserious Grade 3 back pain. At the time of the back pain, the subject was receiving 20 mg BID mitapivat in the Dose Optimization Period. On Study Day 62, computed tomography of the abdomen and pelvis showed iron deposition in the liver and nonobstructing right renal calculi. On Study Days 66 and 67, study treatment was reduced to 20 mg QD and 5 mg BID, respectively, due to back pain. On Study Day 91, CT of the abdomen and pelvis revealed a nonserious Grade 2 thoracic vertebral fracture. No action was taken with study treatment due to the event. Treatment for the event of thoracic vertebral fracture included morphine sulfate and sacral orthosis. On Study Day 109, the subject was discharged. The nonserious AEs of back pain and thoracic vertebral fracture were considered not resolved at the end of the study. The events of back pain and thoracic vertebral fracture were not considered related to study treatment. On Study Day 101, the subject began a dose taper, with the final dose

received on Study Day 114. The subject discontinued treatment during the Optimization Dose Period and did not enter the Fixed-Dose Period due to withdrawal by the subject.

Patient (b) (6) was a 55-year-old female who participated in Study 006 and continued treatment with mitapivat in Study 011 and experienced nonserious Grade 1 upper limb fracture and Grade 2 osteoporosis. On Study Day 1 (March 21, 2019), the patient received 20 mg QD mitapivat. Relevant medical conditions included osteopenia (diagnosed in 2018). Baseline DXA scan results in 006 were a total femoral T-score -1.1, Z-score -0.42, and BMD 0.808 g/cm^2 and adjusted spine T-score -1.85, Z-score -0.73, and BMD 0.844 g/cm^2. On Study Day 163, DXA scan results were a total femoral T-score -1.25, Z-score -0.51, and BMD 0.79 g/cm^2 and adjusted spine T-score -2.46, Z-score -1.27, and BMD 0.777 g/cm^2. On Study Day 335, the subject experienced a nonserious AE of Grade 2 osteoporosis. DXA scan results were a total femoral T-score -1.19, Z-score -0.42, and BMD 0.797 g/cm^2 and adjusted spine T-score -2.74, Z-score -1.52, and BMD 0.746 g/cm^2. At the time of the osteoporosis, the subject was receiving 20 mg BID mitapivat. No action was taken with study treatment due to the event. The nonserious AE of osteoporosis was considered not resolved as of the data cutoff date. The investigator considered the event of osteoporosis to be related to study treatment. The Applicant assessed the event of osteoporosis not related to use of mitapivat. The adjusted spine score decreased within the first 6 months of therapy, but then stabilized for 1.5 years. Importantly, this subject had a past medical history of osteopenia and had a follicle-stimulating hormone level of 39.91 mIU/mL at the start of Study 006, indicating postmenopausal status. On Study Day 566, the subject experienced a nonserious AE of Grade 1 upper limb fracture in addition to Grade 1 bone contusion (bruising of the right ribs). At the time of the upper limb fracture, the subject was receiving 20 mg BID mitapivat. No action was taken with study treatment due to the event. The nonserious AE of upper limb fracture was considered not resolved as of the data cutoff date. The investigator considered the event of upper limb fracture to be not related to study treatment.

Patient (b) (6) was a 63-year-old white male who participated in Study 006 and continued to receive treatment with mitapivat in the extension Study 011. On Study Day 1 (April 2, 2019), the patient received 50 mg BID mitapivat. Relevant medical conditions included osteopenia (diagnosed 2018). Baseline DXA scan results in 006 were a total femoral T-score -0.95, Z-score -0.37, and BMD 0.904 g/cm^2 and adjusted spine T-score -1.58, Z-score -0.86, and BMD 0.917 g/cm^2. On Study Day 137, the patient was chopping down trees when a log fell on his leg. The patient was subsequently hospitalized due to SAEs of Grade 3 femur fracture and Grade 3 tibia fracture, and also reported Grade 1 bone pain. At the time of the femur fracture and tibia fracture, the patient was receiving 50 mg BID mitapivat. No action was taken with study treatment due to the event. On Study Day 139, a proximal tibial open-reduction internal fixation was performed, and the SAEs of femur fracture and tibia fracture were considered resolved. On Study Day 141, the subject was discharged. The investigator considered the events of femur fracture and tibia fracture to be not related to study treatment. The events were caused by a log falling on the subject's leg while chopping wood. On Study Day 168, the patient experienced a nonserious AE of Grade 1 osteopenia. DXA scan results were a total femoral T-score -1.4, Z-score -0.81, and BMD 0.839 g/cm^2; adjusted spine results were not reported. At the time of the osteopenia, the subject was receiving 50 mg BID mitapivat. No action was taken with study treatment due to the event. No treatment was reported for the event. On Study Day 350, DXA scan results were a total femoral T-score -1.62, Z-score -1.01, and BMD 0.808 g/cm^2 and adjusted spine T-score -1.68, Z-score -0.92, and BMD 0.907 g/cm^2. On Study Day 511, DXA scan results were a total femoral T-score -1.67, Z-score -1.06, and BMD 0.8 g/cm^2 and adjusted

spine T-score -1.85, Z-score -1.08, and BMD 0.887 g/cm^2. The nonserious AE of osteopenia was considered not resolved as of the data cutoff date. The investigator considered the event of osteopenia to be related to study treatment. The subject was continuing in the extension study as of the data cutoff date. The Applicant assessed the event of osteopenia not related to the use of mitapivat because the subject had a medical history of osteopenia and alcoholic hepatitis.

17.4. Study 011

Exposure

In the Extension Study 011, the median duration of treatment with mitapivat for Cohorts 1, 2, and 3 was 23.1 weeks (range: 4.3, 84.4 weeks), 28.1 weeks (range: 2.1, 86.1 weeks), and 50.3 weeks (0.3, 78.3 weeks), respectively. This does not include exposure to mitapivat in the antecedent studies. For the design and patient disposition of Study 011, see Section 16.1.

Table 143. Duration of Exposure, Safety Analysis Set, Study 011

Exposure Duration	Cohort 1 (n=36)	Cohort 2 (n=35)	Cohort 3 (n=17)
Duration of exposure (weeks)			
Mean	33.3	39.1	38.7
Median	23.1	28.1	50.3
Range	4.3, 84.4	2.1, 86.1	0.3, 78.3
By duration category, n (%)			
≤12 weeks	5 (13.9%)	2 (5.7%)	3 (17.6%)
>12 to ≤24 weeks	14 (38.9%)	10 (28.6%)	4 (23.5%)
>24 to ≤48 weeks	6 (16.7%)	10 (28.6%)	1 (5.9%)
>48 to ≤72 weeks	9 (25.0%)	9 (25.7%)	8 (47.1%)
>72 to ≤87 weeks	2 (5.6%)	4 (11.4%)	1 (5.9%)
>87 weeks	0	0	0

Source: adex.xpt
Abbreviation: n, number of subjects in each category

Safety Results

Table 144 summarizes the overall safety results of Study 011. The incidence of Grade ≥3 TEAEs was highest in Cohort 1 (patients who received placebo in Study 006 and started treatment with mitapivat in Study 011).

Table 144. Overall Summary of Safety, Safety Analysis Set, Study 011

Safety Analysis, n (%)	Cohort 1 (n=36)	Cohort 2 (n=35)	Cohort 3 (n=17)	Total (n=88)
Deaths	0	0	0	0
Any TEAEs	35 (97.2%)	26 (74.3%)	11 (64.7%)	72 (81.8%)
Serious TEAEs	3 (8.3%)	3 (8.6%)	1 (5.9%)	7 (8.0%)
Grade ≥3 TEAEs	6 (16.7%)	4 (11.4%)	1 (5.9%)	11 (12.5%)
TEAEs leading to discontinuation of study drug	0	1 (2.9%)	0	1 (1.1%)
TEAEs leading to dose reduction of study drug	0	1 (2.9%)	0	1 (1.1%)
TEAEs leading to interruption of study drug	1 (2.8%)	2 (5.7%)	0	3 (3.4%)

Source: adae.xpt
Abbreviations: n, number of subjects in each cohort; TEAE, treatment-emergent adverse event

No deaths were reported in Study 011. Overall, serious TEAEs occurred in seven patients (8%) in Study 011: pyrexia, cytomegalovirus infection, erysipelas, varicella zoster virus infection, gastroenteritis, COVID-19 pneumonia, femur fracture, tibia fracture, syncope, and nephrolithiasis, with each event occurring once. All serious TEAEs were reported as recovered, except for one patient in Cohort 1 who developed Grade 3 cytomegalovirus infection that led to interruption of study treatment.

Across the three cohorts, Grade ≥3 TEAEs occurred in 11 patients (12.5%) in Study 011. All were Grade 3 TEAEs; no Grade 4 TEAEs were reported. The events were arthralgia, AST increased, blood triglycerides increased, cytomegalovirus infection, femur fracture, gastroenteritis, nephrolithiasis, panic attack, pyrexia, syncope, tibia fracture, and varicella zoster virus infection. All events occurred in one patient each, except for AST increased, which occurred in three patients (Cohort 1: two patients [5.6%], Cohort 3: one patient [5.9%]).

In Study 011, a TEAE of Grade 1 insomnia that led to discontinuation of study treatment was reported in one patient in Cohort 2. No treatment was given for insomnia; the patient reportedly recovered.

One patient in Cohort 2 experienced Grade 3 arthralgia that led to dose reduction and interruption of study treatment that was ongoing at the time of the clinical cut-off date. In addition, two patients (Cohort 1: one patient [Grade 1 postprocedural inflammation], Cohort 2: one patient [Grade 3 cytomegalovirus infection]) had TEAEs that resulted in interruption of study treatment.

In Study 011, the incidences of TEAEs in Cohorts 1, 2, and 3 were 97.2%, 74.3%, and 64.7%, respectively. Across the three cohorts, the most frequently reported TEAEs (by FDA Medical Dictionary for Regulatory Activities Query, narrow) (≥10%) were headache, insomnia, nasopharyngitis, and pyrexia. The TEAEs that occurred in Study 011 were generally consistent with those in Studies 006 and 007.

