EU RISK MANAGEMENT PLAN
FOR
PYRUKYND (MITAPIVAT)

RMP version to be assessed as part of this application:

Data lock point for this RMP	19-November-2020	Version number	1.0
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Rationale for submitting an updated RMP: Not applicable

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List of abbreviations

Abbreviation	Explanation
2,3-DPG	2,3-diphosphoglycerate
Ab	antibody
ADAM	advanced dissolution, absorption and metabolism
ADME	absorption, distribution, metabolism and excretion
AE	adverse event
AESI	adverse event of special interest
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ATP	adenosine triphosphate
AUC	area under the concentration-curve
$AUC_{0\text{-}12hr}$	AUC from time 0 to 12 hours
$AUC_{0\text{-}t}$	AUC from hour 0 to the last time point
AUC_{∞}	AUC from hour 0 to infinity
AUC_{last}	AUC from hour 0 to the last measurable concentration
AUC_{τ}	AUC from hour 0 to tau, where τ is the dosing interval
BCRP	breast cancer resistance protein
BID	twice daily
BMD	bone mineral density
$C_{avg,ss,free}$	average free concentration
$C_{avg,ss,tot}$	average total concentration
CL_{hep}	hepatic clearance
CL_{int}	intrinsic clearance
C_{max}	maximum concentration
CSR	clinical study report
CTFG	Clinical Trial Facilitation Group
CTCAE	Common Terminology Criteria for Adverse Events
CV	cardiovascular
CYP	cytochrome P450
DDI	drug-drug interaction
DLT	dose-limiting toxicity
DXA	dual energy x-ray absorptiometry
EC_{50}	half-maximal effective concentration

Abbreviation	Explanation
EMA	European Medicines Agency
EPO	erythropoietin
EPAR	European Public Assessment Report
EU	European Union
Fa	fraction absorbed
Fg	fraction of drug passing though the gut wall without metabolism
GD	Gestation Day
GI	gastrointestinal
GLP	Good Laboratory Practice
H2RA	H2-receptor antagonist
Hb	hemoglobin
HDL	high-density lipoprotein
hERG	human ether-à-go-go related gene
HLT	High Level Term
IC_{50}	half-maximal inhibitory concentration
ICH	International Council for Harmonisation
IV	intravenous
LD	Lactation Day
LLN	lower limit of normal
M/M	missense/missense
M/NM	missense/nonmissense
MATE	multidrug and toxin extrusion protein
MedDRA	Medical Dictionary for Regulatory Activities
MELD	model for end-stage liver disease
ms	millisecond(s)
NADPH	nicotinamide adenine dinucleotide phosphate
NCI	National Cancer Institute
NM/NM	nonmissense/nonmissense
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
NTBI	non-transferrin-bound iron
OAT	organic anion transporter
OATP	organic anion transporting polypeptide
OCT	organic cation transporter

Abbreviation	Explanation
ODWG	Organ Dysfunction Working Group
PEP	phosphoenolpyruvate
P-gp	P-glycoprotein
PIP	paediatric investigation plan
PK deficiency	pyruvate kinase deficiency
PKL	liver-specific form of pyruvate kinase
PKLR	liver-specific and red blood cell-specific forms of pyruvate kinase
PKM	pyruvate kinase muscle isozyme
PKR	red blood cell–specific form of pyruvate kinase
PL	Package Leaflet
PND	Postnatal Day
pQCT	quantitative computed tomography
PT	Preferred Term
QD	once daily
QOL	quality of life
QTc	corrected QT interval
QTcF	Fridericia-corrected QT interval
RBC	red blood cell
RMP	risk management plan
SAE	serious adverse event
SCD	sickle cell disease
SmPC	Summary of Product Characteristics
SMQ	Standardised MedDRA Query
SOC	System Organ Class
TK	toxicokinetic
t_{max}	time to reach C_{max}
UGT	uridine 5’-diphospho-glucuronosyl transferase
ULN	upper limit of normal
US	United States

Part I: Product Overview

Table Part I.1: Product Overview

Active substance (INN or common name):	mitapivat
Pharmacotherapeutic groups (ATC Code):	ATC code: B06AX04
Name of Applicant:	Agios Netherlands B.V.
Medicinal products to which this RMP refers:	1
Invented name in the European Economic Area (EEA):	Pyrukynd
Marketing authorization procedure:	Centralized
Brief description of the product:	Chemical class: pyruvate kinase activator
	Summary of mode of action: Pyruvate kinase deficiency (PK deficiency) is characterized by diminished activity of the red blood cell (RBC)-specific form of the pyruvate kinase (PKR) enzyme, which leads to reduced adenosine triphosphate (ATP) levels, shortened RBC lifespan, and chronic hemolysis. Mitapivat is a pyruvate kinase activator and acts by directly binding to the pyruvate kinase tetramer. Mitapivat improves RBC energy homeostasis by increasing PKR activity and ATP concentrations, while decreasing 2,3-diphosphoglycerate (2,3-DPG).
	Important information about its composition: Mitapivat drug product is supplied as 5 mg, 20 mg, and 50 mg tablet strengths (free-base equivalent) for oral administration. Mitapivat drug substance is a synthetically derived, small molecule. Each film-coated tablet contains lactose monohydrate equivalent to 0.3, 1.4, and 3.4 mg lactose in mitapivat tablets, 5 mg, 20 mg, and 50 mg, respectively.
Hyperlink to the Product Information:	Pyrukynd product information (Module 1.3.1)
Indication in the EEA:	Current: Mitapivat is indicated for the treatment of pyruvate kinase deficiency (PK deficiency) in adult patients.
	Proposed: Not applicable
Dosage in the EEA	Current: The recommended starting dose of Pyrukynd is 5 mg taken orally twice daily. To gradually increase hemoglobin levels and maximize the effect, Pyrukynd should be titrated through sequential doses of 5 mg twice daily, 20 mg twice daily, and 50 mg twice daily with dose increases to the next dose level every 4 weeks.
	Proposed: not applicable

Pharmaceutical form and strengths	Current: film-coated tablet Mitapivat Tablets, 5 mg and 20 mg, are blue, round, film-coated tablets printed with “M5” or “M20” in black ink on one side of the tablet, respectively, for product identification. The approximate diameters for the Mitapivat Tablets, 5 mg and 20 mg, are 5 mm and 8 mm, respectively. The Mitapivat Tablets, 50 mg, are blue, oblong, film-coated tablets printed with “M50” in black ink on one side of the tablet for product identification. The approximate tablets dimensions are length of 16 mm and width of 6.8 mm. Mitapivat drug product is supplied as 5 mg, 20 mg, and 50 mg tablet strengths (free-base equivalent).
	Proposed: not applicable
Will the product be subject to additional monitoring in the EU?	Yes

Part II: Safety Specification

Part II: Module SI – Epidemiology of the indication and target population

Indication:

Mitapivat (also known as mitapivat sulfate, AG-348, and AG-348 sulfate hydrate and formerly known as AGI-1480 and AGX-0841) is indicated for the treatment of pyruvate kinase deficiency (PK deficiency) in adult patients.

Incidence and prevalence:

Pyruvate kinase deficiency is currently classified as the most frequent cause of congenital nonspherocytic hemolytic anemia with a prevalence estimated at 1/20,000 in the general White population on Orphanet (Orphanet, 2020). However, as with many rare genetic diseases, the true prevalence of PK deficiency is not well understood (Beutler and Gelbart, 2000). A systematic review and critical appraisal of the literature was recently undertaken to better understand the epidemiology of the disease (Secrest et al, 2020). With no established international databases designed to ascertain the prevalence of PK deficiency, the scope of the systematic review included peer-reviewed publications from indexed literature search platforms and hand searches of relevant conferences.

Searches were conducted in Medline, Embase, PubMed, Ovid, a single conference year (2018) of the Deutsche Gesellschaft für Hämatologie und Medizinische Onkologie, and other publicly available sources of information to estimate the prevalence of PK deficiency. Pyruvate kinase deficiency and PKLR terms and keywords were combined with epidemiology and gene frequency terms and keywords in queries. Search terms used included, but were not limited to, pyruvate kinase, pyruvate metabolism, PK deficiency, PKLR, gene mutation, prevalence, and incidence.

The systematic review concluded that the best estimate of diagnosed prevalence of PK deficiency is between 3.2 and 8.5 per million in Western populations, while the prevalence of diagnosed and undiagnosed PK deficiency may be as high as 51 per million (Beutler and Gelbart, 2000; Carey et al, 2000; de Medicis et al, 1992). These findings suggest that most patients with PK deficiency remain undiagnosed (Secrest et al, 2020).

Demographics of the population in the proposed indication – age, gender, racial and/or ethnic origin, and risk factors for the disease:

Pyruvate kinase deficiency occurs equally in men and women and most often presents within the first month of life; however, diagnoses in adulthood are frequent, particularly in patients with compensated hemolysis, mild anemia, or misdiagnosed with another hemolytic disorder (Grace and Barcellini, 2020). Overall, patients with more severe anemia are diagnosed at an earlier age, but diagnosis may also be delayed until late adulthood (Grace et al, 2019; Zanella et al, 2005). Higher frequency of the recessively inherited disease reflects consanguinity or, more commonly, a founder effect. This can be seen in the Pennsylvania Amish community where a particularly high frequency exists, associated with a homozygous 1436G→A mutation that results in the production of histidine instead of arginine, which can be traced back to a single immigrant couple (Kanno et al, 1994). Similarly, this can also be seen in the Romani communities, associated with a 1,149 base pair deletion and loss of exon 11 (Baronciani and Beutler, 1995). Higher onset of symptoms leading to diagnosis is also seen in females aged 21 to 30 years. This preponderance might be due to pregnancy as pregnancy

itself precipitates hemolysis, and blood counts are more likely to be obtained during this time (Hirono et al, 1988). Furthermore, recent data suggest the genetic epidemiology of PK deficiency may also be influenced by selection. Evidence of a protective effect has been obtained from murine malaria models and reduced replication of plasmodium falciparum in the red cells of pyruvate kinase deficient patients ex vivo (Min-Oo et al, 2003; Qidwai et al, 2014). A high frequency of PK deficiency is found in populations in the Middle East and Sub-Saharan Africa that have been subject to selective pressure from malaria (Machado et al, 2012; van Bruggen et al, 2015).

The main existing treatment options:

Currently there are no approved disease-specific therapeutic agents for the treatment of patients with PK deficiency. Historic attempts to develop pharmacologic treatments, such as riboflavin and sulphydryl compounds, have been unsuccessful (Blume et al, 1976; Staal et al, 1975; Zanella et al, 1976). Patients' treatment options are supportive, treating the symptoms of lifelong hemolytic anemia and associated complications. These supportive care options may lead to additional complications by themselves and further compound complications of the existing disease as well. To treat the anemia common interventions include splenectomy and transfusions that increase hemoglobin (Hb) concentrations while iron chelation and cholecystectomy treat the common complications of the hemolytic anemia, described in more detail below. Hematopoietic allogeneic stem cell transplantation, although potentially curative, is rarely performed because of a relatively high morbidity and mortality rate compared with supportive care (Grace and Barcellini, 2020; van Straaten et al, 2018). Mitapivat, a pharmacologic activator of PKR, would be the first product indicated for the treatment of PK deficiency in adult patients if approved.

Approximately half (59%) of all patients diagnosed with PK deficiency undergo total splenectomy, with median age at splenectomy of 4.1 years, either to reduce the need for transfusion (in patients who are regularly receiving transfusions) or to increase Hb (in patients who are not regularly receiving transfusions but who tolerate their anemia poorly). Splenectomy may not ameliorate anemia or alleviate the need for transfusions because hemolysis continues at other anatomical sites (Grace et al, 2019). In addition, splenectomy exposes patients to the risk of infection, which can require prolonged prophylactic antibiotic therapy and strict vaccination compliance, and increases the risk of thrombosis, pulmonary hypertension, and iron overload (Crary and Buchanan, 2009; Evans, 1985; Jones et al, 2016).

Partial splenectomy has been considered as an alternative to total splenectomy in multiple types of congenital hemolytic anemias due to the emergence of antibiotic-resistant pneumococci and the risk of post-splenectomy thrombosis. The goal of partial splenectomy, in which at least 80%-90% of the spleen is removed, is to decrease hemolysis while leaving behind a functioning splenic remnant for phagocytic function. There are a few case reports documenting the results of partial splenectomy in patients with PK deficiency with 2 documented failures and 1 documented success with an increase in the baseline Hb and reduction in transfusions (Diesen et al, 2008; Rice et al, 2003; Sandoval et al, 1997). In practice, most clinicians do not recommend partial splenectomy for PK deficiency (Grace et al, 2019).

Another common intervention is RBC transfusion. The decision to transfuse a patient with PK deficiency is based on each patient's tolerance of anemia, their lifestyle, and quality of life (QOL) considerations; there are no guidelines defining recommended transfusion regimens (Al-Samkari et al, 2020; Grace et al, 2019). Patients with similar Hb concentrations and symptom severity can differ in their chosen transfusion frequency; therefore, transfusion

frequency should not be used as an indicator of disease severity (Al-Samkari et al, 2020). Although transfusions can temporarily increase Hb, they have been shown to lead to increases in iron overload, which may result in further complications and negatively affect QOL. In the PK Deficiency Natural History Study, patients who were regularly receiving transfusions after splenectomy had higher median ferritin and were more likely to require chelation therapy (94% vs 44%) than patients who discontinued regular transfusions after splenectomy (Grace et al, 2018). Patient surveys indicate that patients are concerned about the risks of iron overload and the need for chelation therapy associated with regular transfusions, as well as the prospect of being dependent on transfusions for their entire lives (Al-Samkari et al, 2020; White et al, 2020).

Iron chelation is commonly used because of iron overload caused by PK deficiency itself and by other supportive care treatments such as transfusion. However, compliance is often poor, limiting its benefit, and there are risks associated with its use. For example, decreases in serum creatinine clearance and increases in transaminases, requiring frequent monitoring, are common with deferasirox. Cases of hepatitis and serious hypersensitivity reaction have also been reported (Exjade (deferasirox) Summary of Product Characteristics, 2016; Exjade (deferasirox) Package Insert, 2019; Jadenu (deferasirox) Package Insert, 2019).

Besides the supportive treatments targeting improvement of hemolytic anemia, there are other treatments focused on additional complications of the disease. For instance, another frequent complication of PK deficiency is gallstones due to increased levels of unconjugated bilirubin; these are frequently treated with cholecystectomy (Grace et al, 2019; Grace et al, 2015). However, patients who have undergone cholecystectomy can still develop biliary lithiasis, which can result in chronic liver damage. Furthermore, patients with PK deficiency often have osteopenia and osteoporosis and endocrine dysfunction, such as thyroid disease, which all need to be appropriately managed.

Natural history of the indicated condition in the untreated population, including mortality and morbidity:

An observational study to obtain critical information regarding the natural history of PK deficiency and the range and incidence of related symptoms, treatments, and complications—known as the PK Deficiency Natural History Study (NCT02053480)—has been developed by Boston Children's Hospital (Boston, Massachusetts, United States [US]) and was funded and supported by Agios. This multicenter, global Natural History Study was designed as a longitudinal cohort study with retrospective, baseline, and annual collection of data over a 2-year period and has identified 254 pediatric and adult subjects. Patients with PK deficiency present with anemia and a range of comorbidities, such as splenectomy, thrombosis, sepsis, iron overload with secondary organ damage, gallstones, extramedullary hematopoiesis, and bone abnormalities (Grace et al, 2018).

Important comorbidities:

Splenomegaly is present in 80%-85% of individuals with PK deficiency, with variable degrees of enlargement (Grace et al, 2019). Possible causes of splenomegaly include deposition of iron and dead RBCs in the spleen from hemolysis and extramedullary erythropoiesis. Hypersplenism can occur in the setting of splenomegaly and should be suspected in patients with an increasing transfusion burden, mild thrombocytopenia, or neutropenia. Individuals with palpable splenomegaly may be at risk for splenic injury with trauma to the abdomen (Grace et al, 2019).

Splenectomy is a common procedure in subjects with PK deficiency, which causes a loss of immunological defense, placing patients at risk for infections and sepsis with encapsulated organisms (Zahid and Bains, 2017). Antibiotics for infection prophylaxis and a more intensive immunization schedule are indicated after splenectomy. The ideal duration for prophylactic antibiotics is not clear. Some physicians recommend a lifetime of prophylactic antibiotics, and others will recommend discontinuation after 1 year if patients live close to a medical center and agree to seek urgent medical care for all fevers (Grace et al, 2019). In the PK deficiency Natural History Study, post-splenectomy sepsis was reported in 7% of patients (Grace et al, 2018).

Because PK deficiency causes severe hemolytic anemia, patients are more susceptible to developing jaundice and gallbladder disease. In healthy adults, RBCs are phagocytized and broken down by macrophages, with the heme group in Hb being converted to unconjugated bilirubin. The unconjugated bilirubin is then exported from the macrophage, binds to albumin, and is taken up by hepatocytes. Once in the hepatocyte, the bilirubin is conjugated and excreted into bile. In cases of hemolysis, increasing amounts of unconjugated bilirubin are produced and unconjugated bilirubin builds up in the blood, leading to high total bilirubin values. Severity of hemolysis and modifiers of bilirubin metabolism determine the degree of indirect hyperbilirubinemia. Nearly every patient with PK deficiency will have an elevated indirect bilirubin level, due to increased unconjugated bilirubin, and will experience frequent complications of jaundice and gallstones. Gallstones are a frequent complication of PK deficiency, occurring at all ages and reported in 30%-45% of patients (Grace et al, 2019; Zanella et al, 2007).

Another common comorbidity of PK deficiency is iron overload. Iron overload in PK deficiency, similar to other hemolytic anemias, develops because of ineffective erythropoiesis, which causes the suppression of hepcidin, the key systemic regulator of iron metabolism, which leads to increased intestinal iron absorption and release of iron from iron stores (Finkenstedt et al, 2009; Rider et al, 2011a). Furthermore, iron overload can be secondary to regular transfusions, which has been used to treat severe forms of the disease such as hemolytic episodes, severe symptoms of anemia, and aplastic crisis. The human body lacks a physiologic mechanism for removal of the excess iron load resulting from blood transfusion. Each unit of transfused packed RBCs contains approximately 200 to 250 mg of elemental iron. In the presence of iron overload, after transferrin binding is saturated, non-transferrin-bound iron (NTBI) is readily transported through calcium channels into the hepatocytes, cardiac myocytes, and endocrine glands. The accumulation of iron in different organs leads to the different clinical complications of iron overload. Reactive oxygen species produced by the metabolism of NTBI contribute to the cellular dysfunction, apoptosis, and necrosis in target organs. Iron overload has been associated with multiple comorbidities including heart failure secondary to cardiac siderosis, cardiac arrythmia, liver fibrosis, chronic viral hepatitis, pulmonary hypertension, leg ulcers, and endocrine disease (hypothyroidism, hypoparathyroidism, growth retardation, hypogonadism, osteoporosis/osteopenia, and diabetes). Patients with transfusion-dependent treatment tend to develop clinical iron overload after 10-20 transfusions while non-transfusion-dependent patients will develop clinical iron overload later in life at approximately 10-15 years of age (Grace et al, 2019).

Patients with PK deficiency are likely to develop osteopenia throughout their lifetimes. The pathogenesis of PK deficiency osteopenia is multifactorial. Anemia itself, along with iron overload and its associated complications, can all have detrimental effects on bone health. Ineffective erythropoiesis can cause an exponential increase in the number of erythroid

precursors produced, resulting in bone marrow expansion. Iron overload directly disrupts bone formation, has toxic effects on osteoblasts, and induces a decrease in the recruitment of cells of the osteoblastic lineage, and transfusion-related iron deposition in endocrine glands can cause impaired growth (Balogh et al, 2018). Patients with PK deficiency are at risk for low bone mineral density (BMD), fractures, and bone pain. Adults with PK deficiency have a 15.6% higher chance of developing osteoporosis compared with the general population, with the chances increasing to 21.2% in patients who have ever received a transfusion (Boscoe et al, 2020). Correspondingly, in a large cohort study, universal dual-energy X-ray absorptiometry (DXA) scanning has revealed that over three-quarters of adults with PK deficiency had osteopenia or osteoporosis (median age of 36 years), irrespective of transfusion requirements (Al-Samkari, Grace, et al, 2020). Bone fractures have been reported in 17% of adult subjects with PK deficiency (Grace et al, 2018).

Similar to other hemolytic disorders, pulmonary hypertension is a complication of PK deficiency. The dysfunction and destruction of erythrocytes leads to numerous changes in the cardiovascular system, both as compensatory mechanisms and as pathologic consequences of the disease. Anemia results in reduced blood viscosity, elevated cardiac output, decreased cardiac filling pressures, and reduced systemic and pulmonary vascular resistance. In addition to changes resulting directly from anemia, organ dysfunction may contribute to pathologic changes in the cardiovascular system. Liver disease, cardiac dysfunction, renal impairment, and splenic changes may all be confounding factors when examining the mechanisms by which pulmonary hypertension develops in chronic hemolytic anemia (Fraidenburg and Machado, 2016). Pulmonary hypertension, diagnosed by echocardiogram and confirmed with cardiac catheterization, is a complication in 3% of patients with PK deficiency after splenectomy (Bachmeyer et al, 2009; Grace et al, 2018).

Extramedullary hematopoiesis, driven by ineffective erythropoiesis, occurs in approximately 10% of patients (Grace et al, 2018). This occurs most commonly in the liver and spleen but also occurs in the paravertebral area and mediastinum (Aizawa et al, 2003; Plensa et al, 2005). Rarely, extramedullary hematopoiesis occurs in the central nervous system, eye, lymph nodes, lung, or pleura.

Leg ulcers have also been reported in patients with PK deficiency. The etiology is multifactorial, although it is important to note that erythrocytes in hemoglobinopathies are rigid and inflexible and can cause occlusion of smaller blood vessels, vascular obstruction, and subsequently tissue necrosis. Other potential contributing factors to consider are infections, abnormal autonomic control with excessive vasoconstriction, thrombosis, decrease in oxygen carrying capacity due to anemia, and impaired endothelial function (Alavi and Kirsner, 2015).

Part II: Module SII – Nonclinical part of the safety specification

A series of nonclinical pharmacology, absorption, distribution, metabolism and excretion (ADME), pharmacokinetic, and toxicology studies have been conducted to support the Marketing Authorisation Application for mitapivat for the treatment of PK deficiency in adult patients.

Key Safety Findings (from nonclinical studies)	Relevance to Human Use
Toxicity	
Single- or Repeat-Dose Toxicity Studies The main toxicities from the nonclinical single- or repeat-dose toxicity studies were hypersensitivity (anaphylactoid reactions) observed in dogs, reproductive findings in rats, and emesis observed in monkeys. No evidence of liver injury (unrelated to hypertrophy) was observed in any of the toxicity studies.	
Hypersensitivity: In a non–Good Laboratory Practice (GLP)-compliant, single-dose study of mitapivat in beagle dogs, clinical observations consistent with anaphylactoid reactions, including swelling (ear, eyelid, mouth, paw) and/or discoloration (reddening of the mouth, thoracic region, abdominal region, or whole body) were observed at ≥30 mg/kg. Emesis was observed at ≥62.5 mg/kg. Lethargy, prostration, and reduced activity were noted in dogs given 125 mg/kg on Day 1. The maximum tolerated dose was 62.5 mg/kg, which correlated with area under the concentration-time curve (AUC) from time 0 to 12 hours ($AUC_{0\text{-}12hr}$) and maximum (peak) concentration (C_{max}) values of 8,580 hr•ng/mL (2.4-fold the human $AUC_{0\text{-}12hr}$ value [3,580 hr•ng/mL] at 50 mg twice daily [BID]) and 4,890 ng/mL, respectively. The no-observed-effect level (NOEL)/no-observed-adverse-effect level (NOAEL) in dogs was 10 mg/kg, which correlated with mitapivat $AUC_{0\text{-}12hr}$ and C_{max} values of 1,200 hr•ng/mL (0.34-fold the human $AUC_{0\text{-}12hr}$ value at 50 mg BID) and 447 ng/mL (0.46-fold the human C_{max} value at 50 mg BID), respectively. Based on the results of the single-dose studies, the rat and the monkey were chosen as the toxicology species for further evaluation. The dog was considered the less appropriate higher species because of anaphylactoid reactions, to which dogs are known to be more sensitive than humans. These reactions would have limited the exposure in subsequent dog studies, thereby compromising the full evaluation of toxicity at high exposure	Hypersensitivity: The clinical signs seen in the single-dose dog study were typical of anaphylactoid reactions described in dogs (Dowling, 2009; Hacker et al, 1981; Lorenz et al, 1977; Lorenz et al, 1982; Waddell, 2010), which have been noted after administration of lipid and nonlipid detergents, radiocontrast media, nonsteroidal anti-inflammatory drugs, analgesics, and morphine (Szebeni, 2005). Anaphylactoid reactions have been observed after oral administration of drugs in dogs in early development programs. Importantly, it is well known that dogs are very sensitive to anaphylactoid reactions compared with rats or humans (Lorenz et al, 1977; Lorenz et al, 1982). Additionally, the liquid formulation administered in dogs was different from the tablet or capsule formulation administered to humans. No events suggestive of an anaphylactoid reaction have been reported with mitapivat in clinical studies in healthy adult subjects and subjects with PK deficiency. Hypersensitivity adverse events (AEs) were observed in subjects with PK deficiency who received mitapivat. In the clinical development program, hypersensitivity AEs were reported for 26 subjects (26 of 155, 16.8%) with PK deficiency and 6 subjects (6 of 155, 3.9%) had hypersensitivity AEs assessed as related to treatment by the Investigator (Table 19-3.1, Table 18.3.1-10.6b). There were no Grade ≥3 AEs of hypersensitivity or serious adverse events (SAEs) of hypersensitivity in studies with mitapivat (Table 19-3.1, Table 19-4.1). The incidence of events of hypersensitivity was higher in subjects who received placebo in Study AG348-C-006, a Phase 3, randomized,

Key Safety Findings (from nonclinical studies)	Relevance to Human Use
multiples over those expected clinically, and dogs were not used in any further toxicology or safety pharmacology studies (Module 2.6.6, Section 2.2.1).	double-blind, placebo-controlled clinical trial of adult patients with PK deficiency who were not regularly transfused, compared with subjects who received mitapivat. During the 24-week period, hypersensitivity AEs were reported for 10 subjects (10 of 39, 25.6%) with PK deficiency who received placebo and 4 subjects (4 of 40, 10%) with PK deficiency who received mitapivat (Table 18.3.1-10.6a). Hypersensitivity was observed in humans; however, the observed AEs were mild in severity, nonserious, and resolved without treatment discontinuation. Hypersensitivity is a potential risk that is not considered important for inclusion in the list of safety concerns in the risk management plan (RMP). Hypersensitivity is a potential risk with minimal clinical impact on patients (in relation to the severity of the indication treated) (Module SVII.1.1).
Gastrointestinal (emesis) and hepatic findings: In a 28-day GLP-compliant repeat-dose monkey study, administration of mitapivat resulted in nonadverse higher liver weights in the 150 mg/kg/day group males and females with no correlating microscopic observations. The NOEL was determined to be 50 mg/kg/day and the NOAEL was 150 mg/kg/day for both sexes. The dosage level of 150 mg/kg/day resulted in Day 27 mitapivat $AUC_{0\text{-}12hr}$ and C_{max} values of 13,400 hr•ng/mL (3.7-fold the human $AUC_{0\text{-}12hr}$ value at 50 mg BID) and 3,510 ng/mL, respectively, in males and 12,700 hr•ng/mL (3.5-fold the human $AUC_{0\text{-}12hr}$ value at 50 mg BID) and 3,260 ng/mL, respectively, in females (Module 2.6.6, Section 3.2.4). In a 13-week GLP-compliant repeat-dose monkey study, administration of mitapivat resulted in nonadverse clinical observations of emesis at all dose levels, transient body weight loss during Week 0 in the 100 and 200 mg/kg/day group males and females, and microscopic changes of hepatocellular hypertrophy correlating with higher liver weights in the ≥50 mg/kg/day group males and females. Based on these results, the NOAEL was 200 mg/kg/day. The dosage level of 200 mg/kg/day resulted in Day 90 mean C_{max} values of 2,420 ng/mL and 3,180 ng/mL, and mean $AUC_{0\text{-}12hr}$ values of 10,400 hr•ng/mL and 14,600 hr•ng/mL (2.9- and 4.1-fold the human $AUC_{0\text{-}12hr}$ value at 50 mg BID), for males and	Hepatic findings: No evidence of liver injury (unrelated to hypertrophy) was observed in any of the single or repeat-dose toxicity studies. This is important as (quantitative) structure-activity relationship models have predicted mitapivat to have hepatotoxic potential due to the presence of the sulfonamide substructure, as well as the known link to hepatotoxicity of sulfonamide class of antibiotic drugs (4-aminobenzene-sulfonamides). These agents are characterized by a 5- or 6-membered nitrogen-containing ring attached to the nitrogen (N1) of the sulfonamide group and an arylamine group (N4) at the para position from the sulfonamide moiety (Brackett, 2007; Brackett et al, 2004; Choquet-Kastylevsky et al, 2002; Hemstreet and Page, 2006; Knowles et al, 2001; Smith and Jones, 2008; Strom et al, 2003; Tornero et al, 2004; Verdel et al, 2006). The liver toxicity associated with the sulfonamide antibiotics is part of a spectrum of adverse reactions believed to be caused by metabolites of the antibiotics, which are specific to the N1 and N4 structural context (Brackett, 2007; Brackett et al, 2004; Choquet-Kastylevsky et al, 2002; Hemstreet and Page, 2006; Knowles et al, 2001; Naisbitt et al, 1999; Tornero et al, 2004). Mitapivat is structurally distinct from the sulfonamide antibiotics. Mitapivat does not belong to the 4 aminobenzene-sulfonamide class and, more importantly, does not have the N1 or the N4 moieties highlighted previously. Mitapivat will be metabolized distinctly and cannot produce the metabolites thought to be responsible for sulfonamide antibiotic-related liver toxicity.

Key Safety Findings (from nonclinical studies)	Relevance to Human Use
females, respectively (Module 2.6.6, Section 3.2.5). In a 9-month GLP-compliant repeat-dose monkey study, a minimal nonadverse increase in emesis was noted. At necropsy, increased liver and adrenal weights, correlating with hepatocellular hypertrophy and hypertrophy of the zona fasciculata, respectively, at or above 50 mg/kg/day. Minimal subcapsular hepatocellular pressure necrosis, secondary to adaptive hepatocellular hypertrophy, was observed in the 100 and 200 mg/kg/day males and 200 mg/kg/day females and was considered adverse. Increased incidence of pigmented macrophages in lungs, liver, and lymph nodes in all mitapivat groups was considered a nonadverse exacerbation of what was seen in control animals. Based on the results of this study, the NOAEL was considered to be 50 mg/kg/day for males and 100 mg/kg/day for females. These dosage levels corresponded to mean $AUC_{0\text{-}12hr}$ values of 4,370 and 8,040 hr•ng/mL (1.2- and 2.2-fold the human $AUC_{0\text{-}12hr}$ value at 50 mg BID) and mean C_{max} values of 1,570 and 2,350 ng/mL for males and females, respectively, on Day 270 (Module 2.6.6, Section 3.2.6). Reproductive findings: In a GLP-compliant 28-day rat study, histologic effects consistent with off-target aromatase inhibition were observed in the male and female reproductive tract at the mid- and high-dosage levels, which were associated with $AUC_{0\text{-}12hr}$ values ≥79,000 hr•ng/mL. The NOAEL was considered to be 600 mg/kg/day for males and 20 mg/kg/day for females. Dosage levels of 600 mg/kg/day for males and 20 mg/kg/day for females resulted in Day 27 mitapivat $AUC_{0\text{-}12hr}$ values of 118,000 and 10,400 hr•ng/mL (33- and 2.9-fold the human $AUC_{0\text{-}12hr}$ value at 50 mg BID), respectively, and mitapivat C_{max} values of 27,500 and 4,730 ng/mL, respectively (Module 2.6.6, Section 3.1.3). In a 13-week GLP-compliant repeat-dose rat study, adverse microscopic findings included spermatid retention, tubular degeneration, and increased incidence and/or severity of Leydig cell hypertrophy in males at ≥150 mg/kg/day and uterine atrophy in females at 100 mg/kg/day. Based on the results of this study, the NOAEL was considered to be	In Study AG348-C-001, a Phase 1 single-ascending dose clinical study in healthy subjects, 1 subject who received a single dose of mitapivat 120 mg experienced a Grade 2 alanine aminotransferase (ALT) and Grade 1 aspartate aminotransferase (AST) increase without increased bilirubin on Day 17 that resolved without treatment. An additional 6 subjects experienced transient Grade 1 laboratory increases in ALT and/or AST (CSR AG348-C-001). In Study AG348-C-002, a Phase 1 multiple-ascending dose clinical study in healthy subjects, 1 subject who received mitapivat 700 mg BID experienced a dose-limiting toxicity (DLT) of Grade 3 increase in ALT and AST and an increase of bilirubin to 1.5 times the upper limit of normal (ULN) after 11 days. Treatment was stopped, and the subject's liver test results returned to normal. The sponsor and Investigator assessed the elevated liver tests as related to mitapivat and they were considered a DLT. An additional 10 subjects had transient Grade 1 laboratory increases in ALT and/or AST across dosing levels (CSR AG348-C-002). Transaminase increases were observed in subjects with PK deficiency who received mitapivat. In the clinical development program, transaminase increased AEs were reported for 24 subjects (24 of 155, 15.5%) with PK deficiency and 10 subjects (10 of 155, 6.5%) had transaminase increased AEs assessed as related to treatment by the Investigator (Table 19-3.1, Table 18.3.1-10.6b). There were no SAEs of transaminase increased reported in subjects with PK deficiency treated with mitapivat (Table 19-4.1). The incidence of events of transaminase increased was higher in subjects who received placebo in Study AG348-C-006 compared with subjects who received mitapivat, possibly due to untreated hemolysis associated with PK deficiency. During the 24-week period, transaminase increased AEs were reported for 6 subjects (6 of 39, 15.4%) with PK deficiency who received placebo and only 1 subject (1 of 40, 2.5%) with PK deficiency who received mitapivat (Table 18.3.1-10.6a). A higher percentage of subjects in Study AG348-C-007, an open-label clinical trial in regularly transfused adult subjects with PK deficiency, who received mitapivat had events of transaminase increase compared with subjects who received mitapivat or placebo in Study AG348-C-006 (Table 18.3.1-10.6a, Table 18.3.1-10.6b). Subjects in Study AG348-C-007 were regularly receiving transfusions and had a higher rate

Key Safety Findings (from nonclinical studies)	Relevance to Human Use
60 mg/kg/day for males and 50 mg/kg/day for females. Dosage levels of 60 mg/kg/day for males and 50 mg/kg/day for females resulted in Day 90 mitapivat $AUC_{0\text{-}12hr}$ values of 16,800 and 39,400, respectively (4.7 and 11-fold the human $AUC_{0\text{-}12hr}$ value at 50 mg BID), and mitapivat C_{max} values of 5,550 and 13,600, respectively (Module 2.6.6, Section 3.1.4). In a 6-month GLP-compliant repeat-dose rat study, potentially adverse microscopic findings included retained spermatids and occasional vacuolation of the seminiferous epithelium in males at ≥150 mg/kg/day and uterine atrophy and increased vaginal mucification in the 200 mg/kg/day group females. All test article-related findings recovered or partially recovered during the recovery period except for mean body weights, which remained similar to the end of the dosing period; however, body weight gains during the recovery period were similar to those of the control group animals. Based on the results of this study, the NOAEL was considered to be 60 mg/kg/day in males and 50 mg/kg/day in females. The 60 mg/kg/day dosage level in males corresponded to a mean $AUC_{0\text{-}12hr}$ value of 23,900 hr•ng/mL (6.7-fold the human $AUC_{0\text{-}12hr}$ value at 50 mg BID) and a mean C_{max} value of 9,770 ng/mL, and in females, the 50 mg/kg/day dosage level corresponded to a mean $AUC_{0\text{-}12hr}$ value of 66,000 hr•ng/mL (18-fold the human $AUC_{0\text{-}12hr}$ value at 50 mg BID) and a mean C_{max} value of 16,700 ng/mL for mitapivat on Study Day 180 (Module 2.6.6, Section 3.1.5).	of chelation therapy than subjects in Study AG348-C-006. Transaminase increases were considered an adverse event of special interest (AESI) for mitapivat in clinical studies with subjects with PK deficiency. The sponsor conducted intensive investigation and assessment of all AESI and transaminase increased AEs reported. There were no clinically relevant trends identified in the events reported that would indicate mitapivat as the perpetrator of the AEs. Transaminase increase is not considered a risk for mitapivat. Gastrointestinal (emesis) findings: Relevance to human usage is detailed below in the local tolerance section. Reproductive findings: Relevance to human usage is detailed below in the reproductive/ developmental toxicity section.
Reproductive/Developmental Toxicity **Embryo-Fetal Development** GLP-compliant definitive embryo-fetal development studies were conducted in Sprague Dawley rats and New Zealand white rabbits. Developmental toxicities were observed in rats but not rabbits. Fetal adverse effects in rats were considered likely due to changes in sex hormones related to aromatase inhibition. In the definitive study in pregnant female Sprague Dawley rats, oral administration of mitapivat to pregnant female rats resulted in maternal toxicity at a dosage level of 200 mg/kg/day as evidenced by moribundity of a single female, as well as test article–related clinical findings of clear nasal discharge, clear material around the mouth, and salivation during the postdosing observations and macroscopic	Embryo-fetal toxicity: The GLP-compliant definitive embryo-fetal development study in Sprague Dawley rats and New Zealand white rabbits demonstrated that fetal adverse effects were observed at $AUC_{0\text{-}12hr}$ values 63-fold (rats) and 3.1-fold (rabbits) the human $AUC_{0\text{-}12hr}$ value at 50 mg BID. In the GLP-compliant combined fertility and early embryonic development study in Sprague Dawley rats, no effect on mating or fertility was observed at any dosage in either males or females. The highest dosages tested were 300 mg/kg/day in males and 200 mg/kg/day in females, correlating with $AUC_{0\text{-}12hr}$ (day last) values [160,000 hr•ng/mL] 45-fold and [174,000 hr•ng/mL] 49-fold the $AUC_{0\text{-}12hr}$ value in humans at 50 mg BID, respectively. The lowest $AUC_{0\text{-}12hr}$ value at which microscopic reproductive findings have been observed in animals was 10,900 hr•ng/mL (Module 2.6.6, Section 3.1.4), and

Key Safety Findings (from nonclinical studies)	Relevance to Human Use
findings of enlarged or fused placentae and a distended amniotic sac. In addition, a test article–related lower mean body weight gain with corresponding reduced mean food consumption was noted at 200 mg/kg/day after the initiation of dose administration. No evidence of maternal toxicity was noted at 10, 20, or 50 mg/kg/day. An increased mean litter proportion of post-implantation loss with a corresponding decrease in the mean number and litter proportion of viable fetuses was noted in the 200 mg/kg/day group. In addition, lower mean fetal weights and test article–related external, soft tissue, and skeletal malformations were noted for fetuses in the 200 mg/kg/day group. Intrauterine growth, survival, and fetal morphology were unaffected by test article administration at 10, 20, and 50 mg/kg/day. Based on these results, teratogenicity consistent with changes in sex hormones due to aromatase inhibition was observed in the embryo-fetal development study at $AUC_{0\text{-}12hr}$ values [of 227,000 hr•ng/mL] 63-fold the human $AUC_{0\text{-}12hr}$ value at 50 mg BID. A dosage level of 50 mg/kg/day was considered the NOAEL for maternal and embryo-fetal developmental toxicity when mitapivat was administered orally by gavage to bred Sprague Dawley rats. A dosage level of 50 mg/kg/day resulted in $AUC_{0\text{-}12hr}$ values of 23,900 and 46,800 hr•ng/mL (6.7- and 13-fold the human $AUC_{0\text{-}12hr}$ value at 50 mg BID) and C_{max} values of 8,400 and 11,400 ng/mL on Gestation Day (GD) 6 and 17, respectively (Module 2.6.6, Section 6.2.1.2). In the definitive reproductive toxicity study in time-mated New Zealand white rabbits, based on adverse clinical findings (decreased defecation), body weight deficits, and corresponding reduced food consumption at 125 mg/kg/day, a dosage level of 60 mg/kg/day was considered to be the NOAEL for maternal toxicity. Lower mean fetal body weights were noted at 125 mg/kg/day; therefore, a dosage level of 60 mg/kg/day was considered to be the NOAEL for embryo-fetal development when mitapivat was administered orally by gavage to time-mated New Zealand white rabbits. A dosage level of 125 mg/kg/day resulted in $AUC_{0\text{-}12hr}$ values of 9,190 and 11,200 hr•ng/mL (2.6- and 3.1-fold the human $AUC_{0\text{-}12hr}$ value at 50 mg BID) and C_{max} values of 4,270 and 5,390 ng/mL on GD 7 and 20, respectively. A dosage level of 60 mg/kg/day	the reproductive organ NOEL in the repeat-dose toxicity study in which this occurred was 5,150 hr•ng/mL (1.4-fold the human $AUC_{0\text{-}12hr}$ value [3,580 hr•ng/mL] at 50 mg BID). All findings with the potential to impair fertility in the Sprague Dawley rats were reversible after discontinuation of the study drug. Women of reproductive potential and men with partners who are women of reproductive potential were included in the clinical studies but were required to be abstinent as part of their usual lifestyle, or agree to use 2 forms of contraception, 1 of which must have been considered highly effective, from the time of giving informed consent, during the study, and for at least 1 month after the last dose of study treatment for women and 90 days after the last dose of study treatment for men. This is in accordance to the clinical trial facilitation group (CTFG) guidance because mitapivat is classified as an investigational medical product with possible human teratogenicity/fetotoxicity in early pregnancy (CTFG, 2014). In Study AG348-C-003, a Phase 2, open-label, randomized, dose-ranging study in adult subjects with PK deficiency, 1 subject, a [redacted]-year-old female, became pregnant after receiving mitapivat 25 mg BID for approximately 11 months. Upon report of the pregnancy, treatment with mitapivat was immediately discontinued by the Investigator. The subject received mitapivat 25 mg BID for 39 days between her last menstrual period and the positive home pregnancy test. The pregnancy proceeded normally with no events reported in the mother or fetus. The subject gave birth to a normal healthy baby [redacted] by scheduled caesarian section. The projected exposure of the subject was 19 times lower than the exposure in the rat at the dose where no adverse effects were observed on the fetus and the mother (ie, the NOAEL) (CSR AG348-C-003, Section 14.3.3). Additionally, 1 pregnancy was reported in a partner of a male subject who was receiving mitapivat 25 mg BID in Study AG348-C-003. The subject received their first dose of mitapivat approximately 4 years before the reported pregnancy of the partner with treatment ongoing. The subject's partner was a [redacted]-year-old female who was on a contraceptive pill at the time of conception and had an ultrasound confirm normal pregnancy. The subject's partner gave birth to a normal healthy baby [redacted] by vaginal episiotomy. A complication in delivery was noted with the subject's partner

Key Safety Findings (from nonclinical studies)	Relevance to Human Use
resulted in $AUC_{0\text{-}12hr}$ values of 3,930 and 5,360 hr•ng/mL (1.1- and 1.5-fold the human $AUC_{0\text{-}12hr}$ value at 50 mg BID) and C_{max} values of 2,320 and 2,430 ng/mL on GD 7 and 20, respectively (Module 2.6.6, Section 6.2.2.3). **Fertility and Early Embryonic Development** A GLP-compliant, combined fertility and early embryonic development study was conducted to evaluate oral administration of mitapivat in male and female Sprague Dawley rats before cohabitation, through mating and implantation, and through recovery. The results of the combined fertility and early embryonic development study demonstrated effects on maternal and paternal parameters; however, no effects on mating or fertility were observed. The paternal NOEL for mitapivat was 60 mg/kg/day. Reduced body weights and body weight gains were observed in the males at 300 mg/kg/day and reduced food consumption was observed at 150 and 300 mg/kg/day. At 300 mg/kg/day, a mitapivat-related macroscopic observation of bilateral small testes was observed, and mitapivat-related microscopic findings were observed at ≥150 mg/kg/day in the testes and epididymides, including degeneration of the seminiferous tubules, spermatid retention, and atypical residual bodies in the testes as well as an increased incidence of cellular debris in the epididymides. These pathologic findings correlated with decreased sperm motility and density and increased numbers of abnormal sperm (detached or no head). The findings observed in the treated male rats did not affect mating parameters at doses up to and including 300 mg/kg/day and were determined to be reversible during the 10-week recovery period. The paternal NOAEL was determined to be the high dose of 300 mg/kg/day as no effect on mating and fertility parameters was observed up to an including this dose. The Day 84 $AUC_{0\text{-}12hr}$ value correlating with this dose was 160,000 hr•ng/mL (45-fold the human $AUC_{0\text{-}12hr}$ value at 50 mg BID). The maternal NOEL for mitapivat was 50 mg/kg/day. Reductions in food consumption and decreases in progesterone levels were observed at 200 mg/kg/day. There were also decreases in the number of estrous stages during the precohabitation period at 200 mg/kg/day, but this change was not considered adverse because	experiencing postpartum hemorrhage, which led to prolongation of hospitalization. There was a manual removal of placenta [redacted] (CSR AG348-C-003, Section 14.3.3). The SmPC Section 4.6 states that women of childbearing potential should avoid becoming pregnant while receiving Pyrukynd. Women of childbearing potential should use contraception during treatment with Pyrukynd and for at least 1 month after the last dose. Mitapivat may decrease the systemic exposure of hormonal contraceptives that are sensitive substrates of CYP3A4 (see SmPC Sections 4.4 and 4.5). Additional or alternative methods of contraception should be considered. The SmPC Section 4.6 states that there are no or a limited amount of data from the use of mitapivat in pregnant women. Studies in animals have shown reproductive toxicity (see SmPC Section 5.3). Pyrukynd is not recommended during pregnancy and in women of childbearing potential not using contraception. The SmPC Section 4.6 states that there are no human data on the effect of mitapivat on fertility. Animal studies have shown reversible effects on reproductive organs of males and females (see SmPC Section 5.3). While taking mitapivat, there may be an impact on the ability of a woman and a man to conceive. Section 5.3 of the SmPC describes the relevant nonclinical findings pertinent to effects on male and female reproductive organs and sperm quality, considered related to the sex hormone changes due to the inhibition of aromatase by mitapivat. Although human data on pregnancies are limited, there is no evidence of human teratogenicity based on genotoxic potential and the 2 successful pregnancies. Although nonclinical reproductive toxicity studies did not demonstrate teratogenicity/fetotoxicity at maternal exposures comparable to those in humans administered mitapivat at 50 mg BID (proposed clinical dose), these studies demonstrated embryo-fetal toxicity at high dose levels. Based on these nonclinical findings, embryo-fetal toxicity is considered an important potential risk (Module SVII.3.1).

Key Safety Findings (from nonclinical studies)	Relevance to Human Use
there were no effects on the number of females that mated or were pregnant. There were no test article–related histopathologic findings observed in the female rats at doses up to 200 mg/kg/day. All findings in females resolved during the 4-week recovery period. The maternal NOAEL for mitapivat was determined to be the high dose of 200 mg/kg/day as no effect on mating and fertility parameters was observed up to and including this dose. The Day 29 $AUC_{0\text{-}12hr}$ value correlating with this dose was 174,000 hr•ng/mL (49-fold the human $AUC_{0\text{-}12hr}$ value at 50 mg BID) (Module 2.6.6, Section 6.1.1). **Developmental and Perinatal/Postnatal Reproduction** A GLP-compliant oral gavage developmental and perinatal/postnatal reproduction study was conducted in Sprague Dawley rats to detect adverse effects of mitapivat on maternal rats and development of the offspring consequent to exposure of the maternal rats from implantation through lactation and weaning (Module 2.6.6, Section 6.3.1). Mitapivat was administered to females (F0 generation) beginning on GD 7 and continuing through Lactation Day (LD) 20. Administration of ≥50 mg/kg/day mitapivat was not tolerated in F0 generation females and resulted in unscheduled mortality due to dystocia/prolonged parturition. Maternal mitapivat-related clinical observations were observed at ≥50 mg/kg/day, primarily in animals that experienced unscheduled mortalities or dams euthanized due to no surviving pups. Reduced mean maternal body weights, body weight gains, and food consumptions, and increases in mean duration of gestation and percentage of dams with stillborn pups or no liveborn pups were observed only at 200 mg/kg/day. Reduced postpartum pup viability was observed at 200 mg/kg/day, resulting in significant reductions in offspring (F1 generation) up to postpartum Day 10, and because of excessive mortality, no F1 generation rats in the 200 mg/kg/day group were weaned or included for postweaning assessment. There were no mitapivat-related effects on any reflex and development parameter in preweaning F1 pups at any dose; however, the limited number of litters surviving at 200 mg/kg/day for evaluation precluded accurate interpretation of findings at that dose. Maternal doses ≤50 mg/kg/day did not result in postweaning F1	

Key Safety Findings (from nonclinical studies)	Relevance to Human Use
generation mortality, clinical observations, body weight, food consumption, or alterations on the day of preputial separation or vaginal opening. Maternal doses of mitapivat as high as 50 mg/kg/day did not affect learning and memory, mating and fertility, macroscopic observations, sperm evaluation, or any ovarian and uterine parameter in the F1 generation rats or naïve female rats mated with F1 generation males. Based on these results, the NOAEL for general toxicity in the F0 generation females was 20 mg/kg/day, and reproductive toxicity in the F1 generation males and females was considered to be 50 mg/kg/day. Per protocol, toxicokinetic (TK) parameters were determined in this study; however, based on GD 17 exposures observed in the definitive rat embryo-fetal development study, the maternal NOAEL would correspond with maternal $AUC_{0\text{-}12hr}$ values of 12,500 hr•ng/mL (3.5-fold the human $AUC_{0\text{-}12hr}$ value at 50 mg BID), and the NOAEL for reproductive toxicity in F1 rats would correspond with maternal $AUC_{0\text{-}12hr}$ values of 46,800 hr•ng/mL (13-fold the human $AUC_{0\text{-}12hr}$ value at 50 mg BID).	
Lactation There are no nonclinical data on excretion of mitapivat in milk.	Lactation: Safety in this population is currently not known. The SmPC Section 4.6 states that it is unknown whether mitapivat and/or its metabolites are excreted in human milk. A risk to newborns/infants cannot be excluded. A decision must be made whether to discontinue breast-feeding or to abstain from Pyrukynd therapy taking into account the benefit of breast-feeding for the child and the benefit of therapy for the woman. Use during lactation is not considered part of the safety concerns in the RMP.
Studies in Juvenile Animals In a non–GLP-compliant 28-day range-finding oral gavage toxicity study in juvenile Sprague Dawley rats, mitapivat was administered from Postnatal Day (PND) 7 through 35 or PND 21 through 49 (Report AG348-N-101) (Module 2.6.6, Section 6.4.1). Regardless of dose or the period of dose administration, oral administration of mitapivat did not produce mortality, adverse clinical observations, differences in mean body weights, mean body weight gains, mean food consumption values, or macroscopic observations in males or females. Mean testes weights, and testes weights relative	Findings in the GLP-compliant juvenile toxicology study largely recapitulated findings observed in previous toxicology and reproductive and development studies using adult Sprague Dawley rats, with only few novel findings as outlined below. Most findings were attributable to changes in sex hormones due to aromatase inhibition and/or perturbations in CYP enzyme expression. Consistent with studies in adult rats, reproductive effects were observed upon sexual maturity in juvenile rats administered mitapivat; all evaluable reproductive effects outlined below were reversible after a non-dosing recovery period. Delayed sexual maturity was observed in males at $AUC_{0\text{-}12hr}$ values

Key Safety Findings (from nonclinical studies)	Relevance to Human Use
to mean body weights and brain weights were increased in males in the 20 and 100 mg/kg/day dose groups on PND 21. Based on these results, the NOAEL in males and females after mitapivat administration from PND 7 to 35 was 100 mg/kg/day ($AUC_{0\text{-}12hr}$ range of 20,900 to 76,300 hr•ng/mL and C_{max} range of 6,690 to 20,500 ng/mL). The NOAEL for males and females administered mitapivat from PND 21 to 49 was 300 mg/kg/day ($AUC_{0\text{-}12hr}$ 39,400 hr•ng/mL and C_{max} 7,500 ng/mL) and 100 mg/kg/day ($AUC_{0\text{-}12hr}$ 71,800 hr•ng/mL and C_{max} 22,200 ng/mL), respectively. In a GLP-compliant 13-week study in juvenile Sprague Dawley rats (Report AG348-N-102) (Module 2.6.6, Section 6.4.2), mitapivat was administered from PND 7 through PND 97 (in main study animals) or from PND 7 through PND 125/LD 6 (in reproductive subset animals) to juvenile Sprague Dawley rats, with a Recovery Phase of approximately 11 weeks (males) and approximately 6 weeks (females). Reproductive effects were observed upon sexual maturity in juvenile rats administered mitapivat; all evaluable reproductive effects were reversible after a nondosing recovery period. Delayed sexual maturity was observed in males at ≥150 mg/kg/day; there was no effect of mitapivat on sexual maturity in females. Lower percentage of motile sperm and epididymal sperm density were observed at 300 mg/kg/day. Negative effects of mitapivat on male mating and fertility parameters and litter and uterine examinations after mating with untreated female cohorts at ≥150 mg/kg/day and longer precoital interval and lower mating, fertility, and pregnancy indices were observed, as well as lower pregnancy rates and lower implantation sites and live pups/litter. In females, longer estrous cycle length was observed at 200 mg/kg/day. Mitapivat administration to juvenile female rats resulted in dystocia/ prolonged parturition upon sexual maturation at ≥50 mg/kg/day, manifesting as adverse clinical observations, mortality, prolonged gestation, higher numbers of stillborn pups and poor pup viability/survival. Reversible hematology findings of lower reticulocytes and/or higher red cell distribution width were observed in males and females at all doses of mitapivat. Reversible higher urine pH	≥79,000 hr•ng/mL, or ≥22-fold the human $AUC_{0\text{-}12hr}$ value at 50 mg BID; there was no effect of mitapivat on sexual maturity in females. Unlike the fertility study, there were negative effects of mitapivat on male mating and fertility parameters and litter and uterine examinations after mating with untreated female cohorts at $AUC_{0\text{-}12hr}$ values ≥79,000 hr•ng/mL, or ≥22-fold the human $AUC_{0\text{-}12hr}$ value at 50 mg BID; longer precoital interval and lower mating, fertility, and pregnancy indices were observed, as well as lower pregnancy rates and lower implantation sites and live pups/litter. Clinical pathology findings in juvenile rats administered mitapivat were similar to those in adult rats with a few exceptions as outlined below. Reversible hematology findings of lower reticulocytes and/or higher red cell distribution width were observed in juvenile males and females at all doses of mitapivat and $AUC_{0\text{-}12hr}$ values ≥5,430 hr•ng/mL, or ≥1.5-fold the human $AUC_{0\text{-}12hr}$ value at 50 mg BID; in studies in adult rats, this finding was most prominent in female rats and occurred only sporadically in males. Reversible higher urine pH was observed in males at all doses at $AUC_{0\text{-}12hr}$ values ≥5,430 hr•ng/mL, or ≥1.5-fold the human $AUC_{0\text{-}12hr}$ value at 50 mg BID, and this finding was not previously observed in adult rats. Reversible or partially reversible lower triglycerides were observed in females at all doses and $AUC_{0\text{-}12hr}$ values ≥6,480 hr•ng/mL, or ≥1.8-fold the human $AUC_{0\text{-}12hr}$ value at 50 mg BID, and in males only at the high dose and $AUC_{0\text{-}12hr}$ values 223,000 hr•ng/mL, 62-fold the human $AUC_{0\text{-}12hr}$ value at 50 mg BID; lower triglycerides were not commonly observed in adult rat toxicology studies and were seen only in males at high doses. A unique finding in juvenile rats was lower cholesterol observed only in the recovery period in males and females at all doses. In females, higher thyroid and ovary weights were observed at $AUC_{0\text{-}12hr}$ values ≥72,000 hr•ng/mL, or ≥20-fold the human $AUC_{0\text{-}12hr}$ value at 50 mg BID, and weights remained higher after the recovery period. Higher ovary weights were observed in toxicology studies using adult female rats. Higher thyroid weights were a novel finding in juvenile rats; however, the proliferative thyroid findings were attributable to chronic CYP enzyme induction after initiation of dosing and were observed in the rat carcinogenicity study. Higher liver weights with correlative hepatocellular hypertrophy consistent with CYP enzyme induction were a prominent

Key Safety Findings (from nonclinical studies)	Relevance to Human Use
was observed in males at all doses. Reversible or partially reversible lower triglycerides were observed in females at all doses and in males only at the 300 mg/kg/day dose. Lower cholesterol was observed only in the recovery period in males and females at all doses. Organ weight changes in male juvenile rats included higher testis weights correlating histologically with seminiferous tubule dilatation at all doses and higher epididymis weights correlating with epididymal cellular debris 300 mg/kg/day; all findings partially resolved after the recovery period. In females, higher thyroid and ovary weights were observed at ≥50 mg/kg/day, and weights remained higher after the recovery period. Higher liver weights with correlative hepatocellular hypertrophy consistent with cytochrome P450 (CYP) enzyme induction were a prominent finding in adult rat toxicology studies, and both findings were conspicuously absent in juvenile rats. Bone changes including lower bone density and mass were observed at all doses in males and at ≥50 mg/kg/day mg in females; bone changes were at least partially reversible after the recovery period. The NOAEL for general toxicity could not be established in males due to adverse microscopic findings in reproductive organs at all dose levels. In females, the NOAEL for general toxicity was 10 mg/kg/day based on adverse clinical observations in females occurring at the end of the gestation period/during parturition. The NOAEL for F1 growth and development was 30 mg/kg/day in males and 200/100 mg/kg/day in females due to effects on sexual maturation that were observed in males. The NOAEL for mating and fertility was 30 mg/kg/day in males and 200/100 mg/kg/day in females based on effects on multiple mating and fertility parameters in males and the absence of effects on mating and fertility parameters at any dose level in females. The NOAEL for reproductive toxicity in females was 10 mg/kg/day based on mortality and adverse clinical observations as a result of dystocia/prolonged parturition. The NOAEL for F2 generation preweaning growth and development was at least the paternal dose of 30 mg/kg/day in males (higher dose levels could not be assessed due to the low number of viable F2 pups) and 100 mg/kg/day in females (based	finding in adult rat toxicology studies, and both findings were conspicuously absent in juvenile rats. It is likely that initiation of dosing of mitapivat in neonatal rats (PND 7) resulted in persistently altered expression of CYP enzymes in treated animals and is related to the persistent changes in the recovery period of ovarian and thyroid weights, as well as altered cholesterol homeostasis evident in the recovery period (Piekos et al, 2017). Bone changes including lower bone density and mass were observed at all doses and $AUC_{0\text{-}12hr}$ values ≥5,430 hr•ng/mL, or ≥1.5-fold the human $AUC_{0\text{-}12hr}$ value at 50 mg BID, in males and $AUC_{0\text{-}12hr}$ values ≥72,000 hr•ng/mL, or ≥20-fold the human $AUC_{0\text{-}12hr}$ value at 50 mg BID, in females; bone changes were at least partially reversible after the recovery period. The pediatric population is included as a special population in the Pyrukynd SmPC Sections 4.2 and 5.2. Per Section 4.2 of the Pyrukynd SmPC, the safety and efficacy of Pyrukynd in children and adolescents less than 18 years old have not been established. No data are available. Non-clinical studies in juvenile animals have been conducted (see Section 5.3 of the SmPC). In the Pyrukynd SmPC Section 5.2, it states that the pharmacokinetics of mitapivat in children and adolescent patients less than 18 years old have not been studied. Also, the indication statement for Pyrukynd in the SmPC (Section 4.1) clearly states that the medicinal product is for use in adult patients. Use of mitapivat in pediatric subjects has not yet been evaluated in the clinical development program. As the target indication does not cover pediatric patients, pediatric use is not a risk.