Table 145. TEAEs That Occurred in ≥3 Patients in the Sum of Three Cohorts, by FDA Medical Query (Narrow), Safety Analysis Set, Study 011

FMQ, n (%)	Cohort 1 (n=36)	Cohort 2 (n=35)	Cohort 3 (n=17)
All events	35 (97.2%)	26 (74.3%)	11 (64.7%)
Headache	18 (50.0%)	3 (8.6%)	2 (11.8%)
Insomnia	12 (33.3%)	1 (2.9%)	0
Nasopharyngitis	5 (13.9%)	5 (14.3%)	3 (17.6%)
Pyrexia	5 (13.9%)	2 (5.7%)	2 (11.8%)
Nausea	4 (11.1%)	3 (8.6%)	1 (5.9%)
Arthralgia	4 (11.1%)	4 (11.4%)	0
Fatigue	3 (8.3%)	3 (8.6%)	1 (5.9%)
Hepatic injury	3 (8.3%)	1 (2.9%)	1 (5.9%)
Anemia	3 (8.3%)	0	1 (5.9%)
Peripheral edema	3 (8.3%)	0	1 (5.9%)
Abdominal pain	3 (8.3%)	0	0
Dyspepsia	2 (5.6%)	4 (11.4%)	0
Dizziness	2 (5.6%)	3 (8.6%)	0
Diarrhea	2 (5.6%)	2 (5.7%)	0
Arthritis	2 (5.6%)	1 (2.9%)	0

FMQ, n (%)	Cohort 1 (n=36)	Cohort 2 (n=35)	Cohort 3 (n=17)
Vitamin D deficiency	1 (2.8%)	1 (2.9%)	2 (11.8%)
Sinusitis	1 (2.8%)	0	3 (17.6%)
Cough	1 (2.8%)	0	2 (11.8%)
Dysgeusia	0	2 (5.7%)	1 (5.9%)

Source: adae.xpt
Abbreviations: FMQ, FDA medical query; n, number of subjects in each cohort

The safety results of mitapivat in Study 011 appear consistent with those in Studies 006 and 007.

17.5. Cumulative Safety Analysis of Studies 003, 006, 007, and 011 in the Initial NDA and 120-Day Safety Update

This section summarizes the cumulative safety analysis for Studies 003, 006, 007, and 011 reported in the initial NDA and in the 120-Day safety update.

Initial NDA

The initial NDA contained safety data from a total of 155 patients with PKD who received mitapivat at any dose in clinical studies (40 and 27 patients from Studies 006/011 [Cohort 2] and Study 007/011 [Cohort 3], respectively; 52 patients in Study 003; and 36 patients in Study 006/011 [Cohort 1, who received placebo in Study 006]). The NDA contained a pooled cumulative safety analysis of Studies 003, 006, 007, and 011. Since Studies 006 and 007 were the pivotal trials, the safety review was primarily based on these studies and the Extension Study 011. For Study 003, analyses were based on the highest dose that the patient received (i.e., ≤50 mg BID: 18 patients, >50 mg BID: 34 patients). Therefore, the safety analysis of mitapivat at the recommended dose regimen was based on a total of 121 patients.

120-Day Safety Update

The Applicant submitted the 120-day safety update on September 14, 2021. The submission contained additional safety data from the two ongoing studies (the Phase 3 extension Study 011 and the Extension Period of Study 003) in patients with PKD with a clinical cut-off date of March 4, 2021, providing more than 3 and 6 months of additional safety information, respectively. The 120-day safety update contained data from 157 patients who received mitapivat at any dose across the four clinical studies (Study 006/011 [Cohort 2]: 40 patients, Study 007/011 [Cohort 3]: 27 patients, Study 003: 52 patients, Study 006/011 [Cohort 1]: 38 patients) and 123 patients who received mitapivat at the recommended dose regimen. In the 120-day safety update, two additional patients (who received placebo in Study 006) received treatment with mitapivat in Cohort 1 of Study 011.

The review of the cumulative safety data was primarily based on the results of the pivotal Studies 006 and 007 and the extension Study 011. In the initial NDA and the 120-day safety update, a total of 103 and 105 patients, respectively, received treatment with mitapivat from these Phase 3 studies. Safety data were presented for the on-treatment period (cumulative period), i.e., from the date of the start of study treatment to 28 days after the end of study treatment. Study 006/011 (Cohort 1), Study 006/011 (Cohort 2), and Study 007/011 (Cohort 3) are also referred to as Cohort 1, Cohort 2, and Cohort 3 of Study 011, respectively, in this review.

Extent of Exposure

Initial NDA

In the initial NDA, the overall median exposure to mitapivat in Studies 003, 006, 007, and 011 was 11.4 months (range: 1.0, 58.7 months). Totals of 71 patients (45.8%) and 26 patients (16.8%) received mitapivat for >12 and >24 months, respectively. In patients who received mitapivat in Studies 006/007 and continued in the Extension Study 011, the median cumulative exposure to mitapivat in Cohorts 1, 2, and 3 of Study 011 was 5.3 months (range: 1.0, 19.4), 11.6 months (range: 6.0, 25.4 months), and 12.4 months (range: 3.7, 28.6 months), respectively.

Table 146. Initial NDA: Summary of Cumulative Exposure to Mitapivat, Safety Analysis Set, Studies 003, 006, 007, and 011

	Study 003		Study 006/011 Cohort 1	Study 006/011 Cohort 2	Study 007/011 Cohort 3	
Duration	≤50 mg BID (n=18)	>50 mg BID (n=34)	≤50 mg BID (n=36)	≤50 mg BID (n=40)	≤50 mg BID (n=27)	Total (n=155) All Doses
Duration of exposure (months)						
Mean	26.7	23.4	7.7	13.5	14.3	16.0
Median	22.4	15.7	5.3	11.6	12.4	11.4
Range	3.0, 58.7	3.0, 57.4	1.0, 19.4	6.0, 25.4	3.7, 28.6	1.0, 58.7
Patients treated, by duration, n (%)						
>3 months	17 (94.4%)	33 (97.1%)	31 (86.1%)	40 (100%)	27 (100%)	148 (95.5%)
>6 months	14 (77.8%)	28 (82.4%)	17 (47.2%)	40 (100%)	24 (88.9%)	123 (79.4%)
>12 months	10 (55.6%)	21 (61.8%)	8 (22.2%)	18 (45.0%)	14 (51.9%)	71 (45.8%)
>18 months	10 (55.6%)	12 (35.3%)	1 (2.8%)	12 (30.0%)	9 (33.3%)	44 (28.4%)
>24 months	9 (50.0%)	11 (32.4%)	0	2 (5.0%)	4 (14.8%)	26 (16.8%)
>36 months	7 (38.9%)	11 (32.4%)	0	0	0	18 (11.6%)

Source: adex.xpt and SCS.
Abbreviations: BID, twice daily; n, number of subjects in each treatment arm

120-Day Safety Update

At the updated clinical cutoff date, the overall median exposure to mitapivat in Studies 003, 006, 007, and 011 was 13.9 months (range: 1.3, 64.9 months) compared to a median of 11.4 months (range: 1.0, 58.7 months) in the initial NDA. Also, 92 patients (58.6%) had received mitapivat for >12 months and 36 patients (22.9%) for >24 months (an increase from 71 patients [45.8%] and 26 patients [16.8%], respectively, in the initial NDA). In patients who received mitapivat in Studies 006/007 and continued in the Extension Study 011, the median cumulative exposure to mitapivat in Cohorts 1, 2, and 3 of Study 011 was 8.7 months (range: 1.3, 23.1 months), 15.2 months (range: 6.0, 29.1 months), and 13.6 months (range: 3.7, 32.3 months), respectively.

Table 147. 120-Day Safety Update: Cumulative Exposure to Mitapivat, Safety Analysis Set, Studies 003, 006, 007, and 011

	Study 003		Study 006/011 Cohort 1	Study 006/011 Cohort 2	Study 007/011 Cohort 3	Total
Duration	≤50 mg BID (n=18)	>50 mg BID (n=34)	≤50 mg BID (n=38)	≤50 mg BID (n=40)	≤50 mg BID (n=27)	(n=157) All Doses
Duration of exposure (months)						
Mean	29.1	25.4	10.2	16.5	16.2	18.3
Median	22.4	15.7	8.7	15.2	13.6	13.9
Range	3.0, 64.9	3.0, 63.5	1.3, 23.1	6.0, 29.1	3.7, 32.3	1.3, 64.9
Patients treated, by duration, n (%)						
>3 months	17 (94.4%)	33 (97.1%)	35 (92.1%)	40 (100%)	27 (100%)	152 (96.8%)
>6 months	13 (72.2%)	24 (70.6%)	31 (81.6%)	39 (97.5%)	24 (88.9%)	131 (83.4%)
>12 months	10 (55.6%)	21 (61.8%)	11 (28.9%)	33 (82.5%)	17 (63.0%)	92 (58.6%)
>18 months	10 (55.6%)	12 (35.3%)	6 (15.8%)	14 (35.0%)	11 (40.7%)	53 (33.8%)
>24 months	9 (50.0%)	11 (32.4%)	0	8 (20.0%)	8 (29.6%)	36 (22.9%)
>36 months	7 (38.9%)	11 (32.4%)	0	0	0	18 (11.5%)

Source: adex.xpt and SCS.
Abbreviations: BID, twice daily; n, number of subjects in each treatment arm

Dose Modification

Initial NDA

Patients in the Extension Study 011 underwent dose modifications consistent with those implemented in Studies 006 and 007. In the initial NDA, 30 patients underwent dose modifications (reductions) of mitapivat across Studies 006, 007, and 011. Most were due to planned discontinuations (Table 148). The incidence was highest in patients who continued in Cohort 3, followed by Cohort 2 and Cohort 1 of Study 011 (Cohort 1: 6 patients [16.7%], Cohort 2: 11 patients [27.5%], Cohort 3: 13 patients [48.1%]).

120-Day Safety Update

The cumulative incidence of dose modification of mitapivat in Studies 006, 007, and 011 reported in the 120-day safety update was similar to the initial NDA submission. In the 120-day safety update, two further patients in Cohorts 1 and 2 underwent dose modifications (which were due to scheduled dose tapers to discontinue study drug).

Table 148. Initial NDA and 120-Day Safety Update: Summary of Dose Modification, Safety Analysis Set, Studies 006, 007, and 011

	Study 006/011 Cohort 1 ≤50 mg BID		Study 006/011 Cohort 2 ≤50 mg BID		Study 007/011 Cohort 3 ≤50 mg BID	
Dose Modification n, (%)	**Initial NDA (n=36)**	**120-Day Update (n=38)**	**Initial NDA (n=40)**	**120-Day Update (n=40)**	**Initial NDA (n=27)**	**120-Day Update (n=27)**
All patients with ≥1 dose reduction	6 (16.7%)	7 (18.4%)	11 (27.5%)	12 (30.0%)	13 (48.1%)	13 (48.1%)
Reason for dose reduction						
Planned discontinuation	5 (13.9%)	6 (15.8%)	8 (20.0%)	9 (22.5%)	11 (40.7%)	11 (40.7%)
Prescribed dose reduction	0	0	0	0	0	0
Adverse event	1 (2.8%)	1 (2.6%)	2 (5.0%)	2 (5.0%)	1 (3.7%)	1 (3.7%)
Increased Hb	0	0	0	0	0	0
Other	1 (2.8%)	1 (2.6%)	2 (5.0%)	2 (5.0%)	3 (11.1%)	3 (11.1%)

Source: adsl.xpt.
Incidences are based on the number of subjects, not the number of events. The same patient may appear in different categories.
Abbreviations: BID, twice daily; n, number of subjects in each group

Overall Safety Findings

The overall safety results of mitapivat in the 120-day safety update were consistent with those reported in the initial NDA and the results of the pivotal Studies 006 and 007. The incidences of events reported in the Phase 2 dose-ranging Study 003 were higher than those in the pivotal Studies 006 and 007 and the Extension Study 011.