Key Safety Findings (from nonclinical studies)	Relevance to Human Use
on dose level during the mating/gestation period). The NOAEL for memory, learning, ambulation, fine movement, and habituation was 300 mg/kg/day in males and 200/100 mg/kg/day in females. On PND 97, the $AUC_{0\text{-}12hr}$ was 5,430 (1.5-fold the human $AUC_{0\text{-}12hr}$ value at 50 mg BID), 79,000 (22-fold the human $AUC_{0\text{-}12hr}$), and 223,000 (62-fold the human $AUC_{0\text{-}12hr}$) hr•ng/mL in males at 30, 150, and 300 mg/kg/day, respectively, and 6,480 (1.8-fold the human $AUC_{0\text{-}12hr}$), 72,000 (20-fold the human $AUC_{0\text{-}12hr}$), 215,000 (60-fold the human $AUC_{0\text{-}12hr}$) hr•ng/mL in females at 10, 50, and 200/100 mg/kg/day, respectively.	
Genotoxicity Mitapivat was nonmutagenic and nonclastogenic in GLP-compliant assays including bacterial reverse mutation assays, an in vitro micronucleus assay in human peripheral blood lymphocytes, and an in vivo micronucleus assay in Sprague Dawley rats (Reports AG348-N-002-R1, AG348-N-003-R1, AG348-N-004-R1, AG348-N-043-R1, AG348-N-044-R1, and AG348-N-045-R1) (Module 2.6.6, Section 4.1.1).	Mitapivat is not mutagenic or clastogenic based on these in vitro and in vivo studies. Genotoxicity is not considered a risk for mitapivat.
Carcinogenicity In a GLP-compliant 2-year study in juvenile Sprague Dawley rats, mitapivat was administered for at least 104 consecutive weeks to determine the carcinogenicity and TK characteristics of mitapivat. Mitapivat administration was associated with neoplastic lesions in the liver and thyroid of males at 300 mg/kg/day and preneoplastic lesions in the liver of males at all dose levels and females at 200 mg/kg/day, which is most likely related to known effects of mitapivat on CYP enzyme induction and associated hepatocellular changes (centrilobular hypertrophy). Beyond this effect, there was no evidence that mitapivat caused de novo tumor types or promoted rare tumor types within the liver. Additionally, in the pancreas of males, acinar adenoma and hyperplasia were observed at an increased incidence and/or severity at ≥30 mg/kg/day; however, the pancreatic findings were within the range of historical control data at ≤100 mg/kg/day and were outside the historical control data range only at 300 mg/kg/day and $AUC_{0\text{-}12hr}$ values of >168,000 hr•ng/mL, >47-fold the human $AUC_{0\text{-}12hr}$ at 50 mg BID. A NOEL was not	Proliferative and sometimes neoplastic lesions were observed in the liver, thyroid, ovaries, and pancreas in the 2-year study in juvenile Sprague Dawley rats; the findings were not considered to represent a human safety risk nor alter the benefit-risk profile of mitapivat. In the liver, administration of mitapivat was associated with an increased incidence of hepatocellular adenoma/carcinoma in males given 300 mg/kg/day, an increased incidence and/or severity of foci of cellular alteration at ≥30 mg/kg/day in males and at 200 mg/kg/day in females, a dose-related increased incidence and severity of centrilobular hypertrophy in both sexes at all doses, and an increased incidence of biliary cysts in females at 200 mg/kg/day. In the thyroid gland, administration of mitapivat was associated with increased incidence of follicular cell adenoma and diffuse follicular cell hypertrophy in males given 300 mg/kg/day. The higher incidence of hepatocellular adenoma/carcinoma, hepatocellular foci of cellular alteration, and centrilobular hypertrophy, as well as thyroid follicular cell adenoma and diffuse cell hypertrophy, were considered secondary to hepatic CYP induction. Mitapivat induces CYP enzymes in the liver of rats, and hepatocellular hypertrophy has been a consistent

Key Safety Findings (from nonclinical studies)	Relevance to Human Use
determined. At the lowest dose levels in males and females, the mitapivat Day 182 $AUC_{0\text{-}12hr}$ values were 13,000 and 28,600 hr•ng/mL (3.6- and 8.0-fold the human $AUC_{0\text{-}12hr}$ value at 50 mg BID) for males and females, respectively (Module 2.6.6, Section 5.1.1). In a GLP-compliant 26-week carcinogenicity study in CByB6F1/Tg rasH2 hemizygous mice, mitapivat was administered for 26 weeks to determine the potential carcinogenicity and TK characteristics of mitapivat. Oral administration of mitapivat was not carcinogenic in CByB6F1/Tg rasH2 hemizygous males at ≤500 mg/kg/day or females at ≤250 mg/kg/day. After the first daily dose on Day 182 of the highest respective doses, $AUC_{0\text{-}12hr}$ values of 22,800 hr•ng/mL and 9,180 hr•ng/mL in males and females (6.4- and 2.6-fold the human $AUC_{0\text{-}12hr}$ value at 50 mg), respectively, were observed (Module 2.6.6, Section 5.2.1).	feature in previous rat toxicology studies. These findings were considered rodent-specific and not relevant for human safety assessment (Hall et al, 2012). In the ovaries, an increased incidence and/or severity of granulosa and/or luteal/granulosa cell hyperplasia was noted in females at 200 mg/kg/day. This finding occurred only at mitapivat $AUC_{0\text{-}12hr}$ values well above the range observed in humans (>100-fold) and was therefore not considered relevant for human safety assessment. In the pancreas, acinar adenoma and hyperplasia were observed at an increased incidence and/or severity in males from all dose groups; the incidence of acinar hyperplasia was outside the historical control data range for males at 300 mg/kg/day while it was considered equivalent to the maximal historic incidence for males at 100 mg/kg/day (6.67% vs 6.15%) and within the historic control data range for males at 30 mg/kg/day. The pancreatic findings were not considered to represent a significant risk for human safety based on a weight of evidence assessment that considered the following: 1) No malignancies occurred; 2) The incidence of the pancreatic findings was outside the range observed historically in the test strain only at 300 mg/kg/day (47-fold the human $AUC_{0\text{-}12hr}$ at 50 mg BID), and the findings of acinar hyperplasia and adenoma likely reflect an exacerbation of a background finding in the rat test system; 3) The off-target aromatase inhibitory activity of mitapivat creates a hormonal milieu (high testosterone, low estrogen) known to potentiate pancreatic acinar adenomas in rats. Rats are more sensitive to the inhibitory effect of mitapivat on aromatase than humans, suggesting aromatase modulation would not promote proliferative processes in the pancreas at relevant mitapivat exposures in humans; 4) The molecular pathogenesis of ductal adenocarcinoma in humans is very different from that of rat acinar tumors; and 5) Pharmacologic activation of pyruvate kinase is not known to be carcinogenic, and mitapivat itself was nongenotoxic in all assessments of mutagenicity and clastogenicity. In the clinical development program, no events suggestive of mitapivat having a carcinogenic signal have been reported to date. Carcinogenicity is not considered a risk for mitapivat. As the nonclinical carcinogenicity findings are not considered to represent a safety risk in humans and no clinical data suggestive of mitapivat

Key Safety Findings (from nonclinical studies)	Relevance to Human Use
	having a carcinogenic potential have been reported to date.
Safety pharmacology	
Cardiovascular System, Including Potential Effect on the QT Interval Multiple nonclinical studies were conducted to address the potential cardiovascular (CV) effects of mitapivat, including automated and manual patch clamp assays for potential inhibition against currents known to be associated with prolonged heart rate–corrected QT interval (QTc) (Reports AG348-N-006-R1, AG348-N-014-R1, and AG348-N-023-R1) and a single-dose CV safety pharmacology study in cynomolgus monkeys (Report AG348-N-048-R1) (Module 2.7.2, Section 3.5.1). In both the automated and manual patch clamp assays for potential inhibition of human ether-à-go-go related gene (hERG) currents, mitapivat was determined to have low potential for inhibition of the hERG current. Mitapivat was also tested in vivo for CV effects in a GLP-compliant CV safety pharmacology study in cynomolgus monkeys. Under the conditions of this study, the NOEL was 75 mg/kg, the highest level tested. The mitapivat $AUC_{0\text{-}12hr}$ and C_{max} in plasma at 75 mg/kg/dose were 23,600 hr•ng/mL (6.6-fold the human $AUC_{0\text{-}12hr}$ at the recommended dose) and 5,470 ng/mL (5.7-fold the human C_{max} at the recommended dose), respectively.	The clinical relationship of change from baseline in heart rate–corrected QT interval (QTc) and plasma concentrations of mitapivat was characterized with results from Study AG348-C-004 and AG348-C-014. In Study AG348-C-004, the pharmacokinetics and safety of mitapivat after a single dose of 5, 50, or 200 mg were evaluated in healthy Japanese and healthy non-Asian subjects. The results of the study predicted a change from baseline in QTc by Fridericia's method (ΔQTcF) of 0.000707 milliseconds (ms) (relative SE = 51%) per ng/mL increase in mitapivat concentration in the study population. The final model was used to predict mean (90% CI) for ΔQTcF at the observed C_{max} after a single administration of mitapivat 50 mg and 200 mg. At a mitapivat concentration of 1,080 ng/mL, the geometric mean C_{max} for the 50 mg dose in this study (N=20), ΔQTcF was predicted to be 1.28 ms (90% CI -2.03, -0.53 ms). At a mitapivat concentration of 5,052 ng/mL, the geometric mean C_{max} for the 200 mg dose in this study (N=20), ΔQTcF was predicted to be 1.53 ms (90% CI -1.27, 4.32 ms). This concentration-QTc analysis showed that the change from baseline in QTcF with mitapivat was well below the 10 ms threshold established by the International Council for Harmonisation (ICH) E14 guideline (US FDA, 2005) and subsequent E14 Q&A (R3) (ICH, 2015) (Module 2.7.2, Section 3.5.2.1). In Study AG348-C-014, the pharmacokinetics, safety, and tolerability of mitapivat after a single oral dose of mitapivat 100 mg in the fasted state, mitapivat 100 mg in the fed state, mitapivat 300 mg in the fasted state, and placebo were evaluated. In the concentration-QTc analysis (fasted arms only), a full model including both mitapivat and its metabolite (AGI-8702) was selected as the primary model. The estimated slope of mitapivat and AGI-8702 was shallow and not statistically significant: 0.000048 ms per ng/mL (90% CI −0.0004501, 0.0005467) for mitapivat and 0.0046 ms per ng/mL (90% CI −0.00398, 0.01322) for AGI-8702, with a small but statistically significant treatment-effect intercept of 0.96 ms. The predicted effect on placebo-corrected ΔQTcF (ΔΔQTcF) was estimated to 3.17 ms (90% CI 1.94, 4.41) and 3.12 ms (90% CI 1.78, 4.45) at the geometric mean concentration pairs of mitapivat and AGI-8702, respectively, for the mitapivat 300 mg

Key Safety Findings (from nonclinical studies)	Relevance to Human Use
	dose group. Based on this concentration-QTc analysis, a QTcF effect (ΔΔQTcF) exceeding 10 ms can be excluded within the observed plasma concentrations of mitapivat and AGI-8702 up to ~14,200 and ~689 ng/mL, respectively. The observed plasma concentration of mitapivat up to ~14,200 ng/mL is 14.7-fold above the mitapivat median C_{max} at steady state from the population PK analysis at 50 mg BID mitapivat in subjects with PK deficiency (CSR AG348-C-014, Module 2.7.2, Section 3.5.2.2). The cardiovascular system, including the potential to effect the QT interval, is not considered a risk for mitapivat.
Central Nervous System and Respiratory System In a GLP-compliant study to evaluate the effects of mitapivat (30, 150, and 300 mg/kg administered orally) on the gross behavioral, physiological, and neurologic state of male Sprague Dawley rats using a modification of a primary observation test, specifically the Irwin test (Irwin, 1968), mitapivat did not affect the gross behavioral, physiologic, or neurologic state of any animals. Under the conditions of this study, the NOEL was 300 mg/kg mitapivat, the highest level tested. The mitapivat $AUC_{0\text{-}12hr}$ and C_{max} values at 300 mg/kg/dose were 188,000 hr•ng/mL (53-fold the human $AUC_{0\text{-}12hr}$ at the recommended dose) and 31,400 ng/mL (33-fold the human C_{max} at the recommended dose), respectively (Module 2.6.2, Section 4.2.1).	As there were no treatment-related clinical observations in the central nervous system in the animal study, there is no relevance to human use.
Respiratory System A GLP-compliant study was conducted to evaluate the acute respiratory effects of mitapivat when administered orally to male Sprague Dawley rats at doses of 0, 30, 150, or 300 mg/kg. A single oral gavage administration of mitapivat to male Sprague-Dawley rats at 30, 150, or 300 mg/kg did not alter respiratory frequency, tidal volume, or minute volume. Under the conditions of this study, the NOEL was 300 mg/kg, the highest dose tested. The mitapivat $AUC_{0\text{-}12hr}$ and C_{max} values at 300 mg/kg/dose were 188,000 hr•ng/mL (53-fold the human $AUC_{0\text{-}12hr}$ at the recommended clinical dose) and 31,400 ng/mL (33-fold the human C_{max} at the recommended	As there were no treatment-related clinical observations in the respiratory system in the animal study, there is no relevance to human use.

Key Safety Findings (from nonclinical studies)	Relevance to Human Use
dose), respectively (Module 2.6.2, Section 4.2.2).	
Mechanisms For Drug Interactions	
Pharmacodynamic Drug Interactions Not applicable	Not applicable
Pharmacokinetic Drug Interactions Mitapivat metabolism was examined using human liver microsomes and recombinant CYP enzymes (Report AG348-N-096). Results from this study suggest that mitapivat is primarily metabolized by CYP3A4/5, with minor contributions from CYP2C9, CYP2C8, and CYP1A2. In the same superseding study (Report AG348-N-096), in vitro metabolism of mitapivat was studied in human liver microsomes with and without CYP enzyme-specific inhibitors, and recombinant CYP isoforms. The results from this study indicate that mitapivat is mainly metabolized (approximately 94%) by CYP3A4/5. The other CYP enzymes appear to have only minimal contributions to the metabolism of mitapivat. Based on in vitro transporter studies, mitapivat appears to be a substrate and an inhibitor of P-glycoprotein (P-gp) (91% and 99% inhibition at 41 and 411 µM, respectively), but not breast cancer resistance protein (BCRP) (half-maximal inhibitory concentration [IC_{50}] >100 µM) (Report AG348-N-057). The uptake ratio of mitapivat in organic anion transporting polypeptide (OATP)1B1- 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 (Report AG348-N-097). Mitapivat appears to be a weak inhibitor of BSEP, OATP1B1, organic anion transporter (OAT)3, multidrug and toxin extrusion protein (MATE)1, and organic cation transporter (OCT)2, with IC_{50} values of 22.0, 29.0, 12.1, 7.17, and 7.76 µM, respectively (Reports AG348-N-089 and AG348-N-097). In vitro, mitapivat appeared to be a weak inhibitor of CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4/5 enzymes (IC_{50} values >100 µM) (Report AG348-N-054). IC_{50} shift experiments indicated the potential for metabolism-based inhibition of CYP2C19 (largely reversible) and of CYP3A4/5 (largely	Section 4.4 of the SmPC includes a general statement regarding the potential for drug-drug interactions. It states that co-administration of specific medicinal products with mitapivat may result in increased risk of insomnia or changes in efficacy of mitapivat or changes in efficacy of the coadministered medicinal products (See SmPC Section 4.5). Potential drug-drug interactions should be considered whenever beginning or discontinuing treatment with mitapivat or other medicinal products concomitantly administered with mitapivat. In addition, there is a statement in the SmPC Section 4.5 that mitapivat is primarily metabolised by CYP3A4 and is a substrate for P-glycoprotein (P-gp). Mitapivat induces CYP3A4 and may also induce CYP2B6, CYP2C8, CYP2C9, CYP2C19, and uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1). Mitapivat may inhibit CYP3A4. Mitapivat may induce and inhibit P-gp (see SmPC Section 5.2). In addition, the SmPC Section 5.2 states that mitapivat exhibits pH-dependent solubility. High solubility is observed up to pH 5.5, with decreasing solubility at higher pH which may decrease mitapivat absorption. **Effect of Other Medicinal Products on Mitapivat** <u>Inhibitors of CYP3A4:</u> A clinical drug-drug interaction (DDI) study was conducted to assess the effect of a strong CYP3A4 and P-gp inhibitor (itraconazole) on the pharmacokinetics of mitapivat (Study AG348-C-012). Systemic exposure of mitapivat increased in the presence of itraconazole compared with mitapivat alone, with the geometric mean AUC from hour 0 to the last time point (AUC_{0-t}), AUC from hour 0 to infinity (AUC_{∞}), and C_{max} ratios of mitapivat in the presence and absence of itraconazole being 4.7, 4.9, and 1.7, respectively. These results show that coadministration of mitapivat with CYP3A4 inhibitors increased mitapivat plasma concentrations (Module 2.7.2, Section 2.4.1). Based on an exposure/response analysis, increased mitapivat plasma concentrations may increase the

Key Safety Findings (from nonclinical studies)	Relevance to Human Use
irreversible). In addition, mitapivat appears to be a weak inhibitor of uridine 5′-diphospho-glucuronosyl transferases (UGTs) 1A3, 1A4, and 1A9, with IC_{50} values of 15.4 ±0.9, 60.6 ±8.5, and 22.6 ±5.8 µM, respectively (Report AG348-N-090). Mitapivat also appears to be a weak inducer of human CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP3A4/5, and UGT1A1 at clinically relevant concentrations (Reports AG348-N-055-R1 and AG348-N-103).	risk of insomnia. The Pyrukynd SmPC Section 4.5 states that the concomitant use of CYP3A4 inhibitors with Pyrukynd should be avoided (see SmPC Section 4.4). If concomitant use of a CYP3A4 inhibitor is unavoidable, patients should be monitored for increased risk of insomnia (see SmPC Section 4.2). <u>Inducers of CYP3A4</u> A clinical DDI study was conducted to assess the effect of a strong CYP3A4 inducer (rifampicin) on the pharmacokinetics of mitapivat (Study AG348-C-012). Systemic exposure of mitapivat in the presence of rifampicin was lower compared with that of mitapivat alone, with the geometric mean AUC_{0-t}, AUC_{∞}, and C_{max} ratios of mitapivat in the presence and absence of rifampicin being 0.09, 0.09, and 0.23, respectively (Module 2.7.2, Section 2.2.1.5.2). This shows that coadministration of mitapivat with CYP3A4 inducers decreased mitapivat plasma concentrations. Decreased mitapivat plasma concentrations may reduce the efficacy of mitapivat. The Pyrukynd SmPC Section 4.5 states that the concomitant use of CYP3A4 inducers with Pyrukynd should be avoided (See SmPC Section 4.4). If concomitant use of a CYP3A4 inducer is unavoidable, patients should be monitored for reduced efficacy of mitapivat. <u>Inhibitors of transporters:</u> Mitapivat is not a substrate for BCRP, OATP1B1, or OATP1B3; hence, coadministration with strong inhibitors of these transporters is not expected to alter mitapivat disposition. Mitapivat is a P-gp substrate, so it is possible that strong inhibitors of P-gp may alter the disposition of mitapivat. In Study AG348-C-009, the ADME and absolute bioavailability of mitapivat were investigated in 8 healthy male subjects after oral administration of a single 120-mg dose of [^{14}C]mitapivat and concomitant single intravenous 0.1 mg microdose of [$^{13}C_6$]mitapivat. The study showed that the total (mean ± SD) recovery of administered radioactive dose over a period of 240 hours was 89.1% ±2.20%, with 49.6% ±3.99% in the urine and 39.6% ±3.38% in the feces. Most (81.9%) of the administered radioactivity was recovered in the first 96 hours postdose. The mean absolute bioavailability of mitapivat estimated from this study was 72.7%. The systemic clearance of mitapivat after intravenous dosing was 9.53 L/h. Because unchanged mitapivat accounted for <1% of the dose in feces, the derived fraction absorbed (Fa) is assumed to be 0.99. Based

Key Safety Findings (from nonclinical studies)	Relevance to Human Use
	on these data, the Fa•Fg (Fg is the fraction of drug passing though the gut wall without metabolism) was calculated to be >0.8. Because mitapivat has a high absorption fraction in the absence of strong P-gp inhibitors (Fa•Fg >0.8), it can be presumed that there will be no more than a 20% increase in exposure (AUC) of mitapivat due to P-gp inhibition in the gastrointestinal (GI) tract (Module 2.7.2, Section 3.3.1.3). The SmPC Section 4.5 states that based on in vitro data, mitapivat may induce and inhibit P-gp (see SmPC Section 5.2) and may alter systemic exposure of substrates (eg, dabigatran etexilate) of this transporter. Concomitant use of Pyrukynd with substrates of P-gp was not evaluated in a clinical drug-drug interaction study. Alternative therapies that are not P-gp substrates should be considered during treatment with Pyrukynd (see SmPC Section 4.4). If co-administration of Pyrukynd with P-gp substrates is unavoidable, patients should be carefully monitored especially for those substrates with a narrow therapeutic index (eg, colchicine, digoxin). Gastric acid–reducing agents: Mitapivat exhibits pH-dependent solubility but has high solubility up to pH 5.5. Proton-pump inhibitors (except for ranitidine) and H2-receptor antagonists do not increase gastric pH above 5.5. Based on this, the exposure of mitapivat is not expected to be altered by gastric acid-reducing agents. The SmPC Section 4.5 states that mitapivat exhibits pH-dependent solubility (see SmPC Section 5.2) and coadministration with gastric acid reducing agents (e.g., famotidine) may decrease mitapivat absorption (see SmPC Section 4.4). Concomitant use of Pyrukynd with medicinal products that elevate the gastric pH was not evaluated in a clinical drug-drug interaction study. If concomitant use of gastric acid-reducing agents is unavoidable, patients should be monitored for reduced efficacy of mitapivat. The SmPC Section 5.2 states that mitapivat exhibits pH-dependent solubility. High solubility is observed up to pH 5.5, with decreasing solubility at higher pH which may decrease mitapivat absorption. **Effect of mitapivat on other medicinal products:** In vitro studies suggest that mitapivat has the potential to induce human CYP2B6, CYP2C8, CYP2C9, CYP2C19 CYP3A4 and UGT1A1. The Pyrukynd SmPC Section 4.4 states that mitapivat may decrease the systemic exposure of hormonal

	contraceptives that are sensitive substrates of cytochrome P450 3A4 (CYP3A4) (eg ethinylestradiol) (see SmPC Section 4.5). Women of childbearing potential should be counselled regarding the use of additional or alternative contraception methods (see SmPC Section 4.6). The SmPC Section 4.5 states that mitapivat induces and may inhibit CYP3A4 and co-administration with sensitive CYP3A4 substrates (e.g. midazolam) may alter systemic exposure of these medicinal products. Concomitant use of Pyrukynd with substrates of this enzyme was not evaluated in a clinical drug-drug interaction study. Alternative therapies that are not sensitive substrates of CYP3A4 should be considered during treatment with Pyrukynd (see SmPC Section 4.4). If co-administration of Pyrukynd with sensitive CYP3A4 substrates is unavoidable, patients should be carefully monitored especially for those substrates with a narrow therapeutic index (eg, alfentanil, carbamazepine, cyclosporine, ergotamine, fentanyl, pimozide, quinidine, sirolimus, tacrolimus). In addition, the SmPC Section 4.5 states mitapivat may alter the systemic exposure of hormonal contraceptives that are sensitive substrates of CYP3A4 (eg, ethinylestradiol) (see SmPC Section 4.4) and may affect their efficacy (see SmPC Section 4.6). The SmPC Section 4.6 states that mitapivat may decrease the systemic exposure of hormonal contraceptives that are sensitive substrates of CYP3A4 (see SmPC Sections 4.4 and 4.5). Additional or alternative methods of contraception should be considered. A clinical DDI study with midazolam to assess the magnitude of interaction between mitapivat and CYP3A4 substrates will be conducted as a post-authorization measure (REC). Based on in vitro data, mitapivat may induce UGT1A1, CYP2B6, CYP2C8, CYP2C9 and CYP2C19 (see SmPC Section 5.2) and may decrease systemic exposure to substrates of these enzymes (eg, irinotecan [UGT1A1]; bupropion [CYP2B6]; omeprazole [CYP2C19]; repaglinide [CYP2C8]; warfarin [CYP2C9]). Concomitant use of Pyrukynd with substrates of these enzymes was not evaluated in a clinical drug-drug interaction study. Alternative therapies that are not UGT1A1 substrates or sensitive substrates of CYP2B6 or CYP2C should be considered during treatment with Pyrukynd (see SmPC Section 4.4). If co-administration is unavoidable, patients should be monitored for loss of therapeutic effect of substates of these enzymes, especially for those with a narrow therapeutic index (eg, irinotecan [UGT1A1]; cyclophosphamide [CYP2B6]; valproic acid [CYP2C19]; paclitaxel

Key Safety Findings (from nonclinical studies)	Relevance to Human Use
	[CYP2C8]; warfarin, phenytoin [CYP2C9]). This is noted in the SmPC Section 4.5. Based on the C_{max} at a 50-mg dose of mitapivat and the IC_{50} values calculated from in vitro data, the risk of DDIs with substrates of OATP1B1, OAT3, MATE1, and OCT2 is expected to be low. Drug-drug interactions is not considered important for inclusion in the list of safety concerns in the RMP. Drug-drug interactions is a known risk that does not affect the benefit-risk profile (Module SVII.1.1).
Other Toxicity-Related Information Or Data	
Local Tolerance The intended route of administration is oral. The GI tract was evaluated in all repeat-dose toxicology studies in Sprague Dawley rats and cynomolgus monkeys. Gastrointestinal findings in the 13-week BID TK study of mitapivat in cynomolgus monkeys found that administration of mitapivat orally (nasogastric intubation) BID to cynomolgus monkeys for at least 3 months was well tolerated at dosage levels of 50, 100, and 200 mg/kg/day (25, 50, and 100 mg/kg/dose) and resulted in nonadverse clinical observations of emesis at all dose levels and transient body weight loss during Week 0 in the 100 and 200 mg/kg/day group for males and females (Module 2.6.6, Section 3.2.5). Gastrointestinal findings in the 9-month oral toxicity and TK study of mitapivat in cynomolgus monkeys with a 28-day recovery period showed a minimal nonadverse increase in emesis (Module 2.6.6, Section 3.2.6).	Consistent with findings in nonclinical studies, mild to moderate GI disorders have been observed in subjects with PK deficiency who received mitapivat. In the clinical development program, GI disorders AEs were reported for 78 subjects (78 of 155, 50.3%) with PK deficiency, and 41 subjects (41 of 155, 26.5%) had GI disorders AEs assessed as related to treatment by the Investigator (Table 18.3.1-10.6b, Table 19-3.1). There were 3 (3 of 155, 1.9%) subjects who had events of Grade 3 severity, all not related to mitapivat (Table 19-3.1). In Study AG348-C-006, during the 24-week period, GI disorders AEs were reported for 17 subjects (17 of 39, 43.6%) with PK deficiency who received placebo and 14 subjects (14 of 40, 35.0%) with PK deficiency who received mitapivat (Table 18.3.1-10.6a). Gastrointestinal disorders were observed in humans; however, they were generally mild in severity, nonserious, and resolved without treatment discontinuation. The SmPC Section 4.8 states Nausea is a very common adverse reaction. Gastrointestinal disorders is a potential risk not considered important for inclusion in the list of safety concerns in the RMP. Gastrointestinal disorders is a potential risk with minimal clinical impact on patients (Module SVII.1.1).
Phototoxicity Mitapivat was tested for phototoxic potential in a GLP-compliant neutral red uptake phototoxicity assay in BALB/c 3T3 mouse fibroblasts. Mitapivat did not demonstrate phototoxic potential in this assay (Module 2.6.6, Section 8.7.1).	The negative 3T3 assay indicates that mitapivat does not form reactive species after absorption of UV-visible light. Mitapivat is not considered to be phototoxic and therefore phototoxicity is not considered a risk for mitapivat.
Receptor Binding Histamine H3 receptor, antagonist/inverse agonism: Mitapivat was evaluated for the potential to inhibit binding and enzymatic	Histamine H3 receptor, antagonist/inverse agonism: The anticipated effects of histamine H3 antagonism/inverse agonism are modulation of

Key Safety Findings (from nonclinical studies)	Relevance to Human Use
activity in a panel of 89 receptors, ion channels, and enzymes (Reports AG348-N-016-R1 and AG348-N-011-R1). Screening was done at a concentration of 10 µM and/or 30 µM. For mitapivat, significant results (percent inhibition >50%) at 10 µM were noted for histamine H2 (64%), histamine H3 (72%), muscarinic M2 (64%), muscarinic M3 (69%), muscarinic M4 (53%), and serotonin 5HT4 (57%). At 30 µM, significant results were noted for histamine H1 (55%), opiate κ (58%), opiate µ (59%), and serotonin 5HT4 (55%). Based on these results, the following IC_{50} determinations were made: histamine H2 (6.4 µM), histamine H3 (1.25 µM), serotonin 5HT4 (4.9 µM), muscarinic M2 (4.4 µM), muscarinic M3 (5.4 µM), muscarinic M4 (1.4 µM) (Reports AG348-N-007-R1 and AG348-N-015-R1). In follow-up functional assays to determine agonist and antagonist activity against all receptors for which IC_{50} determinations were made, mitapivat had no functional activity against any of the receptors except for the histamine H3 receptor, for which antagonist/inverse agonist activity was noted (antagonist IC_{50} activity of 0.102 µM and inverse agonist half-maximal effective concentration (EC_{50}) activity of 0.012 µM) (Report AG348-N-008-R1).	wakefulness and cognition (Schwartz, 2010). Therefore, histamine H3 antagonism/inverse agonism may lead to AEs of insomnia. In the clinical development program, insomnia AEs were reported for 51 subjects (51 of 155, 32.9%) with PK deficiency, and 39 subjects (39 of 155, 25.2%) had insomnia AEs assessed as related to treatment by the Investigator (Table 18.3.1-10.6b, Table 19-3.1). There were 2 (2 of 155, 1.3%) subjects that had events that were Grade 3 in severity (Table 19-3.1). In Study AG348-C-003, there was an apparent relationship between increased dose and occurrence of insomnia AEs, with events of insomnia occurring more frequently in subjects who received mitapivat >50 mg BID (Table 18.3.1-10.6a, Table 18.3.1-10.6b). In Study AG348-C-006, during the 24-week period, insomnia AEs were reported for 7 subjects (7 of 39, 17.9%) with PK deficiency who received placebo and 6 subjects (6 of 40, 15.0%) with PK deficiency who received mitapivat (Table 18.3.1-10.6a). Insomnia AEs were observed in humans; however, they were generally mild in severity, nonserious, and resolved without treatment discontinuation. Insomnia is an identified risk not considered important for inclusion in the list of safety concerns in the RMP. Insomnia is an identified risk with minimal clinical impact on patients (Module SVII.1.1).
Changes in sex hormones due to aromatase inhibition: In an endocrine panel, significant results (percent inhibition >50%) at 10 µM were limited to aromatase (CYP19; 91%). Mitapivat was assessed for the potential to inhibit aromatase from human recombinant insect cells, human placental microsomes, and rat ovarian microsomes (Reports AG348-N-052-R1 and AG348-N-076-R1). Mitapivat inhibited human placental aromatase (IC_{50} 2.05 µM) and rat ovarian aromatase (IC_{50} 0.493 µM).	Changes in sex hormones due to aromatase inhibition: In male subjects treated with mitapivat in Study AG348-C-002, compared with placebo-treated male subjects, the aromatase-dependent hormone studies demonstrated an increase in total and free testosterone mean serum concentrations and decreased concentrations of estradiol and estrone at all doses of mitapivat, including the lowest dose of 15 mg BID. Changes in aromatase-dependent hormone levels in the male subjects treated with mitapivat are consistent with changes in sex hormones due to off-target aromatase inhibition and were reversible within 14 days upon cessation of dosing (CSR AG348-C-002, Section 12.5.5). In clinical studies in subjects with PK deficiency, subjects were monitored for potential clinical effects of changes in sex hormones due to aromatase inhibition by the collection of serial assessments of sex hormone levels. For male subjects during the cumulative period, a decrease in estrone and estradiol levels and increase in testosterone and free testosterone levels have been observed between

Key Safety Findings (from nonclinical studies)	Relevance to Human Use
	baseline and the on-treatment period (Module 2.7.4, Section 2.1.6.2.1). A Medical Dictionary for Regulatory Activities (MedDRA) Version 23.1 pooled list of Preferred Terms (PTs) of endocrinological interest (Table SVII.4) was used to search for AEs potentially due to sex hormone changes caused by aromatase inhibition. In the clinical development program, a total of 25 subjects (25 of 155, 16.1%) with PK deficiency had at least 1 AE of endocrinological interest, and 11 subjects (11 of 155, 7.1%) had AEs that were treatment related as reported by the Investigator (Table 18.3.1-10.6b, Table 19-3.1). There were no Grade ≥3 AEs of endocrinological interest or SAEs of endocrinological interest in studies with mitapivat (Table 19-3.1, Table 19-4.1). In Study AG348-C-006, during the 24-week period, AEs of endocrinological interest were reported for 4 subjects (4 of 39, 10.3%) with PK deficiency who were receiving placebo and 4 subjects (4 of 40, 10.0%) with PK deficiency receiving mitapivat (Table 18.3.1-10.6a). Changes in sex hormones is an identified risk not considered important for inclusion in the list of safety concerns in the RMP. Changes in sex hormones is an identified risk with minimal clinical impact on patients (Module SVII.1.1). Bone mineral density decrease due to aromatase inhibition: Bone mineral density measured by DXA was analyzed in Studies AG348-C-003, AG348-C-006, AG348-C-007, and AG348-C-011 as the worst on-treatment T-score compared with baseline T-score. No clinically meaningful changes in BMD have observed in subjects with PK deficiency who received mitapivat. Most subjects remained within the same baseline category during treatment (Module 2.7.4, Section 2.1.6.2.2). Bone mineral density decrease due to aromatase inhibition is a potential risk not considered important for inclusion in the list of safety concerns in the RMP. Bone mineral density decrease due to aromatase inhibition is a potential risk with minimal clinical impact on patients (Module SVII.1.1).

The safety concerns from nonclinical data are summarized above based on whether the findings have been confirmed by clinical data (identified risk), have not been adequately refuted by clinical data and/or are of unknown significance (potential risk), or require further research (missing information). Important risks are identified risks or potential risks that could have an impact on the risk-benefit balance of the product or have implications for public health.

Safety concerns based on nonclinical findings that have relevance for human use and have not been refuted by the current clinical data will continue to be monitored for any significant clinical consequence.

Part II: Module SIII – Clinical trial exposure

The mitapivat clinical development program was initiated in March 2014 in the US and in July 2015 in Europe. As of the data lock point of 19 November 2020, a total of 16 clinical studies are completed or ongoing, including 4 studies in subjects with PK deficiency, 1 study in subjects with non–transfusion-dependent thalassemia, 2 Investigator-sponsored studies in sickle cell disease (SCD), and 9 clinical pharmacology studies in healthy subjects. A cumulative total of 444 adult subjects have been exposed to mitapivat in Agios-sponsored studies, including 155 subjects with PK deficiency, 269 healthy subjects, and 20 subjects with thalassemia. In addition to the 444 subjects who have been exposed to mitapivat in Agios-sponsored studies, 14 subjects with stable SCD have been enrolled in ongoing investigator-sponsored studies.

Subjects exposed to mitapivat across the clinical development program are summarized in Table SIII.1.

Table SIII.1: Subjects Exposed to Mitapivat in the Clinical Development Program (19 November 2020)

Study	Population	Number of Subjects Exposed to Mitapivat
Pivotal Studies		
AG348-C-006[1,2]	Subjects with PK deficiency who are not regularly transfused	40
AG348-C-007[1]	Subjects with PK deficiency who are regularly transfused	27
Supportive Studies		
AG348-C-003[1,3]	Subjects with PK deficiency	52
AG348-C-011[3,4]	Subjects with PK deficiency	Total: 88 Cohort 1: 36 Cohort 2: 35 Cohort 3: 17
Clinical Pharmacology Studies		
AG348-C-001[5]	Healthy subjects	41
AG348-C-002[5]	Healthy subjects	36
AG348-C-004[5]	Healthy adult subjects of Japanese and non-Asian origin	60
AG348-C-005[5]	Healthy subjects	26
AG348-C-009[5]	Healthy subjects	8
AG348-C-012[5]	Healthy subjects	28
AG348-C-013[5,6]	Healthy subjects	7
AG348-C-014[5]	Healthy subjects	31
AG348-C-019[3]	Healthy subjects	32
Other Studies		
AG348-C-010[3]	Subjects with NTDT	20
NCT04000165[3,7]	Subjects with stable SCD	13
EudraCT Number: 2019-003438-18[3,7]	Subjects with stable SCD	1
	TOTAL	458

Source: Module 2.7.4, Table 1.
Source: Study AG348-C-013, AG348-C-019, and IST data based on study enrollment updates as of 19 November 2020.
Abbreviations: IST = investigator-sponsored trial; NTDT = non–transfusion-dependent thalassemia; PK deficiency = pyruvate kinase deficiency; SCD = sickle cell disease.

[1] Enrollment is complete.
[2] Study is blinded; subjects were randomized in a 1:1 ratio (mitapivat:placebo).
[3] Study is ongoing. For ongoing studies, the numbers of subjects dosed, completed, and ongoing are presented as of final study enrollment or study enrollment updates.
[4] Study enrolls subjects who have completed treatment with placebo (Cohort 1) or mitapivat (Cohort 2) in Study AG348-C-006 or completed treatment with mitapivat in Study AG348-C-007 (Cohort 3) and serves as an extension study; only subjects in Cohort 1 are counted toward total.
[5] Study is completed.
[6] Mitapivat is expectorated for a taste assessment.
[7] Investigator-sponsored study.

The primary studies providing evidence of safety and efficacy of mitapivat in the proposed indication for the treatment of PK deficiency in adult patients are Studies AG348-C-006 and AG348-C-007, with supportive evidence provided from Studies AG348-C-003 and AG348-C-011. Study AG348-C-008 (Peak Registry) will also provide supportive evidence for patients that are receiving mitapivat.

Study AG348-C-003 is an ongoing Phase 2, open-label, 2-arm, multinational, randomized, dose-ranging, safety and tolerability, pharmacokinetics, pharmacodynamics, and clinical activity study in adult subjects with PK deficiency, in which mitapivat was administered as a single agent.

Study AG348-C-006 is a completed Phase 3, randomized, double-blind, placebo-controlled, efficacy, safety, pharmacokinetics, and health-related quality of life study in adult subjects with PK deficiency, in which mitapivat was administered as a single agent.

Study AG348-C-007 is a completed Phase 3, single-arm, efficacy and safety study in regularly transfused adult subjects with PK deficiency, in which mitapivat was administered as a single agent.

Study AG348-C-008 is an ongoing Agios-sponsored, global, retrospective and prospective, longitudinal observational study of pediatric and adult patients with PK deficiency (Category 3 PASS study, See Table III.1).

Study AG348-C-011 is an ongoing Phase 3, multinational, open-label extension study open to subjects who have completed Study AG348-C-006 or AG348-C-007 and meet all other eligibility criteria for Study AG348-C-011 (Category 3 PASS study, See Table III.1).

A total of 155 subjects with PK deficiency were administered mitapivat in Studies AG348-C-003, AG348-C-006, AG348-C-007, and AG348-C-011 at doses ranging from 5 mg 3 times a week to 300 mg BID. Analyses of the data from the 155 subjects with PK deficiency across all doses administered are considered pertinent to the evaluation of the safety profile for the intended indication.

Exposure data for mitapivat across the PK deficiency clinical development program are presented in Table SIII.2 to Table SIII.6. The pooled analyses in studies in subjects with PK deficiency include data as of 19 November 2020, which serves as the data lock point for this RMP. Final data are provided from the pivotal studies (Studies AG348-C-006 and AG348-C-007) in subjects with PK deficiency. Data from the ongoing extension Study 011 and the ongoing Extension Period of Study AG348-C-003 are based on interim data cuts of 12 November 2020 and 28 August 2020, respectively.

Table SIII.2: Duration of Exposure to Mitapivat in Subjects With Pyruvate Kinase Deficiency in the Clinical Development Program (19 November 2020)

Patient Population	Duration of Exposure	Patients, n (%)	Person Time (Years)
Pyruvate kinase deficiency	<1 month	1 (0.6)	0.08
	1 to <3 months	6 (3.9)	1.16
	3 to <6 months	32 (20.6)	13.09
	6 to <12 months	45 (29.0)	33.12
	12 to <24 months	45 (29.0)	64.37
	≥24 months	26 (16.8)	94.86
	≥48 months	11 (7.1)	50.28

Patient Population	Duration of Exposure	Patients, n (%)	Person Time (Years)
	Total	155	206.68

Source: Table 19-1.1.
Notes: The denominator used to calculate percentages is the total number of subjects in the Safety Analysis Set.
Duration of exposure (months) = (date of last dose - date of first dose + 1) / 30.4375.
Person time (years) = sum of person time for each patient in the category / 365.25.

Table SIII.3: Exposure to Mitapivat in Subjects With Pyruvate Kinase Deficiency in the Clinical Development Program, by Age Group and Gender (19 November 2020)

Patient Population	Age Group (yr)	Patients, n (%)			Person Time (Years)		
		Male	Female	Total	Male	Female	Total
Pyruvate kinase deficiency	18 to <65	69 (98.6)	81 (95.3)	150 (96.8)	103.14	96.78	199.93
	65 to <75	1 (1.4)	3 (3.5)	4 (2.6)	1.62	4.84	6.46
	75 to <85	0	1 (1.2)	1 (0.6)	0	0.30	0.30
	≥85	0	0	0	0	0	0
	Total	70	85	155	104.76	101.92	206.68

Source: Table 19-1.2.
Notes: The denominator used to calculate percentages is the total number of subjects in the Safety Analysis Set within each sex.
Person time (years) = sum of person time for each patient in the category / 365.25.

TableSIII.4: Exposure to Mitapivat in Subjects With Pyruvate Kinase Deficiency in the Clinical Development Program, by Dose Level (19 November 2020)

Patient Population	Dose Level	Patients, n (%)	Person Time (Years)
Pyruvate kinase deficiency	>50 mg BID	34 (21.9)	66.29
	≤50 mg BID	121 (78.1)	140.40
	Total	155	206.68

Source: Table 19-1.3.
Abbreviation: BID = twice daily.
Notes: The denominator used to calculate percentages is the total number of subjects in the Safety Analysis Set.
Person time (years) = sum of person time for each patient in the category / 365.25.

Table SIII.5: Exposure to Mitapivat in Subjects With Pyruvate Kinase Deficiency in the Clinical Development Program, by Race (19 November 2020)

Patient Population	Race	Patients, n (%)	Person Time (Years)
Pyruvate kinase deficiency	White	120 (77.4)	176.94
	Black or African American	0	0
	Asian	14 (9.0)	11.00
	Native Hawaiian or other Pacific Islander	1 (0.6)	0.72
	American Indian or Alaska Native	0	0
	Other	4 (2.6)	5.12

Patient Population	Race	Patients, n (%)	Person Time (Years)
	Not provided	16 (10.3)	12.90
	Total	155	206.68

Source: Table 19-1.4.
Notes: The denominator used to calculate percentages is the total number of subjects in the Safety Analysis Set.
Person time (years) = sum of person time for each patient in the category / 365.25.

Table SIII.6: Exposure to Mitapivat in Subjects With Pyruvate Kinase Deficiency in the Clinical Development Program, by Ethnic Group (19 November 2020)

Patient Population	Ethnic Group	Patients, n (%)	Person Time (Years)
Pyruvate kinase deficiency	Hispanic or Latino	3 (1.9)	2.72
	Not Hispanic or Latino	126 (81.3)	175.30
	Not reported	26 (16.8)	28.67
	Total	155	206.68

Source: Table 19-1.5.
Notes: The denominator used to calculate percentages is the total number of subjects in the Safety Analysis Set.
Person time (years) = sum of person time for each patient in the category / 365.25.

Part II: Module SIV – Populations not studied in clinical trials

SIV.1 Exclusion criteria in pivotal clinical studies within the development program

Exclusion criteria have been grouped together when they relate to the same concern. Exclusion criteria that were applied to ensure standardization of the study trial populations and maintaining study integrity that are common to most of the clinical studies are presented in Table SIV.1 and are not relevant to the postmarket population. Other exclusion criteria from Studies AG348-C-006 and AG348-C-007 that were designed to ensure that subjects could participate in the trial or to avoid confounding the study efficacy and safety results are also presented in Table SIV.1 and are not relevant to the postmarket population as the safety profile of mitapivat in these excluded subjects is expected to be similar.

Table SIV.1: Exclusion Criteria in Pivotal Clinical Studies Within the Development Program

Exclusion Criteria	Standardization of Clinical Trials	Confounding Efficacy/Safety	Missing Information
Subjects who have exposure to any investigational drug, device, or procedure within 3 months before the first dose of study drug	X		
Subjects who are currently enrolled in another therapeutic clinical trial involving ongoing therapy with any investigational or marketed product or placebo	X		
Subjects with a known history of allergy to mitapivat or its excipients	X		
Subjects who have a history of major surgery within 6 months of signing informed consent. Note that procedures such as laparoscopic gallbladder surgery are not considered major in this context	X		
Subjects with any other medical or psychological condition, deemed by the Investigator to be likely to interfere with a subject's ability to sign informed consent, cooperate, or participate in the study	X		
Subjects with poorly controlled hypertension (defined as systolic blood pressure >150 mm Hg or diastolic blood pressure >90 mm Hg) refractory to medical management		X	
Subjects who have positive test for hepatitis B surface antigen or hepatitis C virus antibody (Ab) with signs of active hepatitis B or C virus infection		X	

Table SIV.1: Exclusion Criteria in Pivotal Clinical Studies Within the Development Program

Exclusion Criteria	Standardization of Clinical Trials	Confounding Efficacy/Safety	Missing Information
Subjects who have a positive test for HIV-1 or -2 Ab		X	
Subjects who have diabetes mellitus judged to be under poor control by the Investigator or requiring >3 antidiabetic agents, including insulin (all insulins are considered 1 agent); use of insulin per se is not exclusionary		X	
Subjects with a history of recent (within 6 months before 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		X	
Subjects with a 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		X	
Subjects with a 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		X	
Subjects with unstable extramedullary hematopoiesis that could pose a risk of imminent neurologic compromise		X	
Subjects with iron overload sufficiently severe to result in a clinical diagnosis by the Investigator of cardiac (eg, clinically significant impaired left ventricular ejection fraction), hepatic (eg, fibrosis, cirrhosis), or pancreatic (eg, diabetes) dysfunction		X	

Table SIV.1: Exclusion Criteria in Pivotal Clinical Studies Within the Development Program

Exclusion Criteria	Standardization of Clinical Trials	Confounding Efficacy/Safety	Missing Information
Subjects who are currently receiving hematopoietic stimulating agents (eg, erythropoietins, granulocyte colony stimulating factors, thrombopoietins) that have not been stopped for a duration of at least 28 days before the first dose of study drug		X	
Subjects who have a history of potentially curative prior bone marrow or stem cell transplantation		X	
Subjects with a history of drug-induced cholestatic hepatitis		X	
Have a history of transfusions occurring on average more frequently than once every 3 weeks during the 52 weeks before signing informed consent (Study AG348-C-007–specific exclusion criterion)		X	
Subjects who were pregnant or breastfeeding	X		

In the pivotal studies AG348-C-006 and AG348-C-007, the important exclusion criteria and implications with respect to predicting the safety of mitapivat in the postmarketing setting are presented below.

Subjects who are homozygous for the R479H mutation or have 2 nonmissense mutations without the presence of another missense mutation in the *PKLR* gene, as determined per the genotyping performed by the study central genotyping laboratory

Reason for exclusion:

Subjects who were homozygous for the R479H mutation or had 2 nonmissense mutations without the presence of another missense mutation in the *PKLR* gene were excluded to provide an enriched population with the best opportunity to determine treatment response in an ultra-rare disease population.

Is it considered to be included as missing information? No

Rationale:

While for clinical trial enrichment and stratification purposes, patients are sometimes categorized according to their type of gene mutation (ie, missense/missense, missense/nonmissense, nonmissense/nonmissense), there are some important limitations to this categorization. For example, some missense mutations might result in marked protein instability or functional inactivity, and some nonmissense mutations might not have a significant impact on protein structure (Bianchi et al, 2020). In addition, a possible effect of yet unidentified genetic modifiers on the expression of pyruvate kinase might contribute to the clinical variability and could affect potential response to a pyruvate kinase activating

molecule. Current understanding of the genetic heterogeneity of PK deficiency supports that an effect of mitapivat cannot be excluded based on genotype or gene mutation category alone, and that there is a potential for benefit in the real-world setting for all patients regardless of genotype classification. While genotyping for *PKLR* is important to verify disease status, it will not be essential for the safe use of the drug while clinical benefit is being determined in adult patients with PK deficiency in the real-world setting given that mitapivat has been demonstrated to have a manageable safety profile in patients with PK deficiency, regardless of mutation status. Treatment should not be limited by gene mutation status; this is not considered missing information.

Subjects with cardiac dysrhythmias judged as clinically significant by the Investigator or QTcF >450 ms except for subjects with right or left bundle branch block

Reason for exclusion:

Subjects with cardiac dysrhythmias judged as clinically significant by the Investigator or with QTcF >450 ms were excluded because development of studies evaluating drug effects on QT/QTc interval in healthy subjects was ongoing during the start of the pivotal trials. Including these subjects could have interfered with the safety and efficacy evaluation of mitapivat.

Is it considered to be included as missing information? No

Rationale:

In nonclinical studies, mitapivat was determined to have low potential for inhibition of the hERG current (Module SII). In Study AG348-C-004, the results predicted a mean increase in QTcF at the geometric mean C_{max} for the supratherapeutic dose of 200 mg of 3.19 ms and 90% CI upper bound of 5.65 ms. In Study AG348-C-014, based on a concentration-QTc analysis, a QTcF effect (ΔΔQTcF) exceeding 10 ms could be excluded within the observed plasma concentrations of mitapivat and AGI-8702 up to ~14,200 and ~689 ng/mL, respectively. The available nonclinical and clinical data indicate that mitapivat does not have a significant QT/QTc prolongation effect, as the results from this study showed that the effects of mitapivat and its metabolite AGI-8702 on QTcF change from baseline were well below the 10-ms threshold established by the ICH E14 guideline (US FDA, 2005) and subsequent E14 Q&A (R3) (ICH, 2015).

Subjects with 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.

Reason for exclusion:

Pyruvate kinase deficiency can cause severe, ongoing hemolysis that can potentially lead to intrahepatic bile duct obstruction and ultimately progressive cholestatic liver injury. Including subjects with clinically symptomatic cholelithiasis or cholecystitis may have affected the safety assessment of mitapivat.

Is it considered to be included as missing information? No

Rationale:

By nature of the indication, patients with PK deficiency are likely to develop gallbladder disease because during times of hemolysis, increasing amounts of indirect bilirubin are produced, leading to elevated plasma bilirubin values and subsequent complications of

cholelithiasis and cholecystitis. In clinical practice, mitapivat treatment may not be delayed in patients with symptomatic cholelithiasis or cholecystitis given the potential benefit of treatment for the patient. It is likely that in clinical practice, some patients will develop cholelithiasis or cholecystitis, but this should not preclude mitapivat administration because by restoring the underlying defect and reducing hemolysis, mitapivat reduces indirect bilirubin. In the clinical development program, significant improvements in indirect bilirubin levels were observed and maintained in subjects with PK deficiency receiving mitapivat (Module 2.5, Table 12). Treatment should not be limited by clinically symptomatic cholelithiasis or cholecystitis; this is not considered missing information.

Subjects with a diagnosis of any other congenital or acquired blood disorder or any other hemolytic process, except mild alloimmunization, 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 (eg, heterozygous status for certain recessive RBC disorders).

Reason for exclusion:

Including these subjects could have interfered with the safety and efficacy evaluation of mitapivat in the study because the other congenital or acquired blood disorder may confound the clinical and laboratory assessment of the study subjects.

Is it considered to be included as missing information? No

Rationale:

The efficacy and safety of mitapivat is not expected to be altered in patients with PK deficiency who also have other congenital or acquired blood disorders. Available data indicate that because mitapivat specifically targets the pyruvate kinase enzyme, the potential for clinically relevant interactions with other congenital or acquired blood disorders is low. Mitapivat has been administered and well tolerated in 1 subject who was diagnosed with both PK deficiency and sickle cell trait in Study AG348-C-003.

Subjects who have an active infection requiring the use of parenteral antimicrobial agents or Grade ≥3 in severity (per National Cancer Institute [NCI] Common Terminology Criteria for Adverse Events [CTCAE]) within 2 months before the first dose of study drug

Reason for exclusion:

Infection is one of the most commonly reported hemolytic triggers in subjects with PK deficiency. Including subjects who had an active infection requiring the use of parenteral antimicrobial agents or Grade ≥3 in severity within 2 months before the first dose of study drug could have interfered with the evaluation of mitapivat in the study because hemolysis caused by the recent or active infection may have affected the subjects' baseline hematologic lab parameters or impacted the subjects' safety.