Table 149. Initial NDA and 120-Day Safety Update: Overall Summary of Cumulative Safety, Safety Analysis Set, Studies 003, 006, 007, and 011

	Study 003 ≤50 mg BID		Study 006/011 Cohort 1 ≤50 mg BID		Study 006/011 Cohort 2 ≤50 mg BID		Study 007/011 Cohort 3 ≤50 mg BID	
Safety Analysis, n (%)	**Initial NDA (n=18)**	**120-Day Update (n=18)**	**Initial NDA (n=36)**	**120-Day Update (n=38)**	**Initial NDA (n=40)**	**120-Day Update (n=40)**	**Initial NDA (n=27)**	**120-Day Update (n=27)**
Deaths	0	0	0	0	0	0	0	0
Any TEAEs	18 (100%)	18 (100%)	35 (97.2%)	36 (94.7%)	38 (95.0%)	38 (95.0%)	27 (100%)	27 (100%)
Serious TEAEs	9 (50.0%)	9 (50.0%)	3 (8.3%)	3 (7.9%)	6 (15.0%)	6 (15.0%)	3 (11.1%)	3 (11.1%)
Grade ≥3 TEAEs	10 (55.6%)	10 (55.6%)	6 (16.7%)	8 (21.1%)	12 (30.0%)	12 (30.0%)	9 (33.3%)	9 (33.3%)
Treatment-related TEAEs	15 (83.3%)	15 (83.3%)	17 (47.2%)	20 (52.6%)	26 (65.0%)	26 (65.0%)	18 (66.7%)	18 (66.7%)
TEAEs leading to discontinuation	2 (11.1%)	2 (11.1%)	0	0	1 (2.5%)	1 (2.5%)	0	0
TEAEs leading to dose reduction	3 (16.7%)	3 (16.7%)	0	1 (2.6%)	1 (2.5%)	1 (2.5%)	1 (3.7%)	1 (3.7%)
TEAEs leading to interruption	3 (16.7%)	3 (16.7%)	1 (2.8%)	1 (2.6%)	2 (5.0%)	2 (5.0%)	0	0

Source: adae.xpt.
Abbreviations: BID, twice daily; n, number of subjects in each group; TEAE, treatment emergent adverse event

There were no deaths in any study with mitapivat. There were no additional patients who experienced serious TEAEs in the 120-day safety update from the clinical cutoff date of the initial NDA. The incidence of Grade ≥3 TEAEs reported in the 120-day safety update was also consistent with the initial NDA (most of the Grade ≥3 TEAEs were Grade 3 in severity). Two patients had Grade 4 TEAEs (one patient had blood triglycerides increased in Study 007; the other patient in Cohort 1 of Study 011 developed hyperlipidemia). The incidence of treatment modification of study treatment in the 120-day safety update was consistent with the initial NDA; there was one additional patient in Cohort 1 who had the dose of mitapivat reduced due to Grade 2 headache.

In the 120-day safety update, the most frequently reported cumulative TEAEs (by FDA Medical Dictionary for Regulatory Activities Query, narrow) (≥20%) in any cohort in patients who continued in Study 011 from the antecedent studies were headache, insomnia, nasopharyngitis, nausea, fatigue, hepatic injury, back pain, and vomiting. This was consistent with those in Studies 006 and 007.

Table 150. 120-Day Safety Update: TEAEs That Occurred in Five or More Patients in the Sum of Three Cohorts by FDA Medical Query (Narrow), Safety Analysis Set, Study 006, 007, and 011

FMQ, n (%)	Study 006/011 Cohort 1 ≤50 mg BID (n=38)	Study 006/011 Cohort 2 ≤50 mg BID (n=40)	Study 007/011 Cohort 3 ≤50 mg BID (n=27)
All events	36 (94.7%)	38 (95.0%)	27 (100%)
Headache	19 (50.0%)	7 (17.5%)	13 (48.1%)
Insomnia	14 (36.8%)	9 (22.5%)	6 (22.2%)
Nasopharyngitis	8 (21.1%)	10 (25.0%)	10 (37.0%)
Pyrexia	6 (15.8%)	3 (7.5%)	3 (11.1%)
Nausea	5 (13.2%)	10 (25.0%)	6 (22.2%)
Fatigue	5 (13.2%)	8 (20.0%)	8 (29.6%)
Arthralgia	5 (13.2%)	6 (15.0%)	1 (3.7%)
Anemia	5 (13.2%)	1 (2.5%)	2 (7.4%)
Hepatic injury	4 (10.5%)	3 (7.5%)	10 (37.0%)
Suspected COVID-19	4 (10.5%)	0	4 (14.8%)
Abdominal pain	3 (7.9%)	6 (15.0%)	4 (14.8%)
Dizziness	3 (7.9%)	6 (15.0%)	2 (7.4%)
Oropharyngeal pain	3 (7.9%)	3 (7.5%)	4 (14.8%)
Dyspnea	3 (7.9%)	3 (7.5%)	5 (18.5%)
Pain in extremity	3 (7.9%)	4 (10.0%)	0
Peripheral edema	3 (7.9%)	0	2 (7.4%)
Back pain	2 (5.3%)	6 (15.0%)	6 (22.2%)
Diarrhea	2 (5.3%)	6 (15.0%)	3 (11.1%)
Dyspepsia	2 (5.3%)	5 (12.5%)	3 (11.1%)
Cough	2 (5.3%)	4 (10.0%)	3 (11.1%)
Arrhythmia	2 (5.3%)	2 (5.0%)	1 (3.7%)
Rash	2 (5.3%)	1 (2.5%)	2 (7.4%)
Sinusitis	2 (5.3%)	0	3 (11.1%)
Hot flush	1 (2.6%)	3 (7.5%)	2 (7.4%)
Upper respiratory tract infection	1 (2.6%)	2 (5.0%)	4 (14.8%)
Vomiting	1 (2.6%)	1 (2.5%)	6 (22.2%)
Abnormal uterine bleeding	1 (2.6%)	1 (2.5%)	4 (14.8%)

FMQ, n (%)	Study 006/011 Cohort 1 ≤50 mg BID (n=38)	Study 006/011 Cohort 2 ≤50 mg BID (n=40)	Study 007/011 Cohort 3 ≤50 mg BID (n=27)
Gastroenteritis	0	6 (15.0%)	1 (3.7%)
Influenza	0	2 (5.0%)	3 (11.1%)
Urinary tract infection	0	3 (3.7%)	2 (7.4%)
Osteopenia	0	4 (10.0%)	1 (3.7%)

Source: adae.xpt.
Abbreviations: COVID-19, coronavirus disease 2019; FMQ, FDA medical query; BID, twice daily, n, number of subjects in each cohort

In the 120-day safety update, no additional patients had acute hemolysis after withdrawal of mitapivat; there was no apparent increase in the incidence of TEAEs in female patients; there were no observable differences in trends in hormone levels; there were no meaningful differences in decreased bone mineral density in patients with PK deficiency compared with the initial NDA.

With regard to fractures, in addition to the four patients who had fractures with mitapivat treatment (Section 7.6.7.4), one additional patient had Grade 3 rib fracture (ID: (b) (6)) in the 120-day safety update. This was a 70-year-old female who had an underlying medical condition of osteoporosis. The patient received mitapivat in Study 006 and continued in Cohort 2 of Study 011; the rib fracture AE occurred on Day 456. The patient was receiving mitapivat 5 mg BID at the time of onset of the event. The narrative for this patient was not provided.

Laboratory Findings

Initial NDA

Shifts from baseline to Grade 4 chemistry parameters occurred for bilirubin, potassium, triglycerides, and urate. Shifts from baseline (Grade 3) to Grade 4 for bilirubin (high) occurred in two patients in Study 003 who received mitapivat ≤50 mg BID. Shifts from baseline (Grade 0) to Grade 4 for potassium (high) occurred in one patient in Study 011 Cohort 1. Shifts from baseline to Grade 4 for triglycerides (high) occurred in one patient in Study 007 (Grade 2) and one patient in Study 011 Cohort 1 (Grade 3). Shifts from baseline (Grade 3) to Grade 4 for urate (high) occurred in one patient in Study 006 and two patients in Study 007. Hematology shifts from baseline were generally similar to those in Studies 006 and 007 (Section 7.6.8). Changes in the laboratory parameters in the 120-day safety update were generally consistent with the initial NDA.

18. Mechanism of Action / Drug Resistance: Additional Information and Assessment

Not applicable.

19. Other Drug Development Considerations: Additional Information and Assessment

Mitapivat is also under investigation for the treatment of patients with thalassemia and patients with sickle cell disease.

20. Data Integrity-Related Consults (Office of Scientific Investigations, Other Inspections)

Studies 006 and 007 are the pivotal trials to support the efficacy and safety of mitapivat for the proposed indication. Three clinical sites (Study 006: two sites, Study 007: one site) were selected for Office of Scientific Investigations (OSI) inspections. The site selections were based on the enrollment of large numbers of patients and financial disclosure information.

Table 151. Requested OSI Clinical Site Audits for C788-047 and C788-048

Protocol ID	Site ID	Number of Enrolled Patients	Principal Investigator	Location
006	840104	10	Hanny Al-Samkari	Massachusetts General Hospital 55 Fruit Street, MA 02114 USA
006	208101	7	Andreas Birkedal Glenthoj	Department of Hematology Herlev University Hospital Herlev Ringvej 75 2730 Herlev Denmark
007	208101	6	Andreas Birkedal Glenthoj	Department of Hematology Herlev University Hospital Herlev Ringvej 75 2730 Herlev Denmark

Source:FDA compilation.
Abbreviations: OSI, Office of Scientific Investigations

In addition to the two clinical sites (840104 and 208101), Agios Pharmaceuticals (the Applicant) was also inspected. OSI's overall assessment of findings and general recommendations for these sites were as follows:

"The study data derived from the above two clinical investigator sites are considered reliable. Applicant's monitoring and oversight of Study 006 and Study 007 appeared adequate. The study data submitted to the Agency for assessment appeared acceptable in support of the proposed indication."