Is it considered to be included as missing information? No

Rationale:

In clinical practice, mitapivat treatment may not be delayed in patients with an active infection, given the potential benefit of treatment for the patient. Mitapivat may be used in patients with severe infections because infections are common complications in patients with PK deficiency who have had a splenectomy (Grace et al, 2019; Rider et al, 2011b). Infections are likely to occur in clinical practice, as was found in the clinical development program. In

the clinical development program, a total of 96 subjects (96 of 155, 61.9%) with PK deficiency treated with mitapivat experienced an AE of infection (Table 18.3.1-2.1b). Of these subjects, 9 subjects (9 of 155, 5.8%) reported infection AEs with a severity of Grade ≥3 (Table 18.3.1-2.2b). Infection is recognized as a common comorbidity in patients with hemolytic anemias who have been previously splenectomized, requiring patients to undergo prolonged prophylactic antibiotic therapy and maintain strict vaccination compliance (Module SI). It is likely that in clinical practice, some patients will develop infections, but this should not preclude mitapivat administration because mitapivat is not an immunosuppressive agent and was not associated with development or clinically meaningful changes in the severity or rate of infections over time in the clinical development program. Based on the available data of mitapivat use in subjects with active infections in the clinical development program, treatment should not be limited by active infection requiring the use of parenteral antimicrobial agents or Grade ≥3 in severity; this is not considered missing information.

Subjects with a current splenectomy scheduled during the study drug period or who have undergone splenectomy within 12 months before signing informed consent

Reason for exclusion:

Subjects with a current splenectomy scheduled during the study drug period or who had undergone splenectomy within 12 months before signing informed consent were excluded because their inclusion could have affected the safety assessment of mitapivat in the clinical study. Furthermore, because splenectomy usually results in a rise in Hb of 1-3 g/dL in patients with PK deficiency (Zanella et al, 2007; Zanella et al, 2005), a splenectomy scheduled during the study drug period or one that was undergone within 12 months before signing informed consent could have confounded the efficacy assessment of mitapivat.

Is it considered to be included as missing information? No

Rationale:

A total of 118 of 155 subjects with PK deficiency (76.1%) treated with mitapivat in the clinical development program had a history of splenectomy (Table 19-2.3). Splenectomy is a procedure commonly performed in patients with PK deficiency because it has been shown to be an effective therapy for decreasing or eliminating the need for regular transfusions (Grace et al, 2019; Grace et al, 2015; Zanella et al, 2007; Zanella et al, 2005). Therefore, it is likely that in clinical practice, some patients will have current or recent history of a splenectomy procedure, but that should not preclude mitapivat administration because mitapivat was not associated with a different safety profile in patients with or without a history of splenectomy. Based on the available data of mitapivat use in subjects with a history of splenectomy in the clinical development program, treatment should not be limited by recent splenectomy status; this is not considered missing information.

Subjects who are currently receiving medications that are strong inhibitors of CYP 3A4, strong inducers of CYP3A4, strong inhibitors of 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 time frame equivalent to 5 half-lives (whichever is longer) before the first dose of study drug

Reason for exclusion:

At the time of initiating the pivotal clinical studies, metabolism and transporter studies had been conducted only in vitro. In vitro, mitapivat is a substrate of P-gp; therefore, including

patients who required strong inhibitors of P-gp in the study could have increased the exposure of mitapivat. Additionally, mitapivat is a P-gp inhibitor, which could have increased the exposure of digoxin, a sensitive P-gp substrate. Additionally, in vitro studies suggested that mitapivat was primarily metabolized by CYP3A4, so mitapivat pharmacokinetics may be affected by strong inhibitors of CYP3A4 and strong inducers of CYP3A4. Therefore, including patients who required strong inhibitors of CYP3A4 or strong inducers of CYP3A4 in the clinical study could have affected the safety and efficacy of mitapivat, respectively.

Is it considered to be included as missing information? No

Rationale:

In parallel with the pivotal trials, clinical DDI and ADME studies provided additional information on the risk for DDIs with mitapivat. Study AG348-C-012, an open-label, fixed-sequence study in healthy adult subjects, was conducted to evaluate the effect of multiple doses of itraconazole, a strong CYP3A4 and P-gp inhibitor, and rifampicin, a strong CYP3A4 inducer, on the pharmacokinetics and safety of mitapivat. The study found that systemic exposure of mitapivat increased in the presence of itraconazole and decreased in the presence of rifampicin compared with mitapivat alone. Study AG348-C-009 demonstrated that mitapivat has a high absorption fraction in the absence of strong P-gp inhibitors (Fa•Fg >0.8), so it can be presumed that there will be no more than 20% increase in exposure (AUC) of mitapivat due to P-gp inhibition in the GI tract. The new clinical ADME data from Study AG348-C-009 were evaluated after the pivotal studies had finished enrollment, so the exclusion criterion was retained but no longer applies because risk of DDIs with P-gp inhibitors is expected to be low. Based on this new clinical DDI and ADME studies, this is not considered missing information.

Subjects who are currently receiving anabolic steroids, including testosterone preparations, within 28 days before the first dose of study drug

Reason for exclusion:

Mitapivat inhibits human aromatase activity; therefore, mitapivat may increase total and free testosterone mean serum concentrations and decrease concentrations of estradiol and estrone. Including patients who were currently receiving anabolic steroids, including testosterone preparations, within 28 days before the first dose of study drug could have affected the safety assessment of mitapivat, specifically regarding changes in aromatase-dependent hormone levels and downstream effects on BMD. Furthermore, as testosterone stimulates erythropoiesis (Fried and Gurney, 1968), use of anabolic steroids, including testosterone preparations, within 28 days before the first dose of study drug could have confounded the efficacy assessment of mitapivat.

Is it considered to be included as missing information? No

Rationale:

The efficacy and safety of mitapivat are not expected to be altered in patients taking anabolic steroids, including testosterone preparations, as testosterone levels have been observed to remain in the normal range for most subjects treated with mitapivat. Available data from studies in healthy adults and adults with PK deficiency show no reports of AEs that would be suggestive of a signal of clinical consequences associated with hormone changes due to off-target changes in sex hormones due to aromatase inhibition. Furthermore, BMD measured by DXA was analyzed in Studies AG348-C-003, AG348-C-006, AG348-C-007, and AG348-C-011 as the worst on-treatment T-score compared with baseline T-score. No clinically

meaningful changes in BMD were observed in subjects who received mitapivat. Most subjects remained within the same baseline category during treatment (Table 18.3.7-2). Mitapivat is not recognized to cause significant changes in sex hormones due to aromatase inhibition that may lead to BMD decrease and subsequent AEs (Module SVII.1.1). Based on available endocrine data in subjects treated with mitapivat, treatment should not be limited by concurrent or recent use of anabolic steroids, including testosterone preparations; this is not considered missing information.

SIV.2 Limitations to detect adverse reactions in clinical trial development programs

The clinical development program is unlikely to detect certain types of adverse reactions such as rare adverse reactions, adverse reactions with a long latency, or those caused by prolonged exposure.

SIV.3 Limitations in respect to populations typically under-represented in clinical trial development programs

Table SIV.2: Exposure of Special Populations Included or Not in Clinical Trial Development Programs

Type of Special Population	Exposure
Pregnant women Pregnant women were excluded from the clinical development program.	One pregnancy has been reported in a subject receiving mitapivat. This was a [redacted]-year-old female in Study AG348-C-003 who carried and delivered a full-term healthy [redacted] baby [redacted] via scheduled caesarean section [redacted]. The subject received mitapivat 25 mg BID for 39 days between her last menstrual period and the positive home pregnancy test. Additionally, 1 pregnancy was reported in a partner of a male subject who was receiving mitapivat in Study AG348-C-003. The subject received his first dose of mitapivat on [redacted], approximately 4 years before the reported pregnancy of the partner, with treatment ongoing. The subject's partner was a [redacted]-year-old female who was on a contraceptive pill at the time of conception and had ultrasound-confirmed normal pregnancy. The subject's partner gave birth to a normal healthy baby [redacted] by vaginal episiotomy. A complication in delivery was noted with the subject's partner experiencing postpartum hemorrhage, which led to prolongation of hospitalization. There was a manual removal of placenta [redacted] (Module SVII.3.1). Embryo-fetal toxicity is considered an important potential risk (Module SVII.3.2).
Breastfeeding women Breastfeeding women were excluded from the clinical development program.	Not included in the clinical development program.

Table SIV.2: Exposure of Special Populations Included or Not in Clinical Trial Development Programs

Type of Special Population	Exposure
Patients with relevant comorbidities: • Patients with hepatic impairment Study inclusion criteria required subjects to have adequate organ function defined as meeting the following criteria: Serum AST ≤2.5× the ULN, unless the increased AST is assessed by the Investigator as due to hemolysis and/or hepatic iron deposition, and ALT ≤2.5×ULN, unless the increased ALT is assessed by the Investigator as due to hepatic iron deposition. Either normal or elevated levels of serum bilirubin. In subjects with serum bilirubin >ULN, the elevation must be attributed to hemolysis with or without Gilbert's syndrome and must not be attributed to cholecystitis, choledocholithiasis, biliary obstruction, or hepatocellular disease.	Use of mitapivat in patients with hepatic impairment has not been evaluated in the clinical development program. Hepatic elimination plays an important role in the clearance of mitapivat. The metabolic stability of mitapivat was evaluated in rat, dog, monkey, and human liver microsomes (Report AG348-N-066-R1). After 45 minutes of incubation with mitapivat (1 µM) and nicotinamide adenine dinucleotide phosphate (NADPH) (2 mM), liver microsomal intrinsic clearance (Cl_{int}) of mitapivat was 32.1, 22.8, 18.1, and 6.82 L/hr/kg in rats, dogs, monkeys, and humans, respectively. The hepatic clearance calculated based on the CL_{int} was 2.99, 1.75, 2.27, and 1.05 L/hr/kg in rats, dogs, monkeys, and humans, respectively. The hepatic extraction ratio was estimated to be 0.91, 0.92, 0.87, and 0.85 in rats, dogs, monkeys, and humans, respectively. These data suggest that mitapivat liver metabolism clearance was high (Module 2.6.4, Section 5.1.1.2). Mitapivat is known to be metabolized after oral administration with approximately 40% and 50% of administered radioactivity in the human ADME study appearing as metabolites in the feces and urine, respectively (Module 2.7.2, Section 3.1.4). Therefore, there is a potential for hepatic impairment to affect mitapivat exposure. Use of mitapivat in subjects with hepatic impairment has not been evaluated in the clinical development program. By nature of the indication, patients with PK deficiency are likely to experience chronic and acute hemolysis that may lead to increases in unconjugated bilirubin (Module SI). Due to this, analysis of the effect of hepatic parameters such as bilirubin on mitapivat PK is limited. The effect of hepatic parameters on mitapivat PK was assessed as part of a population pharmacokinetics analysis. Most subjects in the dataset had normal AST, ALT, and alkaline phosphatase (ALP) at baseline. No apparent difference in steady-state AUC was observed in subjects with normal AST, ALT, or ALP levels versus those with elevated levels. Most subjects with PK deficiency in the dataset had elevated total bilirubin level at baseline; nevertheless, steady-state AUC appeared to be similar among the subjects with PK deficiency with total bilirubin at the ranges between 1 to 2×ULN, 2 to 3×ULN, and >3×ULN (Module 2.7.2, Section 3.2.2). Additionally, analysis of the degree of hepatic impairment in the PK deficiency clinical development program is unavailable as conventional hepatic dysfunction stratification tools (Child-Pugh, National Cancer Institute-Organ Dysfunction Working Group, and model for end-stage liver disease) all use bilirubin as a criterion of their classifications. Use of mitapivat in patients with hepatic impairment is considered missing information (Module SVII.3.2).

Table SIV.2: Exposure of Special Populations Included or Not in Clinical Trial Development Programs

<table>
<tr><th>Type of Special Population</th><th>Exposure</th></tr>
<tr><td>
Patients with renal impairment
Study inclusion criteria required subjects to have adequate organ function defined as meeting the following criteria:
Serum creatinine ≤1.25×ULN or, if >1.25×ULN, then 24-hour measured or calculated (by Cockcroft-Gault) glomerular filtration rate ≥60 mL/min.
</td><td>
Renal-Impaired Subjects With PK Deficiency at Baseline Treated With Mitapivat in the Clinical Development Program
<table>
<tr><th>Patient Population</th><th>Renal Impaired at Baseline</th><th>Patients n (%)</th><th>Person Time (Years)</th></tr>
<tr><td rowspan="9">PK deficiency</td><td>All renal impaired as defined by creatinine clearance[1]</td><td>15 (9.7)</td><td>18.74</td></tr>
<tr><td>Mild</td><td>15 (9.7)</td><td>18.74</td></tr>
<tr><td>Moderate</td><td>0</td><td>0</td></tr>
<tr><td>Severe</td><td>0</td><td>0</td></tr>
<tr><td>All renal impaired as defined by eGFR[2]</td><td>28 (18.1)</td><td>47.49</td></tr>
<tr><td>Mild</td><td>24 (15.5)</td><td>41.98</td></tr>
<tr><td>Moderate</td><td>4 (2.6)</td><td>5.51</td></tr>
<tr><td>Severe</td><td>0</td><td>0</td></tr>
<tr><td>Total</td><td>155</td><td>206.68</td></tr>
</table>
Source: Table 19-2.2.
Abbreviation: eGFR = estimated glomerular filtration rate.
Notes: The denominator used to calculate percentages is the total the number of subjects in the Safety Analysis Set.
Person time (years) = sum of person time for each patient in the category / 365.25.
[1] Renal impaired at baseline as defined by creatinine clearance is categorized as follows: mild (≥60 and <90 mL/min), moderate (≥30 and <60 mL/min), and severe (≥15 and <30 mL/min). Baseline creatinine clearance is calculated as (140 - age) × baseline weight (kg) × (0.85 if female) / (72 × baseline serum creatinine [mg/dL]).
[2] Renal impaired as defined by eGFR is categorized as follows: mild (≥60 and <90 mL/min/1.73m²), moderate (≥30 and <60 mL/min/1.73m²), and severe (≥15 and <30 mL/min/1.73m²). Baseline eGFR is calculated as eGFR (mL/min/1.73 m²) = 175 × (Scr/88.4)-1.154 × (Age)^(super -0.203) × (0.742 if female) × (1.212 if African American) (SI units) where Scr is SI units of serum creatinine.
Results from the human ADME study showed that <3% of the dose of mitapivat is eliminated unchanged in the urine and that renal clearance accounts for 3.4% of total clearance (Module 2.7.2, Section 3.2.1). These data suggest that renal impairment would not be expected to have a clinically relevant impact on mitapivat exposure.
The effect of renal impairment on mitapivat pharmacokinetics was assessed as part of the population pharmacokinetics analyses. Steady-state AUC was similar between subjects with normal renal functions, mild renal impairment, and for a limited number of subjects with moderate renal impairment. No data are available in subjects with severe renal impairment (Module 2.7.2, Section 3.2.1).
</td></tr>
</table>

Table SIV.2: Exposure of Special Populations Included or Not in Clinical Trial Development Programs

<table>
<tr><th>Type of Special Population</th><th>Exposure</th></tr>
<tr><td>• Patients with CV impairment

Subjects with a history of recent (within 6 months before 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 were excluded from the clinical development program.</td><td>Use of mitapivat in subjects with CV impairment has not been evaluated in the clinical development program.

There are no known safety concerns for use of mitapivat in subjects with a history of CV impairment, as the available nonclinical in vivo data suggest that the potential for clinically relevant CV AEs is low (Module SII). The safety profile of mitapivat in this population is not expected to be different from the subjects without this underlying history.</td></tr>
<tr><td>• Immunocompromised patients

Subjects with a splenectomy scheduled during the study drug period or who had undergone splenectomy within 12 months before signing informed consent were excluded from the clinical development program.

Subjects who had a positive test for hepatitis B surface antigen or hepatitis C virus Ab with signs of active hepatitis B or C virus infection were excluded from the clinical development program.

Subjects who had a positive test for HIV-1 or -2 Ab were excluded from the clinical development program.

Subjects with a history of any primary malignancy, except for 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, were excluded from the clinical development program.</td><td>Immunocompromised Subjects With PK Deficiency at Baseline Treated With Mitapivat in the Clinical Development Program
<table>
<tr><th>Patient Population</th><th>Immunocompromised at Baseline</th><th>Patients n (%)</th><th>Person Time (Years)</th></tr>
<tr><td rowspan="4">PK deficiency</td><td>All asplenia</td><td>118 (76.1)</td><td>149.06</td></tr>
<tr><td>Complete</td><td>117 (75.5)</td><td>148.61</td></tr>
<tr><td>Partial</td><td>1 (0.6)</td><td>0.44</td></tr>
<tr><td>Total</td><td>155</td><td>206.68</td></tr>
</table>
Source: Table 19-2.3.
Notes: The denominator used to calculate percentages is the total the number of subjects in the Safety Analysis Set.
Person time (years) = sum of person time for each patient in the category / 365.25.
Complete: history of full splenectomy; Partial: history of partial splenectomy.

Mitapivat is not an immunosuppressive agent. Mitapivat was not associated with a different safety profile in subjects with a history of splenectomy (Module SIV.1).</td></tr>
</table>

Table SIV.2: Exposure of Special Populations Included or Not in Clinical Trial Development Programs

<table>
<tr><th>Type of Special Population</th><th>Exposure</th></tr>
<tr><td>• Patients with a disease severity different from inclusion criteria in clinical trials</td><td>The severity of the underlying disease in the subjects who were studied in the clinical trials for the target indication is considered to be reflective of the typical subject population that would be expected to receive treatment with mitapivat.</td></tr>
<tr><td>Population with relevant different ethnic origin

There were no ethnic groups that were excluded from the clinical development program.</td><td>The race and ethnic origin of subjects with PK deficiency (N=155) in the clinical development program are presented in Table SIII.5 and Table SIII.6 respectively (Module SIII). Most subjects were white (77.4%), with limited exposure in Asian (9.0%), Native Hawaiian or other Pacific Islander (0.6%), and other (2.6%) subjects. Race was not reported for 16 subjects (10.3%). A greater number of subjects were not Hispanic or Latino (81.3%) compared with Hispanic or Latino (1.9%) or not reported (16.8%).</td></tr>
<tr><td>Subpopulations carrying relevant genetic polymorphisms

Subjects who were homozygous for the R479H mutation or had 2 nonmissense mutations without the presence of another missense mutation in the PKLR gene were excluded from pivotal clinical studies within the development program.</td><td>Subjects in Studies AG348-C-003, AG348-C-006, AG348-C-007, and AG348-C-011 were enrolled based upon clinical laboratory confirmation of PK deficiency, defined as documented presence of at least 2 mutant alleles in the PKLR gene, as determined per the genotyping performed by the study central genotyping laboratory.
Subjects who were homozygous for the R479H mutation or had 2 nonmissense mutations without the presence of another missense mutation in the PKLR gene were included in Study AG348-C-003. There were 10 subjects with nonmissense/nonmissense mutations and 5 subjects who were homozygous for the R479H mutation (code for mutation 1436 G>A) who were included in the study (Listing 16.2.1-3).
Baseline Genetic Polymorphism Characteristics of Subjects With PK Deficiency in the Clinical Development Program
<table>
<tr><th></th><th>Patients, n (%)</th></tr>
<tr><td>PKLR Mutation Category</td><td></td></tr>
<tr><td>Missense/missense</td><td>103 (66.5)</td></tr>
<tr><td>Missense/nonmissense</td><td>42 (27.1)</td></tr>
<tr><td>Nonmissense/nonmissense</td><td>10 (6.5)</td></tr>
</table>
Source: Table 18.1-3.3b.
Current understanding of the genetic heterogeneity of PK deficiency supports that an effect of mitapivat cannot be excluded based on genotype or gene mutation category alone, and that there is a potential for benefit in the real-world setting for all patients regardless of genotype classification While genotyping for PKLR is important to verify disease status, it will not be essential for the safe use of the drug while clinical benefit is being determined in adult patients with PK deficiency in the real-world setting given that mitapivat has been demonstrated to have a manageable safety profile in patients with PK deficiency, regardless of mutation status (Module SIV.1).</td></tr>
</table>

Table SIV.2: Exposure of Special Populations Included or Not in Clinical Trial Development Programs

Type of Special Population	Exposure
Use in pediatric patients Pediatric subjects were excluded from the clinical development program.	Use of mitapivat in pediatric subjects has not been evaluated in the clinical development program. The current proposed indication for mitapivat is for the treatment of PK deficiency in adult patients. One accidental exposure to mitapivat has been reported in a pediatric subject; [redacted]. The subject received mitapivat 100 mg BID for 6 days [redacted]. The subject experienced AEs of Headache, Vomiting, and Rash pustular during mitapivat administration. All the AEs were low grade (Grade 1) and resolved shortly after mitapivat discontinuation. The child's exposure in this case of 100 mg BID is thought to be similar to the exposure of a 60-kg adult receiving 300 mg BID when looking at the exposure on a milligram per kilogram basis, [redacted] (CSR AG348-C-010, Appendix 16.1.8). The 300-mg BID dose was well tolerated in adults for 6 months in Study AG348-C-003 with the most frequently reported AEs being headache and insomnia.

Part II: Module SV – Postauthorization experience

SV.1 Postauthorization exposure

Not applicable; mitapivat was not authorized in any country at the time of the data lock point (19 November 2020).

Part II: Module SVI – Additional EU requirements for the safety specification

Potential for misuse for illegal purposes:

Mitapivat has no pharmacologic properties that would promote its use for abuse or misuse for illegal purposes. No potential for drug dependence or drug abuse has been noted for mitapivat in any of the clinical studies.

Part II: Module SVII – Identified and potential risks

SVII.1 IDENTIFICATION OF SAFETY CONCERNS IN THE INITIAL RMP SUBMISSION

SVII.1.1 Risks not considered important for inclusion in the list of safety concerns in the RMP

Risks with minimal clinical impact on patients (in relation to the severity of the indication treated):

Table SVII.1: Classification of Risks With Minimal Clinical Impact on Patients (in Relation to the Severity of the Indication Treated)

Risk	Identified Risk	Potential Risk
Changes in sex hormones	X	
Bone mineral density decrease due to aromatase inhibition		X
Gastrointestinal disorders		X
Hypersensitivity		X
Insomnia	X	
Triglyceride increases		X

- **Changes in sex hormones (Identified risk)**

Changes in sex hormones is an identified risk associated with mitapivat treatment based on nonclinical and clinical safety findings (Module SII).

In nonclinical studies, mitapivat inhibited human placental and rat ovarian aromatase with an IC_{50} of 2.05 and 0.493 µM, respectively (Module SII). In healthy male subjects treated with mitapivat in Study AG348-C-002, compared with placebo-treated male subjects, the aromatase-dependent hormone studies demonstrated an increase in total and free testosterone mean serum concentrations and decreased concentrations of estradiol and estrone at all doses of mitapivat, including the lowest dose of 15 mg BID. Changes in aromatase-dependent hormone levels in the male subjects treated with mitapivat are consistent with inhibition of human aromatase and were reversible within 14 days upon cessation of dosing (CSR AG348-C-002, Section 12.5.5). Male subjects receiving mitapivat in Study AG348-C-003 experienced an expected increase in total testosterone and free testosterone and a decrease in estradiol and estrone. Testosterone levels have been observed to remain in the normal range for most subjects treated with mitapivat. However, even at the lowest actual dose level of mitapivat, there appears to be a corresponding decrease in male estradiol. Aromatase-dependent hormone levels in the female subjects treated with mitapivat were also collected but were difficult to interpret due to subject age (premenopausal vs postmenopausal) and contraceptive use (hormonal vs nonhormonal contraception); these data were considered not informative for analysis of mitapivat changes in sex hormones due to aromatase inhibition potency.

The in vitro potency of mitapivat and 3 marketed aromatase inhibitors (exemestane, anastrozole, and letrozole) on aromatase inhibition in rat ovarian and human placental tissue is provided in Table SVII.2.

Table SVII.2: Comparison of Aromatase Inhibition Potency With Mitapivat and Marketed Aromatase Inhibitors in Nonclinical Assays

	Rat Ovarian Aromatase IC_{50} (nM)	Mitapivat Fold Lower Aromatase Inhibition (rat)	Human Placental Aromatase IC_{50} (nM)	Mitapivat Fold Lower Aromatase Inhibition (human)
Mitapivat	493	--	2,050	--
Exemestane (Aromasin)	0.672	734	397	5
Anastrozole (Arimidex)	2.87	172	138	15
Letrozole (Femara)	0.392	1,258	8.29	248

Abbreviations: IC_{50} = half-maximal inhibitory concentration.

Mitapivat was observed to inhibit human aromatase in secondary pharmacology in vitro studies, with the in vitro IC_{50} for aromatase inhibition in rat ovarian and human placental tissue being 5- to 248-fold higher than the 3 marketed aromatase inhibitors. This demonstrates that mitapivat is a far less potent inhibitor of aromatase in vitro compared with the 3 marketed aromatase inhibitors.

The area under the curve ($AUC_{\tau,ss}$), average total concentration ($C_{avg,ss,tot}$) and average free concentration ($C_{avg,ss,free}$) of mitapivat and 2 of the 3 marketed aromatase inhibitors at clinically relevant doses are provided in Table SVII.3. Data for anastrozole are not published and available for comparison.

Table SVII.3: Exposure and Total and Free Concentration of Mitapivat Compared With Aromatase Inhibitors at Clinically Relevant Doses

Drug Name	Clinically Relevant Dose	$AUC_{\tau,ss}$ (hr○nmol/L) at Clinically Relevant Dose	$C_{avg,ss,tot}$ (nmol/L)	PPB in Humans	$C_{avg,ss,free}$ (nmol/L)
Mitapivat	50 mg BID[1]	3,580[1]	298.33	97.7%	6.86
Exemestane	25 mg QD[2,6]	104.6[3]	4.36	90%	0.436
Letrozole	2.5 mg QD[4,5]	8,926[3]	371.92	60.1%	148.40

Abbreviations: $AUC_{\tau,ss}$ = area under the plasma concentration versus time curve from 0 to the end of dosing period at steady state; BID = twice daily; $C_{avg,ss,free}$ = average steady-state free concentration; $C_{ave,ss,tot}$ = average steady-state total concentration; QD = once daily; PPB = plasma protein binding.

[1] Source: AG348-PMX-001, Table 9 (units converted where appropriate).

[2] Aromasin (exemestane). Package Insert. Pfizer; 2018.

[3] Lønning P, Pfister C, Martoni A, Zamagni C. Pharmacokinetics of third generation aromatase inhibitors. *Semin Oncol*. 2003;30 (4 suppl 14):23-32.

[4] Femara (letrozole). Package Insert. Novartis; 2014.

[5] Femara (letrozole). Summary of Product Characteristics. Novartis; 2021.

[6] Aromasin (exemestane). Summary of Product Characteristics. Pfizer; 2018.

Letrozole is a reversible aromatase inhibitor. The clinical $C_{avg,ss,free}$ of letrozole at 2.5 mg (approved dose for treatment of postmenopausal women with hormone receptor–positive early breast cancer is 2.5 mg QD; (Femara Package Insert, 2014)) is 148.40 nM, ~18-fold above the in vitro IC_{50} for aromatase inhibition in human placental tissue (Lonning et al, 2003).

Exemestane is an irreversible aromatase inhibitor. The clinical $C_{avg,ss,free}$ of exemestane at 25 mg (approved dose for treatment of postmenopausal women with estrogen

receptor-positive early breast cancer is 25 mg QD; (Aromasin (exemestane) Package Insert, 2018) is 0.436 nM, ~910-fold below the in vitro IC_{50} for aromatase inhibition in human placental tissue. As the drug binds irreversibly, the dose can be a lot lower than reversible aromatase inhibition doses as effect accumulates during treatment (Lonning et al, 2003).

Mitapivat behaves like a reversible aromatase inhibitor in clinical and nonclinical assays. The clinical $C_{avg,ss,free}$ of mitapivat at 50 mg BID (highest dose being administered to adults in the ongoing pivotal trials) is 6.86 nM, ~298-fold below in vitro IC_{50} for human aromatase inhibition, suggesting that the concentration of mitapivat in humans at the highest expected clinically efficacious dose is well below the concentration required for 50% aromatase inhibition; this is confirmed by clinical observations.

In clinical studies in subjects with PK deficiency, subjects were monitored for potential clinical effects of aromatase inhibition by the collection of serial assessments of sex hormone levels. For male subjects during the cumulative period, a decrease in estrone and estradiol levels and increase in testosterone and free testosterone levels was observed between baseline and the on-treatment period. A total of 13 (34.2%) male subjects who had normal estrone levels at baseline had low estrone levels on treatment, 8 (21.1%) male subjects who had high estrone levels at baseline had low levels on treatment, and 10 (26.3%) male subjects with high estrone levels at baseline had normal levels on treatment. Estradiol levels in male subjects decreased but generally did not fall below the lower limit of normal (LLN). A total of 35 (50.0%) male subjects who had normal free testosterone levels at baseline had high levels during the cumulative period. Eight (11.6%) male subjects who had normal testosterone levels at baseline had a high level on treatment, and 2 (2.9%) male subjects who had normal testosterone levels at baseline had testosterone below the LLN on treatment. Changes observed in hormone levels were reversible upon study drug discontinuation (Module 2.7.4, Section 2.1.6.2.1).