Therefore, the overall compliance with good clinical practices is acceptable. For more details, refer to the OSI review by Dr. Anthony Orencia, dated November 15, 2021.

21. Labeling Summary of Considerations and Key Additional Information

Table 152. Proposed Prescribing Information

Section Name	Change Recommended
Highlights of Prescribing Information	Updated to reflect changes made to FPI.
Table of Contents	Updated to reflect changes made to FPI.
Full Prescribing Information	
Indications and Usage	Revised to "indicated for the treatment of hemolytic anemia in adults with pyruvate kinase (PK) deficiency" to specify the aspect of the disease being treated.
Dosage and Administration	Relocated text describing taking drug without regard to food and swallowing table whole to within section 2.1. Text included between headings and numbered subheadings do not get included in the Table of Contents. Revised title of section 2.1 from (b) (4) Recommended Dosage as (b) (4) dosage refers to the administering of a specified amount, number, and frequency of doses over a specified period of time. Added language to advise practitioners to "discontinue PYRUKYND if no benefit has been observed by 24 weeks" This was added because we do not have efficacy information on all of the genetic variants and we would not want treatment given indefinitely in patients who are not benefiting. Added subsection 2.4 for Recommended Dosage for Hepatic Impairment. Added subsection 2.5 Recommended Dosage for Drug Interactions, per the Dosage and Administration Guidance. Added subsection 2.6 Dose Modifications for Adverse Reactions and Hemoglobin Levels Above Normal. Deleted text under (b) (4)
Dosage Forms and Strengths	Deleted "immediate-release" text as tablets are all considered to be immediate-release if not labeled as extended-release.
Contraindications	None.
Warnings and Precautions	Warning 5.1 Acute Hemolysis: Revised name of Warning to add "with Abrupt Treatment Interruption" for clarity. Revised the warning to (b) (4)
Adverse Reactions	Corrected section heading from (b) (4) to Adverse Reactions, per PLR template.

Section Name	Change Recommended
	Revised the format and content of the section to: Provide the basis of the safety population and their exposure (percentage of patients exposed for longer than 24 weeks. Provided the incidence rates of all serious adverse reactions and listed the most common. Added list of most common ARs. Added references to the AR tables in text. Revised name of AR tables for consistency with our current approach. Removed (b) (4) . Removed reference (b) (4) Deleted proposed (b) (4) Left in text below main AR table to discuss variations in reproductive hormones.Deleted (b) (4)
Drug Interactions	Revised format to tabular for clarity. Added more detail on mitigating strategies for interactions. Deleted (b) (4) Revised table to reflect the data submitted and the recommended format.
Use in Specific Populations	Revised section 8.1 to be consistent with PLLR guidance. Nonclinical reproductive toxicity and clinical pregnancy data were negative. Provided risk statements for products with no known risk, but lack of significant trial data in exposed pregnant women. Added subsection for "Clinical Considerations—Disease-Associated Maternal Risk" to describe the risks of the disease in pregnant women. Added required heading for "Data." Revised section 8.2 Lactation to refer to "child" instead of (b) (4) and provide the recommended actions for a product with limited risk to lactating infant due to low rate of ARs in adults. Added recommended statement for products with no identified risks that would preclude breastfeeding. Removed (b) (4) Revised 8.4 Pediatric Use to provide the required verbatim statement per CFR. Removed (b) (4)

Section Name	Change Recommended
	(b) (4) Revised 8.5 Geriatric Use to provide text consistent with the Sept 2020 guidance on Geriatric Use labeling and because there were inadequate numbers of geriatric patients enrolled to identify a difference between groups. (b) (4) Added subsection 8.6 Hepatic Impairment to provide actionable information.
(b) (4)	
Description	Added established nonproprietary name, pharmacological class, and a summary of important physicochemical properties. Asked Applicant to list ingredients using compendial names and in alphabetical order.
Clinical Pharmacology	Edited for clarity and to be consistent with the Clinical Pharmacology labeling guidance. Removed (b) (4) . The proposed text for the Pharmacodynamics section was deleted (b) (4) The Applicant was asked to revise the text. A subsection for Cardiac Electrophysiology was added per the QT-IRT consult review of the QT data. Added drug interaction subsections.
Nonclinical Pharmacology	Edited for clarity and brevity to summarize the carcinogenicity studies in two sentences. Revised the fertility subsection to be consistent with PLLR guidance. Deleted (b) (4)

Section Name	Change Recommended
Clinical Studies	Recommended deletion [redacted] (b) (4) and described the baseline mean laboratory values in Efficacy Results table 6. For ACTIVATE Trial described in tabular format: Hb Response followed by mean change from baseline in Hb, indirect bilirubin, reticulocyte count, LDH, and haptoglobin. In text, described the changes in signs of jaundice, and some symptoms of PKD including tiredeness, shortness of breath as collected in the PKDD. For ACTIVATE-T Trial, described Transfusion Reduction Response and Patients Who Were Transfusion Free in tabular form and median duration of response in text below the table. Removed reference [redacted] (b) (4) per Clinical Studies guidance. Recommended removal of overly promotional text such as [redacted] (b) (4)
How Supplied / Storage and Handling	Deleted [redacted] (b) (4) Because this statement is not applicable to storage conditions for the healthcare practitioner. Revised text to tabular form.
Patient Counseling Information	Updated this section with concepts from Warnings and Precautions and important counseling topics. Information after 17: Manufacturer Info. Asked Applicant to add the street address per 21CFR 201.100(e).
Patient Labeling (MedGuide, IFU)	Revised to reflect changes to USPI.

Abbreviations: AE, adverse event; AR, adverse reactions; CFR, code of federal regulations; FPI, full prescribing information; IRT, interdisciplinary review team; MedDRA, medical dictionary for regulatory activities; PK, pyruvate kinase; PKDD, pyruvate kinase deficiency diary; PKLR, pyruvate kinase liver and red blood cell gene; PLR, physician labeling rule; PLLR, pregnancy and lactation labeling; PRO, patient-reported outcome; PT, preferred term; QT, electrocardiogram interval; SAE, serious adverse event; USPI, United States prescribing information; W&P, warnings and precautions

22. Postmarketing Requirements and Commitments

PMR 1

Complete Trial AG-348-C-011, "An open-label, multicenter, extension Study of AG-348 in adult subjects with pyruvate kinase deficiency previously enrolled in AG-348 studies." In your final study report include safety follow-up data with summaries of AEs, changes in reproductive hormones, changes in blood lipid levels, bone fractures and other AEs from long-term aromatase inhibition. Submit integrated safety datasets and patient level data with the final report.

- Trial Completion: 11/2024
- Final Report Submission: 06/2025

PMR 2

Complete Trial AG-348-C-003, "A Phase 2, Open-Label, Randomized, Dose-Ranging, Safety, Efficacy, Pharmacokinetic and Pharmacodynamic Study of AG-348 in Adult Patients With Pyruvate Kinase Deficiency." In your final study report include safety follow-up data with summaries of AEs, changes in reproductive hormones, changes in blood lipid levels, bone fractures and other adverse events from long-term aromatase inhibition. Submit integrated safety datasets and patient-level data with the final report.

- Trial Completion: 05/2025
- Final Report Submission: 12/2025

PMR 3

Conduct a clinical trial to evaluate the impact of hepatic impairment on the pharmacokinetics of mitapivat in subjects with moderate (Child-Pugh B) hepatic impairment relative to adult healthy subjects, in accordance with the reduced study design described in FDA's May 2003 Guidance for Industry - Pharmacokinetics in Patients with Impaired Hepatic Function: Study Design, Data Analysis, and Impact on Dosing and Labeling (May 2003). Submit the subject-level datasets with the final report. Based on the results of this study, additional subjects with mild (Child-Pugh A) and/or severe (Child-Pugh C) hepatic impairment may need to be enrolled.

- Draft Protocol Submission: 04/2022
- Final Protocol Submission: 09/2022
- Trial Completion: 08/2023
- Final Report Submission: 03/2024

23. Financial Disclosure

Table 153. Covered Clinical Studies: AG-348-C-006 and AG-348-C-007

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from Applicant)
Total number of investigators identified: 289		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): 0		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): 2		
If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c), and (f)): Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: 0 Significant payments of other sorts: 2 Proprietary interest in the product tested held by investigator: 0 Significant equity interest held by investigator: 0 Sponsor of covered study: 0		
Is an attachment provided with details of the disclosable financial interests/arrangements:	Yes ☒	No ☐ (Request details from Applicant)
Is a description of the steps taken to minimize potential bias provided:	Yes ☒	No ☐ (Request information from Applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3): 0		
Is an attachment provided with the reason:	Yes ☐	No ☐ (Request explanation from Applicant)

24. References

Al-Samkari, H, EJ Van Beers, KHM Kuo, W Barcellini, P Bianchi, A Glenthoj, M Del Mar Manu Pereira, R Van Wijk, B Glader, and RF Grace, 2020, The variable manifestations of disease in pyruvate kinase deficiency and their management, Haematologica, 105(9):2229-2239.

Bianchi, P and E Fermo, 2020, Molecular heterogeneity of pyruvate kinase deficiency, Haematologica, 105(9):2218-2228.

Boscoe, AN, Y Yan, E Hedgeman, EJ van Beers, H Al-Samkari, W Barcellini, SW Eber, B Glader, HM Yaish, S Chonat, M Sharma, KHM Kuo, EJ Neufeld, H Wang, M Verhovsek, S Sheth, and RF Grace, 2021, Comorbidities and complications in adults with pyruvate kinase deficiency, Eur J Haematol, 106(4):484-492.

Chioukh, R, MS Noel-Hudson, S Ribes, N Fournier, L Becquemont, and C Verstuyft, 2014, Proton pump inhibitors inhibit methotrexate transport by renal basolateral organic anion transporter hOAT3, Drug Metab Dispos, 42(12):2041-2048.

Fahmi, OA, M Shebley, J Palamanda, MW Sinz, D Ramsden, HJ Einolf, L Chen, and H Wang, 2016, Evaluation of CYP2B6 Induction and Prediction of Clinical Drug-Drug Interactions: Considerations from the IQ Consortium Induction Working Group-An Industry Perspective, Drug Metab Dispos, 44(10):1720-1730.

Gorski, JC, S Vannaprasaht, MA Hamman, WT Ambrosius, MA Bruce, B Haehner-Daniels, and SD Hall, 2003, The effect of age, sex, and rifampin administration on intestinal and hepatic cytochrome P450 3A activity, Clin Pharmacol Ther, 74(3):275-287.

Grace, RF and W Barcellini, 2020, Management of pyruvate kinase deficiency in children and adults, Blood, 136(11):1241-1249.

Grace, RF, D Mark Layton, and W Barcellini, 2019, How we manage patients with pyruvate kinase deficiency, Br J Haematol, 184(5):721-734.

Machavaram, KA, L.; Crewe, K.; Ke, A.; Hatley, O.; Burt, H.; Gardner, I.; Rowland-Yeo, K., 2017, Evaluation of In Vitro-In Vivo Extrapolation (IVIVE) of the induction potential for known CYP2C9 inducers, 14th European ISSX Meeting, GürzenichKöln, Cologne, Germany.

Mao, J, S Tay, CS Khojasteh, Y Chen, CE Hop, and JR Kenny, 2016, Evaluation of Time Dependent Inhibition Assays for Marketed Oncology Drugs: Comparison of Human Hepatocytes and Liver Microsomes in the Presence and Absence of Human Plasma, Pharm Res, 33(5):1204-1219.

Guidance for Industry *Pharmacokinetics in Patients with Impaired Hepatic Function: Study Design, Data Analysis, and Impact on Dosing and Labeling* (May 2003)

Moreau, R, D Tshikudi Malu, M Dumais, E Dalko, V Gaudreault, H Romero, C Martineau, O Kevorkova, JS Dardon, EL Dodd, DS Bohle, and T Scorza, 2012, Alterations in bone and erythropoiesis in hemolytic anemia: comparative study in bled, phenylhydrazine-treated and Plasmodium-infected mice, PLoS One, 7(9):e46101.

Murakami, J and Y Shimizu, 2013, Hepatic manifestations in hematological disorders, Int J Hepatol, 2013:484903.

Obach, RS, RL Walsky, and K Venkatakrishnan, 2007, Mechanism-based inactivation of human cytochrome p450 enzymes and the prediction of drug-drug interactions, Drug Metab Dispos, 35(2):246-255.

Rab, MAE, BA van Oirschot, S van Straaten, BJ Biemond, J Bos, PA Kosinski, C Kung, EJ van Beers, and R van Wijk, 2019, Decreased Activity and Stability of Pyruvate Kinase in Hereditary Hemolytic Anemia: A Potential Target for Therapy By AG-348 (Mitapivat), an Allosteric Activator of Red Blood Cell Pyruvate Kinase, Blood, 134(Supplement_1):3506-3506.

Shen, H, Z Yang, W Zhao, Y Zhang, and AD Rodrigues, 2013, Assessment of vandetanib as an inhibitor of various human renal transporters: inhibition of multidrug and toxin extrusion as a possible mechanism leading to decreased cisplatin and creatinine clearance, Drug Metab Dispos, 41(12):2095-2103.

Simonson, SG, A Raza, PD Martin, PD Mitchell, JA Jarcho, CD Brown, AS Windass, and DW Schneck, 2004, Rosuvastatin pharmacokinetics in heart transplant recipients administered an antirejection regimen including cyclosporine, Clin Pharmacol Ther, 76(2):167-177.

Thorne, C, 2007, Management of arthralgias associated with aromatase inhibitor therapy, Curr Oncol, 14 Suppl 1:S11-19.

Wang, X, A Zhu, J Wang, F Ma, J Liu, Y Fan, Y Luo, P Zhang, Q Li, B Xu, and P Yuan, 2020, Steroidal aromatase inhibitors have a more favorable effect on lipid profiles than nonsteroidal aromatase inhibitors in postmenopausal women with early breast cancer: a prospective cohort study, Ther Adv Med Oncol, 12:1758835920925991.

Yang, B, S Kirby, J Lewis, PJ Detloff, N Maeda, and O Smithies, 1995, A mouse model for beta 0-thalassemia, Proc Natl Acad Sci U S A, 92(25):11608-11612.

Zanella, A, E Fermo, P Bianchi, LR Chiarelli, and G Valentini, 2007, Pyruvate kinase deficiency: the genotype-phenotype association, Blood Rev, 21(4):217-231.

Zanella, A, E Fermo, P Bianchi, and G Valentini, 2005, Red cell pyruvate kinase deficiency: molecular and clinical aspects, Br J Haematol, 130(1):11-25.

25. Review Team

Table 154. Reviewers of Integrated Assessment

Role	Name(s)
Regulatory Project Manager	Courtney Hamilton, PharmD, BCPS
Nonclinical Reviewer	Shaji Theodore, PhD
Nonclinical Team Leader	Federica Basso, PhD
Office of Clinical Pharmacology Reviewer(s)	Xiaomeng Xu, PhD
Office of Clinical Pharmacology Team Leader(s)	Sudharshan Hariharan, PhD
Associate Director for Labeling	Virginia Kwitkowski, MS, ACNP-BC
Clinical Reviewer	Hyon-Zu Lee, PharmD
Clinical Team Leader	Tanya Wroblewski MD
Statistical Reviewer	Xiaoyu Cai, PhD
Statistical Team Leader	Yeh-Fong Chen, PhD
Cross-Disciplinary Team Leader	Tanya Wroblewski MD
Division Director (pharm/tox)	Todd Bourcier, PhD
Division Director (OCP)	Doanh Tran, PhD
Division Director (OB)	Thomas Gwise, PhD
Division Director (clinical)	Ann Farrell, MD
Office Director (or designated signatory authority)	Hylton Joffe, MD, MMSc.

Abbreviations: OB, Office of Biostatistics; OCP, Office of Clinical Pharmacology

Table 155. Additional Reviewers of Application

Office or Discipline	Name(s)
OPQ	Theodore Carver, PhD
Microbiology	Daniel Jansen, PhD
OPDP	Rebecca Falter
OSI	Anthony Orencia
OSE/DEPI	Steve Bird
OSE/DMEPA	Hina Mehta
OSE/DRISK	
Other	Susan Redwood

Abbreviations: DEPI, Division of Epidemiology; DMEPA, Division of Medication Error Prevention and Analysis; DRISK, Division of Risk Management; OPDP, Office of Prescription Drug Promotion; OPQ, Office of Pharmaceutical Quality; OSE, Office of Surveillance and Epidemiology; OSI, Office of Scientific Investigations

Table 156. Signatures of Reviewers

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Clinical	Hyon-Zu Lee, PharmD	OCHEN/DNH	2.1, 3.1, 3.2, 4, 6.2, 6.3, 7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 8.3, 8.4, 10, 11, 15, 16.1, 16.2, 17, 20, 22, 23 ☒ Authored ☐ Contributed ☐ Approved
Reviewer	**Signature:** Hyon Zu Lee -S Digitally signed by Hyon Zu Lee -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Hyon Zu Lee -S, 0.9.2342.19200300.100.1.1=1300389194 Date: 2022.02.03 10:22:06 -05'00'		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved1
Clinical	Tanya Wroblewski, PhD	OCHEN/DNH	All Sections ☒ Authored ☒ Contributed ☒ Approved
Cross-Disciplinary Team Lead	**Signature:** Tanya M. Wroblewski -S3 Digitally signed by Tanya M. Wroblewski -S3 DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=0011605845, cn=Tanya M. Wroblewski -S3 Date: 2022.02.02 11:40:22 -05'00'		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Clinical	Ann Farrell, MD	OCHEN/DNH	All Sections ☐ Authored ☒ Contributed ☒ Approved
Division Director	**Signature:** Ann Farrell Digitally signed by Ann Farrell DN: cn=Ann Farrell, o=Division of Nonmalignant Hematology, ou=FDA/CDER/OND/OCHEN/DNH, email=ann.farrell@fda.hhs.gov, c=US Date: 2022.02.07 09:51:53 -05'00'		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Pharmacology/Toxicology	Shaji Theodore, PhD	OND/DPTCHEN	5.1, 7.1, 8.4, 13 ☒ Authored ☐ Contributed ☐ Approved
Reviewer	**Signature:** Shaji Theodore -S Digitally signed by Shaji Theodore -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Shaji Theodore -S, 0.9.2342.19200300.100.1.1=2002145917 Date: 2022.01.31 13:29:22 -05'00'		

[1] Include "IA" for authors who contributed to the Interdisciplinary Assessment.
Abbreviations: IA, Interdisciplinary Assessment; ES, Executive Summary

Continued: Table 156. Signatures of Reviewers

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Pharmacology/Toxicology	Federica Basso, PhD	OND/DPTCHEN	5.1, 7.1, 8.4, 13 ☐ Authored ☐ Contributed ☒ Approved
Supervisor	**Signature:** Federica Basso -S Digitally signed by Federica Basso -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Federica Basso -S, 0.9.2342.19200300.100.1.1=0011076316 Date: 2022.01.31 15:37:57 -05'00'		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Pharmacology/Toxicology	Pedro DelValle, PhD, FATS	OND/DPTCHEN	5.1, 7.1, 8.4, 13 ☐ Authored ☐ Contributed ☒ Approved
Supervisor	**Signature:** Pedro L. Del Valle -A Digitally signed by Pedro L. Del Valle -A DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2001000884, cn=Pedro L. Del Valle -A Date: 2022.01.31 14:48:55 -05'00'		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Pharmacology/Toxicology	Todd Bourcier, PhD	OND/DPTCHEN	5.1, 7.1, 8.4, 13 ☐ Authored ☐ Contributed ☒ Approved
Division Director	**Signature:** Todd M. Bourcier -S Digitally signed by Todd M. Bourcier -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=1300235462, cn=Todd M. Bourcier -S Date: 2022.01.31 14:44:55 -05'00'		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Clinical Pharmacology	Xiaomeng Xu, PhD	OTS/OCP/DCEP	5, 6.1, 8.1, 8.2, 14.1, 14.2, 14.6 ☒ Authored ☐ Contributed ☐ Approved
Reviewer	**Signature:** Xiaomeng Xu -S Digitally signed by Xiaomeng Xu -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Xiaomeng Xu -S, 0.9.2342.19200300.100.1.1=2003180106 Date: 2022.02.01 15:08:05 -05'00'		

[1] Include "IA" for authors who contributed to the Interdisciplinary Assessment.
Abbreviations: IA, Interdisciplinary Assessment; ES, Executive Summary