Sex hormone data in female subjects treated with mitapivat was difficult to interpret due to physiologic variations in hormone levels expected throughout the normal menstrual cycle, subject biologic age (premenopausal vs postmenopausal), and contraceptive use (hormonal vs nonhormonal contraception). Sex hormone levels were generally unchanged from baseline to the on-treatment period in female subjects who received mitapivat or placebo. Menstrual diaries were collected from menstruating women; no changes in menstrual cycles after initiation of mitapivat treatment were observed from menstrual diaries (Module 2.7.4, Section 2.1.6.2.1).

To monitor for any clinical consequences from sex hormone changes due to off-target aromatase inhibition by mitapivat, a MedDRA Version 23.1 pooled list of PTs of endocrinological interest (Table SVII.4) was used to search for potential AEs due to aromatase inhibition.

Table SVII.4: Pooled List of Preferred Terms of Endocrinological Interest

Medical Concept	Preferred Terms (PTs)	
Adverse events of endocrinological interest	PT – Abnormal withdrawal bleeding PT – Amenorrhoea PT – Anovulatory cycle PT – Bleeding anovulatory PT – Delayed menarche PT – Dysfunctional uterine bleeding PT – Dysmenorrhoea PT – Menstrual discomfort PT – Menstrual disorder PT – Menstruation irregular PT – Metrorrhagia PT – Premature menarche PT – Premenstrual cramps PT – Premenstrual dysphoric disorder PT – Premenstrual headache PT – Premenstrual pain PT – Premenstrual syndrome PT – Retrograde menstruation PT – Withdrawal bleed PT – Hypomenorrhoea	PT – Menstruation delayed PT – Oligomenorrhoea PT – Pituitary amenorrhoea PT – Menometrorrhagia PT – Menorrhagia PT – Polymenorrhagia PT – Polymenorrhoea PT – Mood swings PT – Menopausal symptoms PT – Premature menopause PT – Acne PT – Erectile dysfunction PT – Arthritis PT – Alopecia PT – Vulvovaginal dryness PT – Vulvovaginal discomfort PT – Dysphoria PT – Night sweats PT – Mood altered

In the clinical development program, a total of 25 subjects (25 of 155, 16.1%) with PK deficiency had at least 1 AE of endocrinological interest, and 11 subjects (11 of 155, 7.1%) had AEs that were treatment related as reported by the Investigator (Table 18.3.1-10.6b, Table 19-3.1). The AEs of Dysmenorrhoea, Menstruation irregular, and Night sweats were among the most commonly reported AEs of endocrinological interest (reported in ≥2 subjects) (Table 18.3.1-10.6b). There were no Grade ≥3 or SAEs of endocrinological interest reported in PK deficiency studies with mitapivat (Table 19-3.1, Table 19-4.1). There was a total of 24 treatment-related AEs, with outcomes of recovered (23 of 24, 95.8%) or ongoing (1 of 24, 4.2%) (Table 19-4.1). Among the 25 subjects who reported AEs of endocrinological interest, the median time to the first onset of the AE was 11.14 weeks (range 0.3, 66.6 weeks), and the median AE duration was 3 days (range 1, 699 days) (Table 18.3.1-10.5b). The incidence of AEs of endocrinological interest was comparable in subjects who received placebo in Study AG348-C-006 compared with subjects who received mitapivat during the 24-week period, with 4 subjects (4 of 39, 10.3%) receiving placebo and 4 subjects (4 of 40, 10.0%) receiving mitapivat (Table 18.3.1-10.6a).

In the Pyrukynd SmPC Section 4.8, adverse reactions in the Investigations System Organ Class (SOC) include Oestrone decreased (males) with a frequency of very common (≥1/10) and Blood testosterone increased (males) and Oestradiol decreased (males) with a frequency of common (≥1/100 to <1/10).

Sex hormone changes due to off-target aromatase inhibition with mitapivat were identified preclinically and confirmed with aromatase-dependent hormone changes in healthy male adult subjects and subjects with PK deficiency. The evaluated changes in aromatase-

dependent sex hormone levels have been observed to remain in the normal range for most subjects treated with mitapivat. In the clinical development program, there were no data suggesting that there is an increase in the risk of AEs that could be reasonably associated with sex hormone changes due to off-target aromatase inhibition by mitapivat. Despite this effect on aromatase-dependent hormone levels, changes in sex hormones is not considered important for inclusion in the list of mitapivat safety concerns in the RMP because mitapivat-induced changes in sex hormone levels are not associated with development of AEs. The risk of changes in sex hormones will be monitored and discussed in the PSURs.

- **Bone mineral density decrease due to aromatase inhibition (potential risk)**

Bone mineral density decrease due to aromatase inhibition is a potential risk associated with mitapivat treatment based on nonclinical and clinical safety findings (Module SII).

Patients with PK deficiency have an inherent risk for developing osteopenia and osteoporosis throughout their lifetimes because of anemia and iron overload caused by ineffective erythropoiesis. Patients with PK deficiency are at risk for low BMD, fractures, and bone pain (Module SI).

Bone mineral density measured by DXA was analyzed in Studies AG348-C-003, AG348-C-006, AG348-C-007, and AG348-C-011 as the worst on-treatment T-score compared with baseline T-score. The analysis showed that no clinically meaningful changes in BMD were observed in subjects who received mitapivat. Most subjects remained within the same baseline category during treatment (Module 2.7.4, Section 2.1.6.2.2). Data from the long-term extension period of Study AG348-C-003 including 31 subjects who were treated for more than 12 months suggested no clinically meaningful worsening of BMD. Additionally, long-term data from Study AG348-C-011 showed no worsening in BMD based on worst on treatment T-score compared with baseline T-score.

Bone mineral density decrease AEs were identified by searching the Standardized MedDRA Query (SMQ) (Broad) of Osteoporosis/Osteopenia. In Studies AG348-C-003, AG348-C-006, AG348-C-007, and AG348-C-011, 13 (13 of 155, 8.4%) subjects treated with mitapivat experienced an event of BMD decrease. Of these events, 7 (7 of 155, 4.5%) subjects reported Osteopenia, 4 (4 of 155, 2.6%) subjects reported Osteoporosis, 1 (1 of 155, 0.6%) subject reported Bone density decreased, and 3 (3 of 155, 1.9%) subjects reported a type of bone fracture (Table 18.3.1-10.6b). All subjects who reported an event of BMD decrease had either a medical history or baseline DXA indicating osteopenia or osteoporosis. There were 2 (2 of 155, 1.3%) subjects who reported an event that was Grade ≥3 in severity (PTs Femur fracture, Rib fracture, Tibia fracture) (Table 19-3.1, Table 18.3.1-2.2b). None of the bone fracture AEs were assessed as related to mitapivat by the Investigator as all of the events were either traumatic bone fractures or occurred in subjects who were considered osteoporotic or osteopenic at baseline. Only 1 AE assessed as treatment related by the Investigator (PT Osteoporosis) was considered serious, and it resolved, with the subject continuing to receive mitapivat treatment in Study AG348-C-003 (Table 19-4.1). Among the 13 subjects who reported AEs of BMD decrease, the median time to the first onset of the AEs was 43.71 weeks (range 6.6, 103.4 weeks), and the median AE duration was 211 days (range 3, 734 days) (Table 18.3.1-10.5b). In Study AG348-C-006, during the 24-week period, BMD decreased AEs were reported for 1 subject (1 of 39, 2.6%) who received placebo (PT Osteoporosis) and 2 subjects (2 of 40, 5.0%) who received mitapivat (PTs Osteopenia, Rib fracture) (Table 18.3.1-10.6a).

Bone mineral density decrease due to aromatase inhibition is not considered important for inclusion in the list of mitapivat safety concerns in the RMP because short- and long-term

BMD data based on DXA scans have shown no correlation of sex hormone changes due to aromatase inhibition and BMD, and no treatment-related fractures have been reported. Mitapivat is not recognized to cause significant aromatase inhibition that may lead to BMD decrease and subsequent AEs. The risk of bone mineral density decrease due to aromatase inhibition will be monitored and discussed in the PSURs.

- **Gastrointestinal disorders (potential risk)**

Gastrointestinal disorders are a potential risk associated with mitapivat treatment based on nonclinical and clinical safety findings (Module SII).

Treatment-emergent AEs of GI disorders have occurred in studies with mitapivat in subjects with PK deficiency and in healthy subjects. A dose relationship was observed for GI events in studies with healthy subjects; all GI events occurred in subjects who received mitapivat doses of ≥700 mg BID (Module 2.7.4, Section 2.1.6.4).

Gastrointestinal disorders AEs were identified by searching with the MedDRA Version 23.1 SMQ (Narrow) of Gastrointestinal nonspecific symptoms and therapeutic procedures. In the clinical development program, GI AEs were reported for 78 subjects (78 of 155, 50.3%) with PK deficiency, and 41 subjects (41 of 155, 26.5%) had GI AEs assessed as related to treatment by the Investigator (Table 18.3.1-10.6b, Table 19-3.1). The AEs of Nausea, Diarrhoea, Vomiting, Dyspepsia, and Abdominal pain were among the most commonly reported AEs of GI disorders (reported in ≥5% of subjects) (Table 18.3.1-10.6b). Most treatment-related AEs were Grade 1 or Grade 2. Adverse events with a severity of Grade ≥3 were reported in 3 subjects (3 of 155, 1.9%), and none of these events were assessed as treatment related by the investigator (Table 19-3.1). Only 2 subjects reported SAEs (PTs Colitis, Enteritis); none of the SAEs were considered treatment related by the investigator, and all SAEs resolved (Table 19-4.1). There was a total of 79 treatment-related AEs, with outcomes of recovered (74 of 79, 93.7%) or ongoing (5 of 79, 6.3%) (Table 19-4.1). Among the 78 subjects who reported AEs of GI disorders, the median time to the first onset of a GI AE was 3.93 weeks (range 0.1, 141.7 weeks), and the median AE duration was 3 days (range 1, 1,232 days) (Table 18.3.1-10.5b). In Study AG348-C-006, during the 24-week period, GI disorders AEs were reported for 17 subjects (17 of 39, 43.6%) who received placebo and 14 subjects (14 of 40, 35.0%) who received mitapivat (Table 18.3.1-10.6a). The SmPC Section 4.8 states Nausea is a very common adverse reaction.

Gastrointestinal disorders is not considered important for inclusion in the list of mitapivat safety concerns in the RMP because most of the treatment-related AEs were mild in severity, nonserious, and resolved without treatment discontinuation.

- **Hypersensitivity (potential risk)**

Hypersensitivity is a potential risk associated with mitapivat treatment based on nonclinical and clinical safety findings (Module SII).

Hypersensitivity AEs were identified by searching with the MedDRA Version 23.1 SMQ (narrow) of Hypersensitivity. In the clinical development program, hypersensitivity AEs were reported for 26 subjects (26 of 155, 16.8%) with PK deficiency, and 6 subjects (6 of 155, 3.9%) had hypersensitivity AEs assessed as related to treatment by the Investigator (Table 18.3.1-10.6b, Table 19-3.1). The AEs of Rash, Rhinitis allergic, Hypersensitivity, Eczema, Drug hypersensitivity, Dermatitis atopic, Dermatitis contact, and Rash maculo-papular were among the most commonly reported hypersensitivity AEs (reported in ≥2 subjects) (Table 18.3.1-10.6b). There were no Grade ≥3 or serious hypersensitivity AEs reported in PK deficiency studies with mitapivat (Table 19-3.1, Table 19-4.1). There was a

total of 9 treatment-related AEs with outcomes of recovered (9 of 9, 100%) (Table 19-4.1). Among the 26 subjects who reported Hypersensitivity AEs, the median time to the first onset of hypersensitivity was 11.00 weeks (range 0.1, 204.1 weeks), and the median AE duration was 21.5 days (range 1, 242 days) (Table 18.3.1-10.5b). The incidence of events of hypersensitivity was higher in subjects who received placebo in Study AG348-C-006 compared with subjects who received mitapivat. During the 24-week period, hypersensitivity AEs were reported for 10 subjects (10 of 39, 25.6%) who received placebo and 4 subjects (4 of 40, 10.0%) who received mitapivat (Table 18.3.1-10.6a).

Hypersensitivity is not considered important for inclusion in the list of mitapivat safety concerns in the RMP because most of the AEs were mild in severity, nonserious, and resolved without treatment discontinuation.

- **Insomnia (identified risk)**

Insomnia is an identified risk of mitapivat based on nonclinical and clinical safety findings (Module SII).

The MedDRA Version 23.1 High Level Term of Disturbances in initiating and maintaining sleep was used to search for events of insomnia. In the clinical development program, insomnia AEs were reported for 51 subjects (51 of 155, 32.9%) with PK deficiency, and 39 subjects (39 of 155, 25.2%) had insomnia AEs assessed as related to treatment by the Investigator (Table 18.3.1-10.6b, Table 19-3.1). Of these events, 15 (15 of 155, 9.7%) subjects reported Initial insomnia, 13 (13 of 155, 8.4%) subjects reported Middle insomnia, 26 (26 of 155, 16.8%) subjects reported Insomnia, and 3 (3 of 155, 1.9%) subjects reported Terminal insomnia (Table 18.3.1-10.5b). Most treatment-related AEs were Grade 1 or Grade 2. Adverse events with a severity of Grade ≥3 were reported in 2 subjects (2 of 155, 1.3%). Only 1 AE assessed as treatment related by the investigator (PT Initial insomnia) was considered serious and resolved, with the subject continuing to receive mitapivat treatment in Study AG348-C-003 (Table 19-4.1). There was a total of 56 treatment-related AEs with outcomes of recovered (46 of 56, 82.1%) or ongoing (10 of 56, 17.9%) (Table 19-4.1). Among the 51 subjects who reported AEs of insomnia, the median time to the first onset of an insomnia AE was 6.14 weeks (range 0.1, 164.6 weeks), and the median AE duration was 40 days (range 1, 761 days) (Table 18.3.1-10.5b). In Study AG348-C-003, there was an apparent relationship between increased dose and occurrence of insomnia AEs; 61.8% of AEs occurred at doses >50 mg BID (Module 2.7.4, Section 2.1.6.3). Additionally, based on an exposure-response analysis, increased mitapivat plasma concentrations may increase the risk of insomnia (AG348-PMx-002). In Study AG348-C-006, during the 24-week period, insomnia AEs were reported for 7 subjects (7 of 39, 17.9%) who received placebo and 6 subjects (6 of 40, 15.0%) who received mitapivat (Table 18.3.1-10.6a).

Insomnia is not considered important for inclusion in the list of mitapivat safety concerns in the RMP because most of the treatment-related AEs were mild in severity, nonserious, and resolved without treatment discontinuation.

In the Pyrukynd SmPC Section 4.8, adverse reactions in the Psychiatric disorders SOC include insomnia (pooled PTs of Initial insomnia, Insomnia, Middle insomnia, and Terminal insomnia) with a frequency of very common (≥1/10).

- **Triglycerides increased (potential risk)**

Increased triglycerides is a potential risk of mitapivat based on clinical safety findings. The MedDRA Version 23.1 PTs of Blood triglycerides increased and Hypertriglyceridaemia were used to search for events of triglycerides increased. In the clinical development program, a

total of 17 subjects (17 of 155, 11.0%) with PK deficiency had at least 1 AE of triglycerides increased, and 10 subjects (10 of 155, 6.5%) had AEs assessed as related to treatment by the Investigator (Table 18.3.1-10.6b, Table 19-3.1). Of these events, 13 (13 of 155, 8.4%) subjects reported Hypertriglyceridaemia and 5 (5 of 155, 3.2%) subjects reported Blood triglycerides increased (Table 18.3.1-10.6b). Adverse events with a severity of Grade ≥3 were reported in 9 subjects (9 of 155, 5.8%). One treatment-related SAE of Grade 4 Hypertriglyceridaemia that led to discontinuation of study drug occurred in a subject in Study AG348-C-003 who had Grade 3 triglycerides levels at baseline (CSR AG348-C-003, Section 14.3.3). There was a total of 11 treatment-related AEs with outcomes of recovered (8 of 11, 72.7%) or ongoing (3 of 11, 27.3%) (Table 19-4.1). Among the 17 subjects who reported AEs of triglyceride increased, the median time to the first onset of a triglyceride increased AE was 13.14 weeks (range 0.1, 141.3 weeks), and the median AE duration was 125.5 days (range 7, 1,173 days) (Table 18.3.1-10.5b). Because elevated triglyceride levels may signal risk for acute pancreatitis, acute pancreatitis AEs were reviewed. Acute pancreatitis AEs were identified by searching the MedDRA Version 23.1 SMQ (Narrow) of Acute pancreatitis. There were no AEs of Pancreatitis reported in any subject on study. One subject who received placebo in Study AG348-C-006 experienced a Grade 3 SAE of Obstructive pancreatitis. The subject had increased ALT levels that qualified the event as an AESI. The event was considered to be not related to study treatment (Module 2.7.4, Section 2.1.6.5. In Study AG348-C-006, during the 24-week period, triglycerides increased AEs were reported for 1 subject (1 of 39, 2.6%) who received placebo and 3 subjects (3 of 40, 7.5%) who received mitapivat (Table 18.3.1-10.6a). Additionally, in Study AG348-C-006 during the 24-week period, increased triglyceride labs of any grade occurred in 48.7% of subjects who received placebo and 37.5% of subjects who received mitapivat (Module 2.7.4, Section 3.1.4).

Triglycerides increased events were transient in nature with triglyceride levels returning to baseline without concomitant treatment medications and without mitapivat dose modification. There have been no reported AEs that would indicate a long-term effect on triglycerides. 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. Some laboratory tests were performed without fasting. Additionally, many subjects who experienced Grade 1 AEs of Hypertriglyceridaemia had elevated triglycerides at baseline, and some subjects had severe triglyceride elevations at baseline. Most subjects who had elevated triglyceride levels on study had low high-density lipoprotein (HDL) at baseline. 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, HDL, hypertriglyceridemia, or QT prolongation (Module 2.7.4, Section 2.1.6.5).

Triglycerides increased is not considered important for inclusion in the list of mitapivat safety concerns in the RMP because most of the AEs were mild in severity, nonserious, and resolved without treatment discontinuation. Additionally, there were no identified trends with other lipid analytes or associations with cardiovascular clinical consequences such as hypertension or heart disease. There was no evidence of a sustained effect in triglycerides as most events resolved transiently.

Adverse reactions with clinical consequences, even serious, but occurring with a low frequency and considered to be acceptable in relation to the severity of the indication treated:

- None

Known risks that require no further characterization and are followed up via routine pharmacovigilance namely through signal detection and adverse reaction reporting, and for which the risk minimization messages in the product information are adhered by prescribers (eg, actions being part of standard clinical practice in each EU Member state where the product is authorized):

- None

Known risks that do not impact the benefit-risk profile:

- **Drug-drug interactions**

The potential for DDIs with mitapivat has been comprehensively investigated (Module SII). In vitro studies demonstrate that mitapivat is primarily metabolized by CYP3A4/5.

The pharmacokinetics of mitapivat was evaluated in 14 healthy adult subjects in the presence and absence of itraconazole, a strong CYP3A4/5 and P-gp inhibitor using an open-label, fixed-sequence study design (Study AG348-C-012, Part 1). 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. Systemic exposure of mitapivat increased in the presence of itraconazole compared with mitapivat alone, with the geometric mean $AUC_{0\text{-}t}$, AUC_{∞}, and C_{max} ratios of mitapivat in the presence and absence of itraconazole being 4.7, 4.9, and 1.7, respectively (Module 2.7.2, Section 2.2.1.5.1). This result shows that coadministration of mitapivat with strong CYP3A4 inhibitors increased mitapivat plasma concentrations (Module 2.7.2, Section 2.4.1). Based on an exposure/response analysis, increased mitapivat plasma concentrations may increase the risk of insomnia. The Pyrukynd SmPC Section 4.4 states that co-administration of specific medicinal products with mitapivat may result in increased risk of insomnia or changes in efficacy of mitapivat or changes in efficacy of the co-administered medicinal products (see SmPC Section 4.5). Potential drug-drug interactions should be considered whenever beginning or discontinuing treatment with mitapivat or other medicinal products concomitantly administered with mitapivat. Section 4.5 of the SmPC states that the concomitant use of CYP3A4 inhibitors with Pyrukynd should be avoided (see SmPC Section 4.4). If concomitant use of a CYP3A4 inhibitor is unavoidable, patients should be monitored for increased risk of insomnia (see SmPC Section 4.2).

The pharmacokinetics of mitapivat was evaluated in 14 healthy adult subjects in the presence and absence of rifampicin, a strong CYP3A4/5 inducer, using an open-label, fixed-sequence study design (Study AG348-C-012, Part 2). In Part 2, healthy subjects received a single oral dose of 50 mg mitapivat on Day 1 of Treatment Period 1. In Treatment Period 2, subjects received oral rifampicin (600 mg QD) from Day 1 through Day 12, with a single oral dose of 50 mg mitapivat on Day 8. Systemic exposure of mitapivat in the presence of rifampicin was lower compared with that of mitapivat alone, with the geometric mean $AUC_{0\text{-}t}$, AUC_{∞}, and C_{max} ratios of mitapivat in the presence and absence of rifampicin being 0.09, 0.09, and 0.23, respectively (Module 2.7.2, Section 2.2.1.5.2). This result suggests that strong inducers of CYP3A4 have the potential to decrease the exposure of mitapivat. Decreased mitapivat plasma concentrations may reduce the efficacy of mitapivat. The Pyrukynd SmPC Section 4.5 states that the concomitant use of CYP3A4 inducers with Pyrukynd should be avoided (see SmPC Section 4.4). If concomitant use of a CYP3A4 inducer is unavoidable, patients should be monitored for reduced efficacy of mitapivat.

Based on the C_{max} at a 50-mg dose of mitapivat and the IC_{50} values calculated from in vitro data, the risk of DDIs with substrates of OATP1B1, OAT3, MATE1, and OCT2 is expected to be low.

In vitro, mitapivat induced human CYP2B6, CYP2C8, CYP2C9, CYP2C19, and CYP3A4; therefore, mitapivat has the potential to decrease the exposure of sensitive substrates of these enzymes. The Pyrukynd SmPC Section 4.4 states that mitapivat may decrease the systemic exposure of hormonal contraceptives that are sensitive substrates of cytochrome P450 3A4 (CYP3A4) (eg ethinylestradiol) (see SmPC Section 4.5). The SmPC Section 4.5 states that mitapivat may alter the systemic exposure of hormonal contraceptives that are sensitive substrates of CYP3A4 (e.g., ethinylestradiol) (see SmPC Section 4.4) and may affect their efficacy (see SmPC section 4.6). The SmPC Section 4.6 states that women of childbearing potential should use contraception during treatment with Pyrukynd and for at least 1 month after the last dose. Mitapivat may decrease the systemic exposure of hormonal contraceptives that are sensitive substrates of CYP3A4 (see SmPC Sections 4.4 and 4.5). Additional or alternative methods of contraception should be considered.

The Pyrukynd SmPC Section 4.5 states that mitapivat induces and may inhibit CYP3A4 and co-administration with sensitive CYP3A4 substrates (eg, midazolam) may alter systemic exposure of these medicinal products. Concomitant use of Pyrukynd with substrates of this enzyme was not evaluated in a clinical drug-drug interaction study. Alternative therapies that are not sensitive substrates of CYP3A4 should be considered during treatment with Pyrukynd (see SmPC Section 4.4). If co-administration of Pyrukynd with sensitive CYP3A4 substrates is unavoidable, patients should be carefully monitored especially for those substrates with a narrow therapeutic index (eg, alfentanil, carbamazepine, cyclosporine, ergotamine, fentanyl, pimozide, quinidine, sirolimus, tacrolimus). A clinical DDI study with midazolam to assess the magnitude of interaction between mitapivat and CYP3A4 substrates will be conducted as a post-authorization measure (REC).

Section 4.5 of the SmPC states that based on in vitro data, mitapivat may induce UGT1A1, CYP2B6, CYP2C8, CYP2C9 and CYP2C19 (see SmPC Section 5.2) and may decrease systemic exposure to substrates of these enzymes (eg, irinotecan [UGT1A1], bupropion [CYP2B6]; omeprazole [CYP2C19]; repaglinide [CYP2C8]; warfarin [CYP2C9]). Concomitant use of Pyrukynd with substrates of these enzymes was not evaluated in a clinical drug-drug interaction study. Alternative therapies that are not UGT1A1 substrates or sensitive substrates of CYP2B6 or CYP2C should be considered during treatment with Pyrukynd (see SmPC Section 4.4). If co-administration is unavoidable, patients should be monitored for loss of therapeutic effect of substates of these enzymes, especially for those with a narrow therapeutic index (eg, irinotecan [UGT1A1]; cyclophosphamide [CYP2B6]; valproic acid [CYP2C19]; paclitaxel [CYP2C8]; warfarin, phenytoin [CYP2C9]). Section 5.2 of the SmPC states that mitapivat induces CYP3A4 and may also induce CYP2B6, CYP2C8, CYP2C9, CYP2C19, and UGT1A1. Mitapivat may inhibit CYP3A4.

The SmPC Section 4.5 states that mitapivat exhibits pH-dependent solubility (see SmPC Section 5.2) and coadministration with gastric acid reducing agents (eg, famotidine) may decrease mitapivat absorption (see SmPC Section 4.4). Concomitant use of Pyrukynd with medicinal products that elevate the gastric pH was not evaluated in a clinical drug-drug interaction study. If concomitant use of gastric acid-reducing agents is unavoidable, patients should be monitored for reduced efficacy of mitapivat. Section 5.2 states that mitapivat exhibits pH-dependent solubility. High solubility is observed up to pH 5.5, with decreasing solubility at higher pH which may decrease mitapivat absorption.

Mitapivat is not a substrate for BCRP, OATP1B1, or OATP1B3; hence, coadministration with strong inhibitors of these transporters is not expected to alter mitapivat disposition. Mitapivat is a P-gp substrate, so it is possible that strong inhibitors of P-gp may alter the disposition of mitapivat. In Study AG348-C-009, the ADME and absolute bioavailability of

mitapivat were investigated in 8 healthy male subjects after oral administration of a single 120-mg dose of [14C]mitapivat and concomitant single intravenous 0.1 mg microdose of [13C6]mitapivat. The study showed that the total (mean ±SD) recovery of administered radioactive dose over a period of 240 hours was 89.1% ±2.20%, with 49.6% ±3.99% in the urine and 39.6% ±3.38% in the feces. Most (81.9%) of the administered radioactivity was recovered in the first 96 hours postdose. The mean absolute bioavailability of mitapivat estimated from this study was 72.7%. The systemic clearance of mitapivat after intravenous dosing was 9.53 L/h. Because unchanged mitapivat accounted for <1% of the dose in feces, the derived Fa is assumed to be 0.99. Based on these data, the Fa•Fg was calculated to be >0.8. Because mitapivat has a high absorption fraction in the absence of strong P-gp inhibitors (Fa•Fg >0.8), it can be presumed that there will be no more than 20% increase in exposure (AUC) of mitapivat due to P-gp inhibition in the GI tract (Module 2.7.2, Section 3.3.1.3). The risk of DDIs with P-gp inhibitors is expected to be low. Section 4.5 of the SmPC states that based on in vitro data, mitapivat may induce and inhibit P-gp (see SmPC Section 5.2) and may alter systemic exposure of substrates (eg, dabigatran etexilate) of this transporter. Concomitant use of Pyrukynd with substrates of P-gp was not evaluated in a clinical drug-drug interaction study. Alternative therapies that are not P-gp substrates should be considered during treatment with Pyrukynd (see SmPC Section 4.4). If co-administration of Pyrukynd with P-gp substrates is unavoidable, patients should be carefully monitored especially for those substrates with a narrow therapeutic index (eg, colchicine, digoxin). Section 5.2 states that mitapivat is a substrate for P-gp and may induce and inhibit P-gp.

The DDIs described above are not considered important risks of mitapivat and can be managed by adhering to the guidance in the Pyrukynd SmPC.

Other reasons for considering the risks not important:

- None

SVII.1.2 Risks considered important for inclusion in the list of safety concerns in the RMP

Table SVII.5: Classification of Risks Considered Important for Inclusion in the List of Safety Concerns in the RMP

Risk	Identified Risk	Potential Risk	Missing Information
Acute hemolysis	X		
Embryo-fetal toxicity		X	
Use in patients with hepatic impairment			X
Long-term use			X

Important Identified Risk 1: Acute hemolysis

Acute hemolysis is an important identified risk as it is a severe condition that may lead to acute exacerbation of the patient's underlying health condition if left untreated. Acute hemolysis is the rapid return to pretreatment levels of hemolysis after sudden mitapivat withdrawal. It occurs only in patients who are Hb responders when abrupt withdrawal of mitapivat (without a gradual reduction in dosing) leads to a rapid and marked return to pretreatment Hb levels, accompanied by signs and symptoms of hemolysis. Acute hemolysis has been observed after abrupt mitapivat withdrawal in the clinical development program.