Continued: Table 156. Signatures of Reviewers

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Clinical Pharmacology/Pharmacometrics	Manuela Grimstein, PhD	OTS/OCP/DPM	8.2,14.4 ☒ Authored ☐ Contributed ☐ Approved
Reviewer	**Signature:** Manuela D. Grimstein -S Digitally signed by Manuela D. Grimstein -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2000561102, cn=Manuela D. Grimstein -S Date: 2022.02.01 11:30:30 -05'00'		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Clinical Pharmacology/Pharmacometrics	Xinyuan Zhang, PhD	OTS/OCP/DPM	8.2, 14.4 ☐ Authored ☐ Contributed ☒ Approved
Team Leader	**Signature:** Xinyuan Zhang -S Digitally signed by Xinyuan Zhang -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Xinyuan Zhang -S, 0.9.2342.19200300.100.1.1=2000431943 Date: 2022.02.01 10:57:40 -05'00'		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Clinical Pharmacology/Pharmacometrics	Ye Yuan, PhD	OTS/OCP/DPM	6.1, 8.1, 14.3 ☒ Authored ☐ Contributed ☐ Approved
Reviewer	**Signature:** Ye Yuan -S Digitally signed by Ye Yuan -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Ye Yuan -S, 0.9.2342.19200300.100.1.1=2002006018 Date: 2022.02.02 09:56:45 -05'00'		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Clinical Pharmacology/Pharmacometrics	Liang Li, PhD	OTS/OCP/DPM	6.1, 8.1, 14.3 ☐ Authored ☐ Contributed ☒ Approved
Team Leader	**Signature:** Liang Li -S Digitally signed by Liang Li -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Liang Li -S, 0.9.2342.19200300.100.1.1=2001459144 Date: 2022.02.01 10:47:58 -05'00'		

[1] Include "IA" for authors who contributed to the Interdisciplinary Assessment.
Abbreviations: IA, Interdisciplinary Assessment; ES, Executive Summary

Continued: Table 156. Signatures of Reviewers

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Clinical Pharmacology	Katarzyna Drozda, PharmD, MS	OTS/OCP/DTPM	8.1, 14.5 ☒ Authored ☐ Contributed ☐ Approved
Reviewer	**Signature:** Katarzyna Drozda -S Digitally signed by Katarzyna Drozda -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2001644139, cn=Katarzyna Drozda -S Date: 2022.02.01 13:58:09 -05'00'		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Clinical Pharmacology	Christian Grimstein, PhD	OTS/OCP/DTPM	8.1, 14.5 ☐ Authored ☐ Contributed ☒ Approved
Team Leader	**Signature:** Christian Grimstein -S Digitally signed by Christian Grimstein -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2000357917, cn=Christian Grimstein -S Date: 2022.02.01 11:49:41 -05'00'		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Clinical Pharmacology	Sudharshan Hariharan, PhD	OTS/OCP/DCEP	5, 6.1, 8.1, 8.2, 14.1, 14.2, 14.6 ☐ Authored ☒ Contributed ☒ Approved
Team Leader	**Signature:** Sudharshan Hariharan -S Digitally signed by Sudharshan Hariharan -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2000394743, cn=Sudharshan Hariharan -S Date: 2022.02.02 10:00:36 -05'00'		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Clinical Pharmacology	Jayabharathi Vaidyanathan, PhD	OTS/OCP/DCEP	5, 6.1, 8.1, 8.2, and 14 ☐ Authored ☐ Contributed ☒ Approved
Associate Director	**Signature:** Jayabharath Vaidyanathan -S Digitally signed by Jayabharath Vaidyanathan -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=1300220018, cn=Jayabharath Vaidyanathan -S Date: 2022.02.01 12:54:46 -05'00'		

[1] Include "IA" for authors who contributed to the Interdisciplinary Assessment.
Abbreviations: IA, Interdisciplinary Assessment; ES, Executive Summary

Continued: Table 156. Signatures of Reviewers

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Statistical	Xiaoyu Cai, PhD	OTS/OB/DBIX	6.2.1.4, 6.2.2.2, 6.2.2.3, 6.2.3.4, 6.2.4.2, 6.2.4.3,16.3, 16.5, 16.6, 16.7 ☒ Authored 6.3 ☒ Contributed ☐ Approved
Reviewer	**Signature:** Yeh Fong Chen -S Digitally signed by Yeh Fong Chen -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Yeh Fong Chen -S, 0.9.2342.19200300.100.1.1=1300157970 Date: 2022.01.31 08:48:54 -05'00'		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Statistical	Yeh-Fong Chen, PhD	OTS/OB/DBIX	1,6 &16 ☐ Authored ☒ Contributed ☒ Approved
Team Leader	**Signature:** Yeh Fong Chen -S Digitally signed by Yeh Fong Chen -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Yeh Fong Chen -S, 0.9.2342.19200300.100.1.1=1300157970 Date: 2022.01.31 08:49:42 -05'00'		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Statistical	Thomas Gwise, PhD	OTS/OB/DBIX	1,6 &16 ☐ Authored ☐ Contributed ☒ Approved
Division Director	**Signature:** Thomas E. Gwise -S Digitally signed by Thomas E. Gwise -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=1300369224, cn=Thomas E. Gwise -S Date: 2022.02.04 16:01:49 -05'00'		

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Clinical	Courtney Hamilton, PharmD	ORO/DROCHEN	Table 55 ☒ Authored ☐ Contributed ☐ Approved
Regulatory Project Manager	**Signature:** Courtney Hamilton Digitally signed by Courtney Hamilton DN: cn=Courtney Hamilton, o, ou, email=Courtney.Hamilton@fda.hhs.gov, c=US Date: 2022.02.03 14:49:09 -05'00'		

[1] Include "IA" for authors who contributed to the Interdisciplinary Assessment.
Abbreviations: IA, Interdisciplinary Assessment; ES, Executive Summary

Continued: Table 156. Signatures of Reviewers

Discipline and Title or Role	Reviewer Name	Office/Division	Sections Authored/ Acknowledged/ Approved[1]
Clinical	Charlene Wheeler	ORO/DROCHEN	Table 55 ☐ Authored ☐ Contributed ☒ Approved
Chief, Project Management	**Signature:** Charlene N. Wheeler -S Digitally signed by Charlene N. Wheeler -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2000621384, cn=Charlene N. Wheeler -S Date: 2022.02.03 14:45:17 -05'00'		

[1] Include "IA" for authors who contributed to the Interdisciplinary Assessment.
Abbreviations: IA, Interdisciplinary Assessment; ES, Executive Summary

---

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

COURTNEY V HAMILTON
02/17/2022 12:00:37 PM

HYLTON V JOFFE
02/17/2022 01:32:43 PM

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research
Office of Translational Sciences
Office of Biostatistics

STATISTICAL REVIEW AND EVALUATION

NEW DRUG APPLICATION

NDA #:	NDA 216196
Drug Name:	Mitapivat (Pyrukynd)
Indication(s):	Treatment of Adult Patients With Pyruvate Kinase Deficiency
Applicant:	Agios Pharmaceuticals, Inc.
Measure(s):	Pyruvate Kinase Deficiency Diary (PKDD) Pyruvate Kinase Deficiency Impact Assessment (PKDIA)
Clinical Outcome Assessment (COA) Type:	Patient-reported Outcome (PRO)
Date(s):	Date Submitted: June 17, 2021 PDUFA Due Date: February 17, 2022 Review Completion Date: February 10, 2022
Review Priority:	Priority
Biometrics Division:	DBIII
Patient-Focused Statistical Support (PFSS) Team:	Weimeng Wang, MEd, MA (PFSS Reviewer) Lili Garrard, PhD (PFSS Team Leader)
Statistical Team:	Xiaoyu Cai, PhD (Primary Statistical Reviewer) Yeh-Fong Chen, PhD (Statistical Team Leader)
Division of Clinical Outcome Assessment (DCOA):	Mira Patel, PhD (Reviewer) Selena Daniels, PharmD, PhD (Team Leader)
Medical Division:	Division of Nonmalignant Hematology (DNH)
Clinical Team:	Hyon-Zu Lee, PharmD (Clinical Reviewer) Tanya Wroblewski, MD (Clinical Team Leader)
Project Manager:	Courtney Hamilton, PharmD

Keywords: NDA Review, Patient-reported Outcome (PRO), Scoring Algorithm

Table of Contents

List of Tables

List of Figures

1. Background

On June 17, 2021, The Applicant (Agios Pharmaceuticals, Inc.) submitted an original new drug application (NDA) for mitapivat for the treatment of adult patients with pyruvate kinase (PK) deficiency. PK deficiency is a rare and serious autosomal recessive disorder affecting the function of the PK enzyme. Signs and symptoms of PK deficiency include fatigue, shortness of breath, bone pain, splenomegaly, and jaundice. The Applicant submitted data from two trials: Study AG-348-C-006 (Study 006) entitled, "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" and Study AG-348-C-007 (Study 007) entitled, "An Open-Label Study to Evaluate the Efficacy and Safety of Mitapivat in Regularly Transfused Adult Subjects With Pyruvate Kinase Deficiency."

This abbreviated statistical review is provided in response to a PFSS consult received from the Clinical and DCOA teams regarding the scoring algorithms for the Pyruvate Kinase Deficiency Diary (PKDD; see Appendix 1) and the Pyruvate Kinase Deficiency Impact Assessment (PKDIA; see Appendix 2). In Study 006, PKDD and PKDIA (both patient-reported outcome [PRO] measures) were used to support key secondary endpoints to assess the signs and symptoms of PK deficiency and their associated impacts. This review focuses on data from Study 006. Please refer to the Integrated Review of NDA 216196 for more details on the study designs, review issues, and the DCOA's review on the two PRO measures.

2. Applicant's Proposed Scoring Algorithms

According to the Applicant's submitted clinical outcome assessment (COA) dossier, the proposed scoring algorithms for both PKDD and PKDIA are on a T-scale, which are based on a linear transformation of a standard normal Z-score constructed by item response theory (IRT) scoring.

2.1. PKDD Scoring Algorithm

The PKDD produces a weekly average T-score, ranging from 25 to 76 with higher scores indicating greater symptom severity. The weekly average T-score is calculated using the following steps:

1. Items 6 and 7 are reverse coded first so that higher scores represent worse symptoms.
2. Given that the PKDD includes mixed-format items (i.e., both 0-10 numeric rating scale [NRS] and 0-4 verbal rating scale [VRS] items), the 0-10 NRS items are rescaled to be on the 0-4 VRS using Table 1 below.

Table 1. Numeric Rating Scale to Verbal Rating Scale Rescaling Table, Study 006

Item response on the 0-10 NRS	0	1	2	3	4	5	6	7	8	9	10
Collapsed response on the 0-4 VRS	0	0	1	1	2	2	3	3	4	4	4

Source: PFSS reviewer's table based on Appendix D of the Appendix A of the Applicant's COA dossier.
Abbreviations: NRS, numeric rating scale; VRS, verbal rating scale.