In the clinical development program, 2 subjects (2 of 155; 1.3%) with PK deficiency experienced acute hemolysis upon abrupt withdrawal of mitapivat dosed at 300 mg BID. The treatment-related AEs experienced by the 2 subjects included 2 Grade 3 SAEs of acute hemolysis (PTs Haemolytic anaemia and Haemolysis) and 1 nonserious Grade 2 case of acute hemolysis (PT Haemolysis). Both subjects had a high reticulocyte percent and low Hb at baseline indicating active ineffective erythropoiesis and ongoing hemolysis. In both subjects, a rapid Hb increase and correction in reticulocyte percent during the first 3 weeks of mitapivat treatment at 300 mg BID, followed by an abrupt discontinuation of mitapivat without taper, resulted in acute hemolysis and anemia. These events of hemolysis had an extreme and specific change in Hb, reticulocyte, and bilirubin levels in response to the start and the end of mitapivat dosing, where there is a strong biological rationale and sufficient evidence to consider these events to be caused by the use of mitapivat (Part SVII.3.1).

By contrast, subjects 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, and their Hb concentrations were either not recorded immediately after the short interruption or decreased gradually after the dose reduction. During the Extension Period of Study AG348-C-003, 14 subjects underwent a dose taper to reduce their mitapivat dose per protocol (starting at 300 mg BID and decreasing by 100 mg increments for 3 weeks). Eleven of the 14 subjects subsequently had decreases in their Hb. The rate at which Hb decreased varied by subject, but the most rapid decrease was approximately 1.1 g/dL per week, which stopped when the subject adjusted to a new dose level (CSR AG348-C-003).

Two additional subjects in Study AG348-C-003 experienced treatment-related SAEs of Haemolytic anaemia. One subject was a confirmed nonresponder. It was determined that these events were not due to sudden withdrawal of mitapivat but were more likely attributed to the subjects' ongoing anemia (CSR AG348-C-003, Section 14.3.3).

Since the establishment of this important identified risk, in the clinical development program, subjects are no longer started at a very high dose of mitapivat. The maximum dose in subjects with PK deficiency is 50 mg BID, and subjects are dose escalated to their optimal dose to avoid rapid increase in Hb. Dose taper strategies are recommended when mitapivat dose cessation is planned to reduce the risk of hemolysis and a rapid decrease in Hb. In an emergency setting, mitapivat dosing can be stopped immediately as long as the patient is properly monitored for changes in Hb and signs and symptoms of acute hemolysis. No additional events of acute hemolysis have been reported in subjects with PK deficiency where the dose taper has been followed as per protocol.

Benefit-risk impact:

Patients with PK deficiency experience lifelong hemolytic anemia with subsequent associated comorbidities. These patients have a serious unmet medical need for safe and effective targeted therapies as there are currently no approved products to manage or treat PK deficiency. The benefit of mitapivat as an effective treatment for PK deficiency outweighs the risk of acute hemolysis, which has been observed only in Hb-responding subjects at 6 times the recommended mitapivat dose and can be managed in clinical practice by adhering to the guidance in the SmPC. Acute hemolysis is included as a special warning and precautions for use in the Pyrukynd SmPC Section 4.4 and Package Leaflet (PL) Section 2. The Pyrukynd SmPC Section 4.4 states that acute haemolysis with subsequent anaemia has been observed following abrupt interruption or discontinuation of Pyrukynd (see SmPC Section 4.8). Abrupt interruption or discontinuation of treatment with Pyrukynd should be avoided. A gradual reduction in dosing rather than abrupt cessation is recommended (see SmPC Section 4.2). If

discontinuing treatment abruptly, patients should be monitored for signs of acute haemolysis and anaemia which may include among other symptoms and signs: jaundice, scleral icterus and dark urine. Section 4.2 of the SmPC states that to minimise the risk of acute haemolysis, abrupt interruption or discontinuation of Pyrukynd should be avoided. The dose should be tapered to gradually discontinue the medicinal product over a 1-2 week period (see Table 2 of the SmPC). Patients should be monitored for signs of acute haemolysis with worsening of anaemia (see SmPC Sections 4.4 and 4.8). Acute hemolysis is also included in the SmPC Section 4.8 under Description of Selected Adverse Reactions, which states that abrupt interruption or discontinuation of Pyrukynd can lead to acute haemolysis (see SmPC Section 4.4). For guidance on how to interrupt or discontinue treatment see SmPC Section 4.2.

Important Potential Risk 1: Embryo-fetal toxicity

There are limited data on mitapivat exposure in pregnant women because subjects who were pregnant were not treated with mitapivat in the clinical development program (Module SIV.3). One pregnancy was reported in a subject receiving mitapivat. This was a [redacted]-year-old female in Study AG348-C-003 who carried and delivered a full-term healthy [redacted] baby [redacted] via scheduled caesarean section at 39 weeks. Additionally, 1 pregnancy was reported in a partner of a male subject who was receiving mitapivat in Study AG348-C-003. The subject's partner was a [redacted]-year-old female who was on a contraceptive pill at time of conception and had ultrasound-confirmed normal pregnancy. The subject's partner gave birth to a normal healthy baby [redacted] by vaginal episiotomy (CSR AG348-C-003, Section 14.3.3).

In embryo-fetal development studies, fetal adverse effects were observed at $AUC_{0\text{-}12hr}$ (Day last) values 63-fold (rats) and 3.1-fold (rabbits) the human $AUC_{0\text{-}12hr}$ value at 50 mg BID (3,580 hr•ng/mL).

In a rat embryo-fetal toxicity study, oral administration of mitapivat was associated with fetal adverse events, including a decrease in the mean number and litter proportion of viable fetuses, lower mean fetal weights, and test article-related external, soft tissue, and skeletal malformations. The maternal and fetal NOAEL occurred at a dose of 50 mg/kg/day and correlated with maternal $AUC_{0\text{-}12hr}$ (Day last) values of 46,800 hr•ng/mL. The findings observed in rats are consistent with effects reported for aromatase inhibitors and are therefore likely due to sex hormone changes caused by aromatase inhibition.

In a rabbit embryo-fetal toxicity study, oral administration of mitapivat resulted in lower mean fetal body weights. No effects on fetal morphology were observed. The maternal and fetal NOAEL occurred at a dose of 60 mg/kg/day and correlated with maternal $AUC_{0\text{-}12hr}$ (Day last) values of 5,360 hr•ng/mL.

Although human data on pregnancies are limited, there is no evidence of human teratogenicity based on genotoxic potential and the 2 successful pregnancies. Although nonclinical reproductive toxicity studies did not demonstrate teratogenicity/fetotoxicity at maternal exposures comparable to those in humans administered mitapivat at 50 mg BID (proposed clinical dose), they did demonstrate embryo-fetal toxicity at high dose levels: $AUC_{0\text{-}12hr}$ values 63- and 3.1-fold higher than the human $AUC_{0\text{-}12hr}$ value at 50 mg BID. Based on these non-clinical findings, embryo-fetal toxicity is considered an important potential risk (Module SVII.3.1).

It is proposed that post-marketing monitoring for pregnancy and lactation will occur via routine pharmacovigilance. A pregnancy-, lactation-, and embryo-fetal toxicity-specific follow-up form will be utilized by the case processing team, or extension thereof, when reports of pregnancy are received and then throughout the course of the pregnancy and delivery, as applicable. The follow-up form is attached in Annex 4.

Benefit-risk impact:

Patients with PK deficiency experience lifelong hemolytic anemia with subsequent associated comorbidities. These patients have a serious unmet medical need for safe and effective targeted therapies as there are currently no approved products to manage or treat PK deficiency. The benefit of mitapivat as an effective treatment for PK deficiency outweighs the potential risk of embryo-fetal toxicity that has yet to be confirmed in humans and can be managed by adhering to the recommendations in the Pyrukynd SmPC. The Pyrukynd SmPC Section 4.6 states that there are no or limited amount of data from the use of mitapivat in pregnant women. Studies in animals have shown reproductive toxicity (see SmPC Section 5.3). Pyrukynd is not recommended during pregnancy and in women of childbearing potential not using contraception. Women of childbearing potential should avoid becoming pregnant while receiving Pyrukynd. Women of childbearing potential should use contraception during treatment with Pyrukynd and for at least 1 month after the last dose. Mitapivat may decrease the systemic concentrations of hormonal contraceptives that are sensitive substrates of CYP3A4 (see SmPC Sections 4.4 and 4.5). Additional or alternative methods of contraception should be considered. In addition, SmPC Section 4.4 states that mitapivat may decrease the systemic concentrations of hormonal contraceptives that are sensitive substrates of cytochrome P450 3A4 (CYP3A4) (eg ethinylestradiol) (see SmPC Section 4.5). Women of childbearing potential should be counselled regarding the use of additional or alternative contraceptive methods (see SmPC Section 4.6). Section 5.3 of the SmPC describes the relevant preclinical safety data.

Missing Information 1: Use in patients with hepatic impairment

Hepatic elimination plays an important role in the clearance of mitapivat. The metabolic stability of mitapivat was evaluated in rat, dog, monkey, and human liver microsomes (Report AG348-N-066-R1). After 45 minutes of incubation with mitapivat (1 µM) and NADPH (2 mM), the liver microsomal intrinsic clearance (CL_{int}) of mitapivat was 32.1, 22.8, 18.1, and 6.82 L/hr/kg in rats, dogs, monkeys, and humans, respectively. The hepatic clearance (CL_{hep}) calculated based on the CL_{int} was 2.99, 1.75, 2.27, and 1.05 L/hr/kg in rats, dogs, monkeys, and humans, respectively. The hepatic extraction ratio was estimated to be

0.91, 0.92, 0.87, and 0.85 in rats, dogs, monkeys, and humans, respectively. These data suggest that mitapivat liver metabolism clearance was high (Module 2.6.4, Section 5.1.1.2).

Mitapivat is known to be metabolized after oral administration with approximately 40% and 50% of administered radioactivity in the human ADME study appearing as metabolites in the feces and urine, respectively (Module 2.7.2, Section 3.1.4). Therefore, there is a potential for hepatic impairment to affect mitapivat exposure.

Use of mitapivat in patients with hepatic impairment has not been evaluated in the clinical development program. By nature of the indication, patients with PK deficiency are likely to experience chronic and acute hemolysis that may lead to increases in unconjugated bilirubin (Module SI). Due to this, analysis of the effect of hepatic parameters such as bilirubin on mitapivat pharmacokinetics is limited. The effect of hepatic parameters on mitapivat pharmacokinetics was assessed as part of a population pharmacokinetics analyses. Most subjects in the dataset had normal AST, ALT, and ALP at baseline. No apparent difference in steady-state AUC was observed in subjects with normal AST, ALT, or ALP levels versus those with elevated levels. Most subjects with PK deficiency in the dataset had elevated total bilirubin levels at baseline; nevertheless, steady-state AUC appeared to be similar among the subjects with PK deficiency with total bilirubin at the ranges between 1 to 2×ULN, 2 to 3×ULN, and >3×ULN (Module 2.7.2, Section 3.2.2). Additionally, analysis of the degree of hepatic impairment in the PK deficiency clinical development program is unavailable as conventional hepatic dysfunction stratification tools (Child-Pugh, NCI-Organ Dysfunction Working Group [ODWG], and model for end-stage liver disease [MELD]) all use bilirubin as a criterion of their classifications.

Mitapivat has not been studied in subjects with hepatic impairment. No dose recommendations can be made, and the safety and efficacy of mitapivat have not been established in such subjects. Because there is a potential for hepatic impairment to affect mitapivat exposure, it will be important to assess the findings from the planned hepatic impairment Study AG348-C-0HEP to determine if dose adjustments of mitapivat are required for subjects with hepatic impairment.

Use in subjects with hepatic impairment is considered an area of missing information as mitapivat has not been studied in subjects with hepatic impairment.

Benefit-risk impact:

Patients with PK deficiency experience lifelong hemolytic anemia with subsequent associated comorbidities. These patients have a serious unmet medical need for safe and effective targeted therapies as there are currently no approved products to manage or treat PK deficiency. The benefit of mitapivat as an effective treatment for PK deficiency outweighs any unknown safety concerns in patients with hepatic impairment.

Use in subjects with hepatic impairment will be further characterized in the planned hepatic impairment Study AG348-C-0HEP to evaluate the pharmacokinetics, safety, and tolerability of mitapivat in subjects with moderate hepatic impairment or normal hepatic function (Part III.2) which is aligned with the CHMP guidance on the ‘Evaluation of the pharmacokinetics of medicinal products in patients with impaired hepatic function’ (2005).

Hepatic impairment is included as a special population in the Pyrukynd SmPC Sections 4.2 and 5.2. In the Pyrukynd SmPC Section 4.2, it states that there are no data available in patients with hepatic impairment. No dose recommendations can be made. In the Pyrukynd SmPC Section 5.2, it states that the pharmacokinetics of mitapivat in patients with mild,

moderate, or severe hepatic impairment have not been studied. The SmPC will be updated once data from the study in patients with hepatic impairment are available.

Missing Information 2: Long-term use

The primary studies providing evidence of long-term use of mitapivat in the proposed indication for the treatment of PK deficiency in adult patients are Studies AG348-C-003 and AG348-C-011. Study AG348-C-008 (Peak Registry) will also provide supportive evidence for subjects that are receiving mitapivat.

Study AG348-C-003 is an ongoing Phase 2, open-label, 2-arm, multicenter, randomized, dose-ranging study assessing the safety and tolerability, pharmacokinetics, pharmacodynamics, and clinical activity of mitapivat in adult subjects with PK deficiency. The study consists of a completed 24-week Core Period and an ongoing 8-year Extension Period. This study initiated on 26 June 2015 and last patient last visit is anticipated in May 2025. In this study, subjects have received doses ranging from 5 mg 3 times a week to 300 mg BID. As of 28 August 2020 (the interim data-cut for this study), 52 subjects have initiated treatment with 18 subjects ongoing treatment in the study.

Study AG348-C-011 is an ongoing Phase 3, multicenter, open-label extension study for subjects who have completed Study AG348-C-006 or AG348-C-007 and meet all other eligibility criteria for Study AG348-C-011. The overall duration of the study is approximately 5 years. This study initiated on 21 March 2019 and last patient last visit is currently anticipated in November 2024. As of 12 November 2020 (interim data-cut for this study), 88 subjects had initiated treatment with 78 subjects still ongoing treatment in the study. This is a Category 3 PASS study, see Table III.1 Ongoing and Planned Additional Pharmacovigilance Activities.

The long-term safety profile of mitapivat is consistent with the safety profile during the 24-week on-treatment period. The most commonly reported events during the cumulative period were similar to those reported during the 24-week on-treatment period, and most events continued to be Grade 1 or 2 in severity. The slight increase in percentage of subjects experiencing these events is considered consistent with what would be expected with continued treatment for a longer duration in this patient population.

The Peak Registry was designed to understand better the natural history of PK deficiency, including diagnosis, demographic and clinical characteristics, burden of disease, treatment patterns, and clinical outcomes in a real-world setting. The registry enrolled its first patient in April 2018 and will continue enrolling through Q2 2025. The study is expected to be completed in Q2 2027 with database lock in Q3 2027, providing at least 2 years and up to 9 years of patient follow-up data. As of 08 March 2022, the Peak Registry had 240 patients enrolled from 48 sites in 16 countries, predominantly located in North America and Europe. This is a Category 3 PASS study, see Table III.1 Ongoing and Planned Additional Pharmacovigilance Activities.

The effects of long-term use of mitapivat in subjects with PK deficiency are not known. All risks and associated long-term safety data will continued to be monitored and characterized.

Benefit-risk impact:

Patients with PK deficiency experience lifelong hemolytic anemia with subsequent associated comorbidities. These patients have a serious unmet medical need for safe and effective targeted therapies as there are currently no approved products to manage or treat PK deficiency. The benefit of mitapivat as an effective treatment for PK deficiency outweighs any unknown safety concerns associated with long-term mitapivat treatment.

The Pyrukynd SmPC Section 5.1 states that the median duration of treatment of mitapivat was 24.1 weeks in AG348-C-006 (ACTIVATE) and median duration of treatment in AG348-C-007 (ACTVATE-T) was 40.3 weeks. In addition, the SmPC Section 4.2 states that treatment with Pyrukynd is intended to be long-term. Pyrukynd should be discontinued if a patient does not experience an improvement of haemolytic anaemia at the maximum recommended dose, based on the totality of laboratory results and clinical status of the patient, unless there is another explanation for response failure (eg, bleeding, surgery, other concomitant illnesses).

The effects of long-term use of mitapivat in subjects with PK deficiency will continue to be further characterized in Study AG348-C-011 (Category 3, PASS), AG348-C-008 Peak Registry (Category 3 PASS) and through routine pharmacovigilance (Part III.2).

SVII.2 New safety concerns and reclassification with a submission of an updated RMP

Not applicable

SVII.3 Details of important identified risks, important potential risks, and missing information

SVII.3.1 Presentation of important identified risks and important potential risks

Important Identified Risk: Acute Hemolysis

Potential mechanisms:

Mitapivat is a potent, activator of alleles of PKR. Pyruvate kinase R is 1 of 4 pyruvate kinase isoenzymes expressed in human tissues from 2 separate genes. Both PKR and the liver-specific form of pyruvate kinase (PKL) are splice isoforms of the *PKLR* gene, while pyruvate kinase muscle isozyme (PKM)1 and PKM2 are both expressed from the *PKM* gene. Mitapivat is an allosteric activator of PKR, PKL, and PKM2 isoenzymes, with similar activity for each. Mature RBCs rely almost exclusively on the process of glycolysis to generate the energy carrier molecule ATP. Thus, PKR is a key enzyme for maintaining energy homeostasis in erythrocytes.

Mitapivat acts by directly binding to the PKR tetramer and allosterically enhancing its affinity for PEP. Patients with PK deficiency who harbor mutations of the PKR enzyme experience a disruption in glycolytic pathway activity that leads to abnormal RBC metabolism and a shortened RBC life span, resulting in chronic nonspherocytic hemolytic anemia. In patients with PK deficiency, RBCs and their progenitors are characterized by changes in metabolism associated with defective glycolysis, including a build-up of PEP and the intermediate 2,3-DPG, and lowered levels of ATP. It is hypothesized that AG-348 restores the ability of RBCs to convert PEP + adenosine diphosphate to pyruvate + ATP and thereby normalizes RBC metabolism in patients with PK deficiency.

The potential for rapid hemolysis upon abrupt withdrawal of mitapivat is anticipated from the drug's known mechanism of action to activate the erythrocyte pyruvate kinase enzyme. In Hb responders, it is anticipated that abrupt withdrawal of the drug, which does not permanently cure the enzymatic defect, could result in a return to the pyruvate kinase–deficient state, which would lead to recurrent hemolysis.

Evidence source and strength of evidence:

In the clinical development program, 2 subjects (2 of 155; 1.3%) with PK deficiency experienced acute hemolysis upon sudden withdrawal of mitapivat dosed at 300 mg BID. In both subjects, a rapid Hb increase during the first 3 weeks of mitapivat treatment at 300 mg BID was followed by an abrupt discontinuation of mitapivat without taper, resulting in acute hemolysis and anemia. Two additional subjects in Study AG348-C-003 experienced treatment related SAEs of Haemolytic anaemia. One subject was a confirmed nonresponder. It was determined that these events were not due to sudden withdrawal of mitapivat but were more likely attributed to the subjects' ongoing anemia. Since the establishment of this important identified risk, in the clinical development program, subjects are no longer started at a high dose of mitapivat. The maximum dose in subjects with PK deficiency is 50 mg BID. Subjects are dose escalated to their optimal dose to avoid rapid increase in Hb as well as rapid decrease in case drug therapy needs to be stopped for any reason. No additional events of acute hemolysis have been reported in subjects with PK deficiency where the dose taper has been followed as per protocol. Clinical trials can provide an estimation of the frequency and nature of an adverse reaction that is expected to occur in clinical practice.

Characterization of the risk:

Acute hemolysis is a severe condition that may lead to acute exacerbation of the patient's underlying health condition if left untreated. Acute hemolysis occurs in patients who are Hb responders when upon subsequent withdrawal of mitapivat without a gradual reduction in dosing, subjects experience a rapid and marked decrease in Hb accompanied by signs and symptoms of hemolysis. Clinical signs and symptoms of acute hemolysis are synonymous with those of a hemolytic episode and involve a range of signs and symptoms that may include acute anemia, indirect hyperbilirubinemia, decreased haptoglobin, increased lactate dehydrogenase, hemoglobinuria, back pain, fatigue, pallor, tachycardia, dyspnea, scleral icterus, and jaundice. Back pain and hemoglobinuria are key symptoms of acute hemolysis because they signal the onset of renal failure.

Hemolysis treatment-related AEs were identified by searching with the MedDRA Version 23.1-PTs Haemolysis and Haemolytic anaemia were used to search for events of acute hemolysis. Treatment-related hemolysis AEs were manually evaluated to identify events of acute hemolysis after abrupt mitapivat withdrawal.

In the clinical development program, 6 (6 of 155, 3.9%) subjects with PK deficiency experienced acute hemolysis AEs, and of these subjects, 4 subjects (4 of 155, 2.6%) experienced at least 1 treatment-related event of acute hemolysis (Table SVII.6). There were only 2 subjects (2 of 155; 1.3%) identified after manual evaluation who had hemolysis AEs that were classified as Acute hemolysis after sudden mitapivat discontinuation.

Table SVII.6: Investigator-Reported Hemolysis and Overall AEs by Frequency and Severity in the Clinical Development Program

	N=155	
	Related	**Overall**
No. (%) of subjects with AE	4 (2.6)	6 (3.9)
(95% CI)	(0.7, 6.5)	(1.4, 8.2)
Grade 1, n (%)	0	1 (0.6)
Grade 2, n (%)	2 (1.3)	3 (1.9)
Grade 3, n (%)	2 (1.3)	2 (1.3)
Grade 4, n (%)	0	0
Grade 5, n (%)	0	0

Source: Table 19-3.1.
Abbreviations: AE = adverse event; CTCAE = Common Terminology Criteria for Adverse Events; MedDRA = Medical Dictionary for Regulatory Activities.
Notes: The denominator used to calculate percentages is the total number of subjects in the Safety Analysis Set. For subjects with multiple occurrences of an AE, the AE with the maximum CTCAE grade is included in the summary.
MedDRA Version 23.1 and CTCAE Version 4.03 are used.

A total of 3 hemolysis treatment-related AEs in 2 subjects (2 of 155; 1.3%) subjects were classified as acute hemolysis upon sudden mitapivat discontinuation after manual evaluation. These cases included 2 Grade 3 SAEs of acute hemolysis (PTs Haemolytic anaemia and Haemolysis) and 1 nonserious Grade 2 case of acute hemolysis (PT Haemolysis) (CSR AG348-C-003, Section 14.3.3). Both subjects recovered within 2 to 3 weeks and returned to mitapivat treatment at lower doses without sequelae (Table SVII.7).

Table SVII.7: Investigator-Reported Hemolysis-Related AEs and Overall AEs by Outcome and Seriousness in in the Clinical Development Program

	N=155	
	Related	**Overall**
Number of subjects with at least 1 AE[1]	4	6
Number of AEs	5	7
Recovered, n (%)	5 (100%)	7 (100%)
Ongoing, n (%)	0	0
Death, n (%)	0	0
Number of subjects with at least 1 SAE	3	3
Number of SAEs	4	4
Recovered, n (%)	4 (100%)	4 (100%)
Ongoing, n (%)	0	0
Death, n (%)	0	0

Source: Table 19-4.1.
Abbreviations: AE = adverse event; MedDRA = Medical Dictionary for Regulatory Activities; PT = Preferred Term; SAE = serious adverse event.
Notes: The denominator used to calculate percentages is the total number of subjects in the Safety Analysis Set. Events are considered to be recovered if the outcome is "recovered/resolved" or "recovered/resolved with sequelae" and are considered to be ongoing if the outcome is "recovering/resolving" or "not recovered/not resolved" or unknown or missing.
MedDRA Version 23.1 is used.
[1] Based on AE episodes, AEs of interest where records with the same PT are combined as 1 episode if the dates overlap or the start date of a subsequent AE is within 1 day of the end date of an earlier AE. An AE episode is considered as related if any AE within the episode is reported as related. The outcome of the AE episode is the outcome of the last (end date) AE record.

In both subjects, a similar clinical course was observed for the onset and laboratory presentation of the AEs. In both cases, the subject's high baseline reticulocyte counts rapidly decreased over the first few days of mitapivat exposure, and bilirubin decreased, suggesting a rapid decrease in the subject's hemolysis. At the same time the stability of the RBCs was confirmed by the rapid increase in Hb. When the drug was stopped, a sharp increase in bilirubin, suggesting increased hemolysis, matched with a sharp decrease in Hb was observed. In both subjects, the decrease in Hb occurred rapidly over 48 hours. The reticulocyte count did not increase as rapidly or return to predose levels during the period when the subjects did not receive drug. Both subjects recovered without sequelae and continued to participate in the clinical study at lower treatment doses than originally initiated. By contrast, subjects 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, and their Hb concentrations were either not recorded immediately after the short interruption or decreased gradually after the dose reduction.

This risk will continue to be monitored via routine pharmacovigilance. Adverse events of acute hemolysis will be identified by using the MedDRA PTs of Haemolysis and Haemolytic anaemia as the search strategy.

Risk factors and risk groups:

All patients treated with mitapivat for PK deficiency who experience sustained increases in Hb are at risk of acute hemolysis upon subsequent interruption or withdrawal of mitapivat without a gradual reduction in dosing.

Preventability:

Acute hemolysis due to mitapivat withdrawal can be prevented. The highest recommended dose of 50 mg BID for adult patients with PK deficiency is lower than the 300-mg BID dose taken by the subjects in the clinical development program who experienced acute hemolysis upon abrupt withdrawal of mitapivat. Acute hemolysis is included as a special warning and precautions for use in the Pyrukynd SmPC Section 4.4. The Pyrukynd SmPC Section 4.4 states that acute haemolysis with subsequent anaemia has been observed following abrupt interruption or discontinuation of Pyrukynd (see SmPC Section 4.8). Abrupt interruption or discontinuation of treatment with Pyrukynd should be avoided. A gradual reduction in dosing rather than abrupt cessation is recommended (see SmPC Section 4.2). If discontinuing treatment abruptly, patients should be monitored for signs of acute haemolysis and anaemia, which may include among other symptoms and signs: jaundice, scleral icterus and dark urine.

The SmPC Section 4.2 states that if a dose reduction is required for adverse event management and/or tolerability, the dose may be reduced to the next lower dose level, 20 mg twice daily or 5 mg twice daily. If a patient needs to discontinue the medicinal product due to an adverse event, the dose taper schedule (SmPC Table 2) should be followed. In situations where the risk to the patient due to the adverse event is greater than the risk of acute haemolysis due to sudden withdrawal of the medicinal product, treatment may be stopped without taper and patients should be monitored for signs of acute haemolysis with worsening of anaemia.

In addition, in Section 4.2 of the SmPC it states that to minimize the risk of acute haemolysis, abrupt interruption or discontinuation of Pyrukynd should be avoided. The dose should be tapered to gradually discontinue the medicinal product over a 1-2 week period (see SmPC Table 2 [and see Table SVII.8 below]). Patients should be monitored for signs of acute haemolysis with worsening of anaemia (see SmPC Section 4.4 and 4.8).

Acute hemolysis is also described in the SmPC Section 4.8 under description of selected adverse reactions stating abrupt interruption or discontinuation of Pyrukynd can lead to acute haemolysis (see SmPC Section 4.4). For guidance on how to interrupt or discontinue treatment see SmPC Section 4.2.

Table SVII.8: Dose Taper Schedule

Current Dose	Dose Taper Schedule		
	Day 1-7	**Day 8-14**	**Day 15**
5 mg twice daily	5 mg **once** daily	Discontinue	N/A
20 mg twice daily	20 mg **once** daily	5 mg **once** daily	Discontinue
50 mg twice daily	50 mg **once** daily	20 mg **once** daily	Discontinue

Abbreviations: N/A = not applicable

A mitapivat dose taper pack, that follows the dose taper schedule, is provided to subjects when they are discontinuing mitapivat (Module 1.3.2, Annex 5.17). The Pyrukynd SmPC section 6.5 details the available mitapivat dose taper packs. If a patient or provider intends to discontinue mitapivat treatment, there are 3 individual separate taper packs that can be provided depending on what dose the patient is currently receiving. For patients receiving

mitapivat 50 mg BID, the Pyrukynd 20 mg film-coated tablets + Pyrukynd 50 mg film-coated tablets pack would be provided. This is a carton containing 14 tablets in 2 blister wallets containing 7 × 50 mg and 7 × 20 mg tablets. The blister cards will have Days 1-7 (in this case on blister cards of 50 mg) and Days 7-14 (in this case on blister cards of 20 mg tablets) marked against each blister pocket so that the patient is aware of which tablet needs to be taken each day (thereby minimizing risk of medication errors).