3. A raw daily sum score is calculated by summing all item scores from step 2.
4. The raw daily sum score is transformed to a daily T-score based on the PKDD scoring table (see Appendix 3).
5. The weekly average T-score is calculated as the mean of the daily T-scores within a given week if at least 4 out of 7 days are non-missing.

2.2. PKDIA Scoring Algorithm

The PKDIA T-score ranges from 30 to 76 with higher scores indicating greater negative impact. Given that the PKDIA is administered on a weekly basis, a T-score is calculated for a given week using the following steps:

1. The 0-10 NRS items are rescaled to be on a 0-4 VRS using Table 1.
2. A raw sum score is computed by summing all item scores from step 1.
3. The raw sum score is transformed to a T-score based on the PKDIA scoring table (see Appendix 4).

3. Agency's Evaluation

3.1. PKDD and PKDIA T-Scores

On October 28, 2021, a joint information request (IR) was issued by PFSS, DCOA and the Statistical teams to help facilitate review of the PKDD and PKDIA endpoint results. Specifically, PFSS asked the Applicant to (1) confirm the Agency's understanding of the scoring algorithms for PKDD and PKDIA, (2) provide justification for rescaling the 0-10 NRS items to be on the 0-4 VRS, and (3) provide rationale and justification for the PKDD and PKDIA scoring tables. The Applicant provided a written response on November 10, 2021 to the joint IR.

Upon reviewing the Applicant's responses, PFSS has major concerns regarding the scoring algorithms for both PKDD and PKDIA, as the scoring algorithms adopt multiple score transformations, which are unjustified and result in difficulties in the final T-score interpretation. Our reasonings are provide in detail below:

1. The rescaling from the 0-10 NRS to a 0-4 VRS appears to be arbitrary.
2. The T-score is a linear transformation of the Z-score, which is constructed using IRT scoring. While we acknowledge the benefit and advantage of using IRT scoring as compared with the conventional classical test theory (CTT) scoring, we cannot agree with using IRT scoring to support the PKDD- and PKDIA-based endpoint calculations due to the small sample size (n=80), the lack of detailed information on IRT scoring, and the unclear rationale of the scoring tables. Specifically,
    a. We note that small sample size could result in large measurement error in the IRT calibration, which can further contribute to pervasive uncertainties in IRT scoring. This is a well-known limitation for IRT scoring. For example, as shown in Table 10 on page 62/3673 of the COA dossier, item slopes for some of the items are very large (e.g., item 1 a1 = 5.65), which may indicate large standard

errors. Note that the Applicant did not provide standard error estimates or Fisher information at each score level in the COA dossier.

b. Based on the Applicant's justification of the scoring tables, the Z-score represents expected a posterior (EAP) score derived from the R package *mirt* (i.e., each response pattern has one corresponding EAP score). The Applicant further states that "*each EAP score in column 2 of the [scoring] table represents the most probable theta level across all possible response pattern combinations for that single scale-level sum score*". This IRT scoring method of transforming a raw sum score to a latent factor score appears to resemble a PROMIS scoring table[1]. However, without details on the exact transformation, the Agency cannot fully assess the Applicant's methodology. Furthermore, the PFSS reviewer was unable to find relevant literature to support the Applicant's scoring approach. Therefore, the resulting latent factor scores (Z-scores) and corresponding T-scores are challenging to interpret.

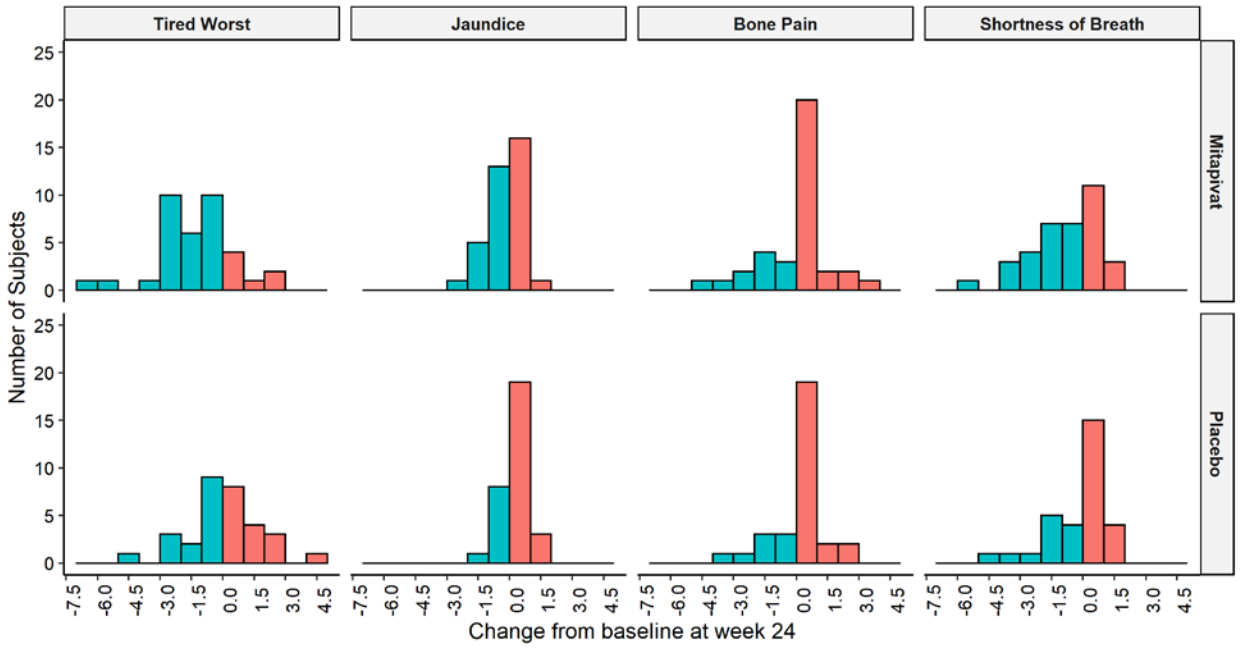
Given these concerns, PFSS recommends against the use of T-scores for efficacy analyses and potential labeling. The raw scores are more interpretable.

3.2. Exploratory Item-Level Descriptive Analyses

To help understand the observed changes at the item-level for the PKDD items, PFSS conducted exploratory descriptive analyses using the item-level raw scores (see Figure 1 and Figure 2). Note that DCOA's review on PKDIA concludes that the PKDIA includes concepts that might be influenced by factors other than treatment (e.g., interference with leisure activities, negative impact on relationships, difficulty with physical activity, interference with household activities), and may not accurately describe clinical benefit. Therefore, PFSS focused on the PKDD items.

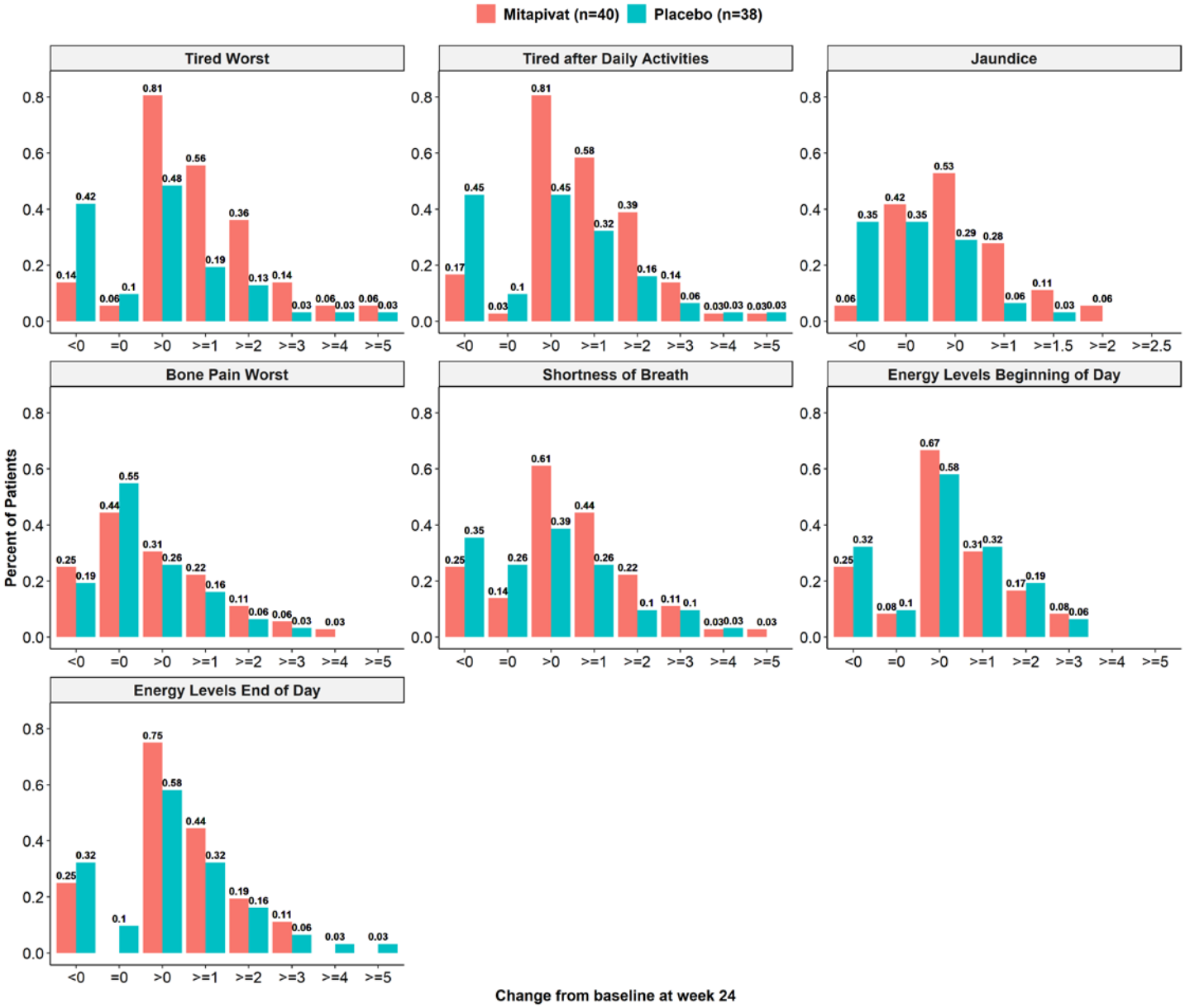
[1] https://www.healthmeasures.net/index.php?option=com_content&view=article&id=180&Itemid=994

Figure 1. Change from Baseline to Week 24 for Core Sign and Symptom Items of PKDD, Full Analysis Set, Study 006



Source: PFSS reviewer's analysis.
The histograms are based on the observed data without any imputation.
Note that blue bars are for improvements and red bars are for worsening.

Figure 2. Percentage of Patients Who Experienced Various Levels of Change (Improvement and Deterioration) from Baseline to Week 24 for Items of PKDD, Full Analysis Set, Study 006

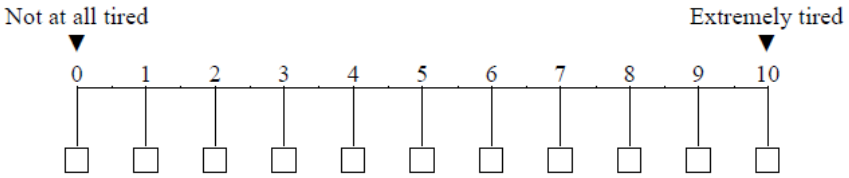


Source: PFSS reviewer's analysis.
The bar-plots are based on the observed data without any imputation. The percent of patients is calculated using the number of non-missing responses in each treatment arm.
Jaundice is measured on a 0-4 verbal rating scale. All other items are measured on a 0-10 numeric rating scale.

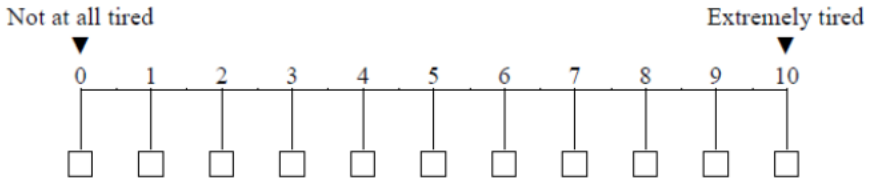
Appendix 1: Pyruvate Kinase Deficiency Diary (PKDD)

Instructions: Please answer the following questions based on your experience with Pyruvate Kinase (PK) Deficiency **today (from the time you woke up this morning to the time you are completing this questionnaire)**. Please select the box corresponding to the number that best describes you.

1. Describe how **tired** you were at its worst today.



2. Describe how **tired you felt after finishing your daily activities (e.g., work, social, leisure, physical or household activities)** today.



3. Describe your **jaundice** (how **yellow your eyes and/or skin** appeared) when you looked in the mirror today.

   ☐0 No yellow eyes and/or skin

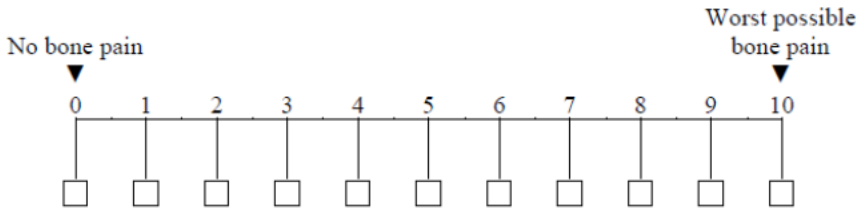
   ☐1 Mild yellow eyes and/or skin

   ☐2 Moderate yellow eyes and/or skin

   ☐3 Severe yellow eyes and/or skin

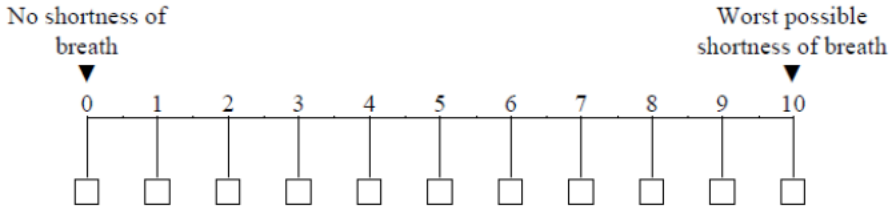
   ☐4 Very severe yellow eyes and/or skin

4. Describe your **bone pain** at its worst today.



   ☐ I have never experienced bone pain

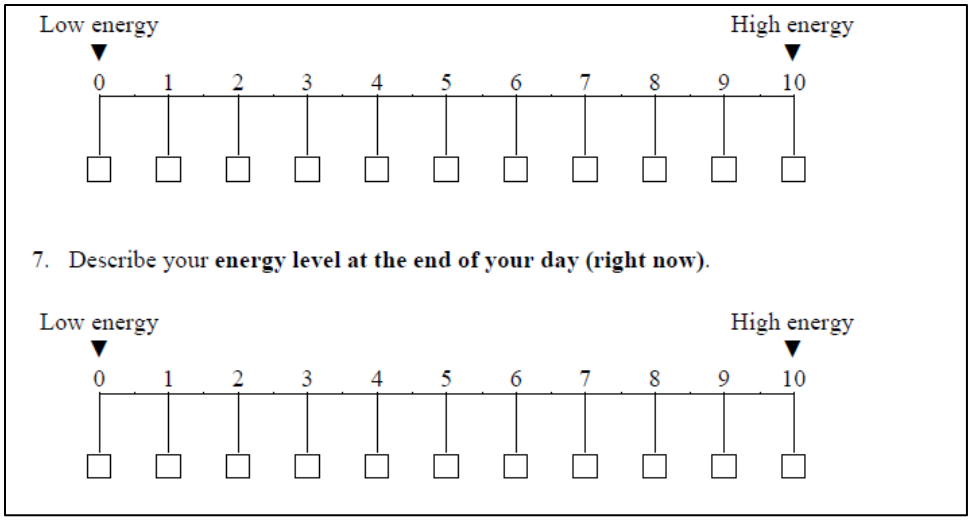
5. Describe your **shortness of breath** during moderate (e.g., walking on an incline or upstairs) physical activity you did today.



   ☐ I avoided this activity because it was too difficult for me to do moderate physical activity

   ☐ Not applicable, because I did not have the opportunity to do moderate physical activity

6. Describe your **energy level at the beginning of your day (after being awake for one hour)**.



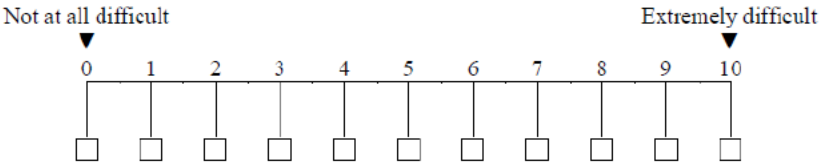
Source: Applicant's COA Dossier.

Appendix 2: Pyruvate Kinase Deficiency Impact Assessment (PKDIA)

Describe how difficult it was for you to do the following **because of how tired you felt** due to your PK deficiency over the past 7 days:

(b) (4)

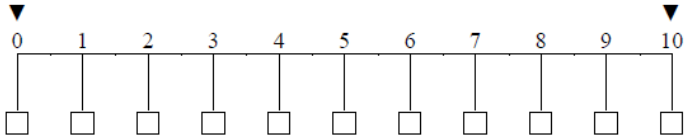
2. **Finish things you wanted to get done**



(b) (4)

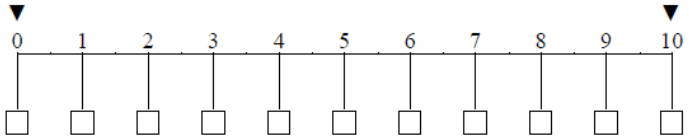
5. How often did your PK deficiency interfere with your ability to do **household activities (e.g., chores, cleaning, laundry)** over the past 7 days?

None of the time — All of the time



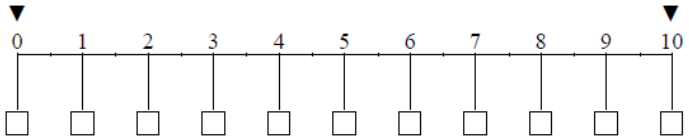
6. How often did a lack of energy due to your PK deficiency interfere with participating in **social activities (e.g., doing something together with friends)** over the past 7 days?

None of the time — All of the time

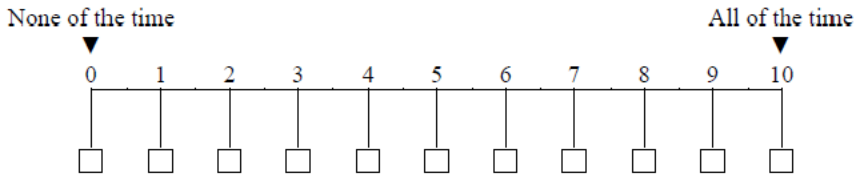


7. How often did your PK deficiency interfere with **leisure activities (i.e., hobbies or things you do for fun in your free time) over the past 7 days**?

None of the time — All of the time

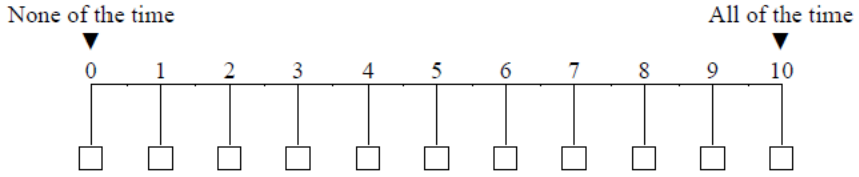


8. How often did you feel **your relationships with friends or family were negatively affected** because of your PK deficiency over the past 7 days?



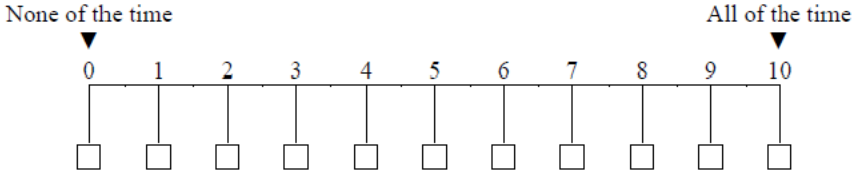
(b) (4)

10. How often did you have **difficulty concentrating** because of your PK deficiency over the past 7 days?



(b) (4)

11b (b) (4) How often did you have difficulty performing **moderate (e.g., walking on an incline or up stairs) physical activity** because of your PK deficiency over the past 7 days?



12. How much **additional rest or sleep did you feel you needed** because of your PK deficiency over the past 7 days?

- ☐0 No additional rest or sleep
- ☐1 A little additional rest or sleep
- ☐2 Some additional rest or sleep
- ☐3 Quite a bit additional rest or sleep
- ☐4 A lot of additional rest or sleep

Source: Applicant's COA Dossier.
Items not included in scoring are presented in gray.

2 Pages have been Withheld in Full as b4 (CCI/TS) immediately following this page

---

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

WEIMENG WANG
02/11/2022 09:08:29 AM

LILI GARRARD
02/11/2022 09:11:02 AM