For patients receiving mitapivat 20 mg BID the Pyrukynd 20 mg film-coated tablets + Pyrukynd 5 mg film-coated tablets pack would be provided. This is a single carton containing 14 tablets in 2 blister wallets containing 7 × 20 mg and 7 × 5 mg tablets. The blister cards will have Days 1-7 (in this case on blister cards of 20 mg) and days 7-14 (in this case on blister cards of 5 mg tablets) marked against each blister pocket so that the patient is aware of which tablet needs to be taken each day (thereby minimizing risk of medication errors).

For patients receiving mitapivat 5 mg BID the Pyrukynd 5 mg film-coated tablets pack would be provided. This is a single carton containing 7 tablets of 5 mg mitapivat tablets. The blister cards will have Days 1-7 marked against each blister pocket so that the patient is aware which tablet needs to be taken each day (thereby minimizing risk of medication errors).

Impact on the benefit-risk balance of the product:

Acute hemolysis is a serious condition that may lead to acute exacerbation of the patients underlying health condition if left unmanaged. Patients with PK deficiency experience lifelong hemolytic anemia with subsequent associated comorbidities. They have a serious unmet medical need for safe and effective targeted therapies, as currently no product is specifically approved to manage or treat PK deficiency. The benefit of mitapivat as an effective treatment for PK deficiency outweighs the risk of acute hemolysis, which has been observed only in Hb-responding subjects at 6 times the recommended mitapivat dose and can be managed in clinical practice by adhering to the guidance in the Pyrukynd SmPC. Acute hemolysis is included as a special warning and precautions for use in the Pyrukynd SmPC Section 4.4 and PL Section 2. The Pyrukynd SmPC states that abrupt interruption or discontinuation of treatment with Pyrukynd should be avoided. A gradual reduction in dosing rather than abrupt cessation is recommended (see Section 4.2 of the SmPC). If discontinuing treatment abruptly, patients should be monitored for signs of acute haemolysis and anaemia, which may include among other symptoms and signs: jaundice, scleral icterus, and dark urine. The important identified risk will be monitored using routine pharmacovigilance (Part III.1).

Public health impact:

Treatment-related acute hemolysis after abrupt mitapivat withdrawal occurred in 2 (1.3%) subjects with PK deficiency treated with mitapivat in the clinical development program. Acute hemolysis is included as a special warning and precautions for use in the Pyrukynd SmPC Section 4.4 and PL Section 2. Pyruvate kinase deficiency is an extremely rare condition (Module SI); therefore, the overall public health impact is expected to be low.

Important Potential Risk: Embryo-fetal toxicity

Potential mechanisms:

Non-clinical studies

As described in Part II of the RMP, the GLP-compliant definitive embryo-fetal development study in Sprague Dawley rats and New Zealand white rabbits demonstrated that fetal adverse effects were observed at $AUC_{0\text{-}12hr}$ values 63-fold (rats) and 3.1-fold (rabbits) the human $AUC_{0\text{-}12hr}$ value at 50 mg BID. Fetal adverse effects in rats were considered likely due to sex hormone changes caused by aromatase inhibition.

In the GLP-compliant combined fertility and early embryonic development study in Sprague Dawley rats, no effect on mating or fertility was observed at any dosage in either males or females. The highest dosages tested were 300 mg/kg/day in males and 200 mg/kg/day in females, correlating with $AUC_{0\text{-}12hr}$ (day last) values [160,000 hr•ng/mL] 45-fold and [174,000 hr•ng/mL] 49-fold the $AUC_{0\text{-}12hr}$ value in humans at 50 mg BID, respectively. The lowest $AUC_{0\text{-}12hr}$ value at which microscopic reproductive findings have been observed in animals was 10,900 hr•ng/mL (Module 2.6.6, Section 3.1.4), and the reproductive organ NOEL in the repeat-dose toxicity study in which this occurred was 5,150 hr•ng/mL (1.4-fold the human $AUC_{0\text{-}12hr}$ value [3,580 hr•ng/mL] at 50 mg BID). All findings with the potential to impair fertility in the Sprague Dawley rats were reversible after discontinuation of the study drug.

The effects of sex hormone changes due to aromatase inhibition were observed only at AUC values considerably higher than those observed with the 50 mg BID dose in adult humans. The plausible biological mechanism for the potential risk of embryo-fetal toxicity is as a result of blocking the production of estrogens from androgens that could lead to a decrease of fetal implantation, an increase of fetal death, and a variety of non-specified teratogenic effects (Tiboni and Ponzano, 2016).

Evidence source and strength of evidence:

Although human data on pregnancies are limited, there is no evidence of human teratogenicity based on genotoxic potential and the 2 successful pregnancies. Although nonclinical reproductive toxicity studies did not demonstrate teratogenicity/fetotoxicity at maternal exposures comparable to those in humans administered mitapivat at 50 mg BID (proposed clinical dose), they did demonstrate embryo-fetal toxicity at high dose levels: $AUC_{0\text{-}12hr}$ values 63- and 3.1-fold higher than the human $AUC_{0\text{-}12hr}$ value at 50 mg BID. Based on these non-clinical findings, embryo-fetal toxicity is considered an important potential risk (Module SVII.3.1).

Characterization of the risk:

Based on in vitro data, mitapivat is a weak aromatase inhibitor. Furthermore, based on nonclinical and clinical data, sex hormone changes is an identified risk (not considered important) associated with treatment. In clinical and nonclinical studies, mitapivat behaves like a reversible aromatase inhibitor. Analysis of the data suggests that the concentration of mitapivat in humans at the highest expected clinically efficacious dose is well below the concentration required to inhibit aromatase by 50%. In the clinical development program, there were no data suggesting that there is an increase in the risk of AEs that could be reasonably associated with hormone changes due to off-target aromatase inhibition by mitapivat.

In animal studies, harmful effects of sex hormone changes due to aromatase inhibition were observed only at AUC values considerably higher than those observed with the 50 mg BID dose in adult humans. In GLP–compliant definitive embryo-fetal development studies in Sprague Dawley rats and New Zealand white rabbits, effects consistent with sex hormone changes due to aromatase inhibition were only observed in rats. In rats, fetal adverse effects were observed at doses associated with day last AUC from 0 to 12 hours ($AUC_{0\text{-}12hr}$) values 63-fold the steady-state $AUC_{0\text{-}12hr}$ value in subjects with pyruvate kinase deficiency (PK deficiency) administered mitapivat 50 mg BID. This included a decrease in the mean number and litter proportion of viable fetuses, lower mean fetal weights, and test article-related external, soft tissue, and skeletal malformations (Module 2.6.6, Section 6.2.1.2). The maternal and fetal NOAEL was seen at maternal doses associated with day last $AUC_{0\text{-}12hr}$ values 13-fold the steady-state $AUC_{0\text{-}12hr}$ value in subjects administered mitapivat 50 mg BID. The adverse effects observed in rats are consistent with effects reported for aromatase inhibitors and are therefore likely due to sex hormone changes caused by aromatase inhibition.

In clinical studies, aromatase-dependent hormone levels in female subjects treated with mitapivat were collected but were difficult to interpret due to subject menopausal status and contraceptive use (premenopausal vs menopausal, hormonal vs nonhormonal contraception); therefore, these data were considered not informative for analysis of mitapivat changes in sex hormones due to aromatase inhibition effects. Menstrual cycle diaries were originally introduced in clinical studies in 2015 to record any effects on the menstrual cycle of female subjects taking mitapivat. These data were subjective and not collected systematically; however, based on an aggregate review, no meaningful trends were identified that could be attributed to changes in sex hormones due to aromatase inhibition.

There have been 2 pregnancies reported during the mitapivat clinical development program:

1. One subject became pregnant and mitapivat was immediately interrupted. The subject gave birth to a normal healthy baby girl at 39 weeks by planned caesarean section. The subject resumed mitapivat treatment after completion of breastfeeding. The projected exposure of the subject was 19 times lower than the exposure in rats at the dose where no adverse effects were observed on the fetus and the mother (ie, the NOAEL) (CSR AG348-C-003, Section 14.3.3).
2. Paternal exposure during pregnancy was reported for a male subject. The male subject had been receiving mitapivat for approximately 4 years before his partner became pregnant. The subject's partner gave birth to a normal healthy baby at 40 weeks via vaginal episiotomy. The subject's partner experienced postpartum hemorrhage, which led to prolongation of hospitalization and red blood cell

transfusion; the postpartum hemorrhage was not considered to be related to study treatment. The subject's partner subsequently recovered and was discharged (CSR AG348-C-003, Section 14.3.3).

Use of mitapivat during pregnancy has not been formally studied, and a limited number of subjects have reported a pregnancy. Therefore, mitapivat is not recommended for use during pregnancy or in females of childbearing potential who are not using contraception. As the safety and efficacy of mitapivat in this population is not currently known, embryo-fetal toxicity is considered an important potential risk.

Embryo-fetal toxicity will be monitored via routine pharmacovigilance. Adverse events of embryo-fetal toxicity will be identified by using the MedDRA SMQ (Broad) of Pregnancy, labour and delivery complications and risk factors as the search strategy.

Post-marketing monitoring for pregnancy and lactation will occur via routine pharmacovigilance. A pregnancy-, lactation-, and embryo-fetal toxicity-specific follow-up form will be utilized by the case processing team, or extension thereof. A follow-up form is attached in Annex 4.

Risk factors and risk groups:

Risk groups include women of child-bearing potential taking mitapivat that may become pregnant and male partners taking mitapivat where a partner becomes pregnant.

Preventability:

Routine risk minimization is considered adequate to minimize the risk of embryo-fetal toxicity. In the Pyrukynd SmPC Section 4.6, it states that there is no or limited amount of data from the use of mitapivat in pregnant women. Studies in animals have shown reproductive toxicity (see SmPC Section 5.3). Pyrukynd is not recommended during pregnancy and in women of childbearing potential not using contraception. Women of childbearing potential should avoid becoming pregnant while receiving Pyrukynd. Women of childbearing potential should use contraception during treatment with mitapivat and for at least 1 month after the last dose. Mitapivat may decrease the systemic exposure of hormonal contraceptives that are sensitive substrates of CYP3A4 (see SmPC Sections 4.4 and 4.5). Additional or alternative methods of contraception should be considered. In addition, the SmPC Sections 4.4 and 4.5 state that mitapivat may decrease the systemic concentrations of hormonal contraceptives that are sensitive substrates of cytochrome P450 3A4 (CYP3A4) (eg, ethinylestradiol). Therefore, women of childbearing potential should be counselled regarding the use of additional or alternative contraception methods. Section 5.3 of the SmPC describes the relevant preclinical safety data.

Impact on the benefit-risk balance of the product:

It is currently unknown what the impact of embryo-fetal toxicity might be. Clear wording will be included in the SmPC and PL to minimize the risk of pregnancy. The important identified risk will be monitored using routine pharmacovigilance whereby any use in pregnancy in clinical practice will be monitored using routine pharmacovigilance, including the pregnancy-, lactation-, and embryo-fetal toxicity-specific follow-up form (Part III.1).

Public health impact:

Use in pregnant patients is expected to be minimal as PK deficiency is an extremely rare condition (Module SI). With the routine risk minimization, it is considered that the risk will be adequately minimized.

SVII.3.2 Presentation of the missing information

Missing Information 1: Use in patients with hepatic impairment

Evidence source:

Hepatic elimination plays an important role in the clearance of mitapivat. The metabolic stability of mitapivat was evaluated in rat, dog, monkey, and human liver microsomes (Report AG348-N-066-R1). After 45 minutes of incubation with mitapivat (1 µM) and NADPH (2 mM), liver microsomal CL_{int} of mitapivat was 32.1, 22.8, 18.1, and 6.82 L/hr/kg in rats, dogs, monkeys, and humans, respectively. The CL_{hep} calculated based on the CL_{int} was 2.99, 1.75, 2.27, and 1.05 L/hr/kg in rats, dogs, monkeys, and humans, respectively. The hepatic extraction ratio was estimated to be 0.91, 0.92, 0.87, and 0.85 in rats, dogs, monkeys, and humans, respectively. These data suggest that mitapivat liver metabolism clearance was high (Module 2.6.4, Section 5.1.1.2).

Mitapivat is known to be metabolized after oral administration with approximately 40% and 50% of administered radioactivity in the human ADME study appearing as metabolites in the feces and urine, respectively (Module 2.7.2, Section 3.1.4). Therefore, there is a potential for hepatic impairment to affect mitapivat exposure.

Use of mitapivat in subjects with hepatic impairment has not been evaluated in the clinical development program. By nature of the indication, subjects with PK deficiency are likely to experience chronic and acute hemolysis that may lead to increases in unconjugated bilirubin (Module SI). Due to this, analysis of the effect of hepatic parameters such as bilirubin on mitapivat PK is limited. The effect of hepatic parameters on mitapivat pharmacokinetics was assessed as part of a population pharmacokinetics analyses. Most subjects in the dataset had normal AST, ALT, and ALP at baseline. No apparent difference in steady-state AUC was observed in subjects with normal AST, ALT, or ALP levels versus those with elevated levels. Most subjects with PK deficiency in the dataset had elevated total bilirubin levels at baseline; nevertheless, steady-state AUC appeared to be similar among the subjects with PK deficiency with total bilirubin at the ranges between 1 to 2×ULN, 2 to 3×ULN, and >3×ULN (Module 2.7.2, Section 3.2.2). Additionally, analysis of the degree of hepatic impairment in the PK deficiency clinical development program is unavailable as conventional hepatic dysfunction stratification tools (Child-Pugh, NCI-ODWG, and MELD) all use bilirubin as a criterion of their classifications.

Use in subjects with hepatic impairment is considered an area of missing information as mitapivat has not been studied in subjects with hepatic impairment and the potential for hepatic impairment to affect mitapivat exposure. No dose recommendations can be made, and the safety and efficacy of mitapivat have not been established in such subjects.

The effect of subjects with hepatic impairment who receive mitapivat will be monitored via Study AG348-C-0HEP and via routine pharmacovigilance by review of AEs in subjects with a medical history consistent with hepatic impairment.

Population in need of further characterization:

Mitapivat has not been studied in subjects with hepatic impairment. No dose recommendations can be made, and the safety and efficacy of mitapivat have not been established in such subjects. Because there is a potential for hepatic impairment to affect mitapivat exposure, it will be important to assess the findings from the planned hepatic

impairment Study AG348-C-0HEP to determine if dose adjustments of mitapivat are required for subjects with hepatic impairment

Study AG348-C-0HEP is designed to evaluate the PK, safety and tolerability of mitapivat in subjects each with moderate or normal hepatic function (Part III.2) which is aligned with the CHMP guidance on the 'Evaluation of the pharmacokinetics of medicinal products in patients with impaired hepatic function' (2005).

Hepatic impairment is included as a special population in the Pyrukynd SmPC Sections 4.2 and 5.2. In the Pyrukynd SmPC Section 4.2, it states that there are no data available in patients with hepatic impairment. No dose recommendations can be made. In the Pyrukynd SmPC Section 5.2, it states that the pharmacokinetics of mitapivat in patients with mild, moderate, or severe hepatic impairment have not been studied.

Missing Information 2: Long-term use

Evidence source:

The primary studies providing evidence of long-term use of mitapivat in the proposed indication for the treatment of PK deficiency are Studies AG348-C-003 and AG348-C-011. Study AG348-C-008 (Peak Registry) will also provide supportive evidence for subjects that are receiving mitapivat.

Study AG348-C-003 is an ongoing Phase 2, open-label, 2-arm, multicenter, randomized, dose-ranging study assessing the safety and tolerability, pharmacokinetics, pharmacodynamics, and clinical activity of mitapivat in adult subjects with PK deficiency.

The study consists of a completed 24-week Core Period and an ongoing 8-year Extension Period. This study initiated on 26 June 2015 and last patient last visit is anticipated in May 2025. In this study, subjects have received doses ranging from 5 mg 3 times a week to 300 mg BID. As of 28 August 2020 (the interim data-cut for this study), 52 subjects have initiated treatment with 18 subjects ongoing treatment in the study.

Study AG348-C-008 is an ongoing Agios-sponsored, global, retrospective and prospective, longitudinal observational study of pediatric and adult patients with PK deficiency.

The Peak Registry was designed to understand better the natural history of PK deficiency, including diagnosis, demographic and clinical characteristics, burden of disease, treatment patterns, and clinical outcomes in a real-world setting. The registry enrolled its first patient in April 2018 and will continue enrolling through Q2 2025. The study is expected to be completed in Q2 2027 with database lock in Q3 2027, providing at least 2 years and up to 9 years of patient follow-up data. As of 08 March 2022, the Peak Registry had 240 patients enrolled from 48 sites in 16 countries, predominantly located in North America and Europe. This is a Category 3 PASS study, see Table III.1 Ongoing and Planned Additional Pharmacovigilance Activities

Study AG348-C-011 is an ongoing Phase 3, multicenter, open-label extension study for subjects who have completed Study AG348-C-006 or AG348-C-007 and meet all other eligibility criteria for Study AG348-C-011. The overall duration of the study is approximately 5 years. This study initiated on 21 March 2019 and last patient last visit is currently anticipated in November 2024. As of 12 November 2020 (interim data-cut for this study), 88 subjects had initiated treatment with 78 subjects still ongoing treatment in the study. This is a Category 3 PASS study, see Table III.3.1 Ongoing and Planned Additional Pharmacovigilance Activities.

The long-term safety profile of mitapivat is consistent with the safety profile during the 24-week on-treatment period. The most commonly reported events during the cumulative period were similar to those reported during the 24-week on-treatment period, and most events continued to be Grade 1 or 2 in severity. The slight increase in percentage of subjects experiencing these events is considered consistent with what would be expected with continued treatment for a longer duration in this patient population.

No trends in TEAEs that may be a result of long-term changes in sex hormones were observed, including no overall trend in decreasing BMD, signals pertaining to psychological disorders such as anxiety or depression, or cardiovascular disorders. Furthermore, other events that may be considered a long-term tolerability concern with mitapivat, such as repeated or worsening insomnia, were not frequently reported. Events such as triglyceride increases or transaminase increases were transient, and long-term safety impacts of transient increases in these parameters were not observed.

Population in need of further characterization:

The effects of long-term use of mitapivat in subjects with PK deficiency will be further characterized in ongoing Study AG348-C-011 and the Peak Registry (AG348-C-008) which are both Category 3 PASS studies. Study AG348-C-011 allows for subjects to be treated with mitapivat for an additional 5 years after transitioning from Study AG348-C-006 and Study AG348-C-007. This study has been ongoing since 21 March 2019. The Peak Registry has been ongoing since April 2018 and will provide up to 9 years of patient follow-up data for subjects that are receiving mitapivat.

The Pyrukynd SmPC Section 4.2 of the SmPC states that treatment with Pyrukynd is intended to be long-term. Pyrukynd should be discontinued if a patient does not experience an improvement of haemolytic anaemia at the maximum recommended dose, based on the totality of laboratory results and clinical status of the patient, unless there is another explanation for response failure (eg, bleeding, surgery, other concomitant illnesses).

The SmPC Section 5.1 states that the median duration of treatment of mitapivat was 24.1 weeks in AG348-C-006 and median duration of treatment in AG348-C-007 was 40.3 weeks.

PART II: MODULE SVIII – SUMMARY OF THE SAFETY CONCERNS

Table SVIII.1: Summary of Safety Concerns

Summary of Safety Concerns	
Important identified risks	Acute hemolysis
Important potential risks	Embryo-fetal toxicity
Missing information	Use in patients with hepatic impairment Long-term use

Part III: Pharmacovigilance Plan (including postauthorization safety studies)

III.1 Routine pharmacovigilance activities

Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection:

Specific adverse reaction follow-up questionnaires for the safety concerns

A pregnancy-, lactation-, and embryo-fetal toxicity-specific follow-up form will be used when reports of pregnancy are received and then throughout the course of the pregnancy and delivery, as applicable. A follow-up form is attached in Annex 4.

Other forms of routine pharmacovigilance activities for safety concerns:

None

III.2 Additional pharmacovigilance activities

The target population for mitapivat is treatment of adult patients with PK deficiency, an extremely rare autosomal recessive disease (Module SI).

Study AG348-C-0HEP to evaluate the pharmacokinetics, safety, and tolerability of mitapivat in subjects with moderate hepatic impairment or normal hepatic function will further characterize the safety concern of use in patients with hepatic impairment.

Study AG348-C-011 to evaluate the long-term safety and tolerability of mitapivat in an open-label extension study in subjects with PK deficiency previously enrolled in Studies AG348-C-006 or AG348-C-007.

Study AG348-C-008 (Peak Registry) is an Agios-sponsored, global, retrospective and prospective, longitudinal observational study of pediatric and adult patients with PK deficiency.

Studies

Study short name and title:

Hepatic impairment Study AG348-C-0HEP

A Phase 1, Single-Dose, Open-label Study to Evaluate the Pharmacokinetics, Safety, and Tolerability of Mitapivat in Subjects With Moderate Hepatic Impairment or Normal Hepatic Function

Rationale and study objectives:

To evaluate the pharmacokinetics, safety, and tolerability of mitapivat in subjects with moderate hepatic impairment or normal hepatic function.

Safety concerns addressed:

- Use in patients with hepatic impairment

Study design:

Phase 1, single-dose, open-label study designed to evaluate the pharmacokinetic, safety, and tolerability of a single 50 mg mitapivat dose in subjects with moderate hepatic impairment compared with subjects with normal hepatic function.

Study population:

To be determined

Milestones:

Milestone	Planned Date
Final study report:	31 March 2024

Study short name and title:

Long-term safety and tolerability Study AG348-C-011

A Phase 3, Multicenter, Open-label, Long-term, Extension Study of Mitapivat in Adults with PK Deficiency Previously Treated in Studies AG348-C-006 or AG348-C-007.

Rationale and study objectives:

To evaluate the long-term safety and tolerability of mitapivat in adult subjects with PK deficiency previously enrolled in Study AG348-C-006 or AG348-C-007.

Safety concerns addressed:

- Acute hemolysis
- Long-term use in subjects with PK deficiency

Study design:

Phase 3, Multicenter, Open-label, Long-term, Extension Study of Mitapivat in Adults with PK Deficiency Previously Treated in Studies AG348-C-006 or AG348-C-007.

Subjects are assigned to 1 of the following 3 cohorts, depending on the antecedent study and the previous treatment received in the antecedent study:

- Cohort 1: subjects who received placebo in Study AG348-C-006
- Cohort 2: subjects who received mitapivat in Study AG348-C-006
- Cohort 3: subjects who received mitapivat in Study AG348-C-007

Study population:

Subjects included in the study are ≥18 years of age and have PK deficiency, have completed the applicable antecedent mitapivat study (ie, through the Part 2 Week 24 visit of Study AG348C006 or AG348-C-007). Subjects enrolled in Cohorts 2 and 3 must have demonstrated clinical benefit from mitapivat treatment in the antecedent studies, in the opinion of the Investigator.

Milestones:

Milestone	Planned Date
Final study report:	30 November 2025

Study short name and title:

Longitudinal observational study (Peak Registry)

An ongoing Agios-sponsored, global, retrospective and prospective, longitudinal observational study of pediatric and adult patients with PK deficiency.

Rationale and primary study objectives:

To understand better the natural history of PK deficiency, including diagnosis, demographic and clinical characteristics, burden of disease, treatment patterns, and clinical outcomes in a real-world setting.

Safety concerns addressed:

- Long-term use

Study design:

The Peak Registry is an observational, longitudinal, global registry of adult and pediatric patients with a genetically confirmed diagnosis of PK deficiency. The registry aims to enroll approximately 500 adult and pediatric patients from approximately 60 study centers across up to 20 countries and will be open for enrollment for 7 years with all participants to be followed prospectively for at least 2 years and for up to 9 years.

Study population:

Patients of any age, with a diagnosis of PK deficiency confirmed via genetic testing, are eligible for enrollment in the Peak Registry. Genetic diagnosis includes the presence of biallelic PKLR mutations (either compound heterozygous or homozygous state), including newly described variants. Patients are not required to be on any specific treatment to be eligible for study participation; the final study report will inform on patients that received mitapivat.

Milestones:

Milestone	**Planned Date**
Final study report for patients that received mitapivat:	30 September 2028

III.3 Summary Table of additional pharmacovigilance activities

Table III.3.1: Ongoing and Planned Additional Pharmacovigilance Activities

Title and Study Status	Summary of Objectives	Safety Concerns Addressed	Milestones	Due Dates
Category 1 - Imposed mandatory additional pharmacovigilance activities that are conditions of the marketing authorization				
None				
Category 2 – Imposed mandatory additional pharmacovigilance activities that are specific obligations in the context of a conditional marketing authorization or a marketing authorization under exceptional circumstances				
None				
Category 3 - Required additional pharmacovigilance activities				
AG348-C-0HEP A Phase 1, Single-Dose, Open-label Study to Evaluate the Pharmacokinetics, Safety, and Tolerability of Mitapivat in Subjects With Moderate Hepatic Impairment or Normal Hepatic Function Planned	Evaluate the pharmacokinetics, safety, and tolerability of mitapivat in subjects with moderate hepatic impairment or normal hepatic function	Use in patients with hepatic impairment	Final study report:	31 March 2024
AG348-C-011 A Phase 3, Multicenter, Open-label, Long-term, Extension Study of Mitapivat in Adults with PK Deficiency Previously Treated in Studies AG348-C-006 or AG348-C-007. Ongoing	Evaluate the long-term safety and tolerability of mitapivat.	Acute hemolysis Long-term use	Final study report	30 November 2025
AG348-C-008 (Peak Registry) An ongoing Agios-sponsored, global, retrospective and prospective, longitudinal observational study of pediatric and adult patients with PK deficiency. Ongoing	To understand better the natural history of PK deficiency, including diagnosis, demographic and clinical characteristics, burden of disease, treatment patterns, and clinical outcomes in a real-world setting.	Long-term use for patients receiving mitapivat	Final study report for patients that received mitapivat	30 September 2028

Part IV: Plans for postauthorization efficacy studies

There are no planned or ongoing postauthorization efficacy studies that are conditions of the marketing authorization or that are specific obligations.

Part V: Risk minimization measures (including evaluation of the effectiveness of risk minimization activities)

Risk Minimization Plan

V.1 Routine risk minimization measures

Table V.1.1: Description of Routine Risk Minimization Measures by Safety Concern

Safety Concern	Routine Risk Minimization Activities
Acute hemolysis (Important identified risk)	Routine risk communication: • *Acute haemolysis is listed as a special warnings and precautions for use in the Summary of Product Characteristics (SmPC) Section 4.4* • *Acute haemolysis is described as a selected adverse reaction in the SmPC Section 4.8* • *Acute haemolysis is listed as a warning and precaution in Package Leaflet (PL) Section 2* • *Acute haemolysis, after abrupt interruption or discontinuation of Pyrukynd, is described in PL Section 4* Routine risk minimization activities recommending specific clinical measures to address the risk: • *Warning and precaution that acute haemolysis with subsequent anaemia has been observed following abrupt interruption or discontinuation of Pyrukynd in SmPC Section 4.4* • *Warning that to minimise the risk of acute haemolysis, abrupt interruption or discontinuation of Pyrukynd should be avoided in SmPC Sections 4.2 and 4.4* • *Advice on the dose taper schedule to be followed when discontinuing Pyrukynd in SmPC Section 4.2* • *Warning to monitor patients for signs of acute haemolysis with worsening of anaemia if discontinuing treatment in SmPC Sections 4.2 and 4.4* • *Warning and precaution for the patient to talk to their doctor if they develop symptoms of acute haemolysis in PL Section 4* Other routine risk minimization measures beyond the Product Information: • *Pack size: Dose taper blister packs that follow the dose taper schedule when discontinuing mitapivat* • *Description of the dose taper blister packs in SmPC Section 6.5 and PL Section 6*

Table V.1.1: Description of Routine Risk Minimization Measures by Safety Concern

Safety Concern	Routine Risk Minimization Activities
Embryo-fetal toxicity (Important Potential Risk)	Routine risk communication: • *Information on nonclinical findings in SmPC Section 5.3* Routine risk minimization activities recommending specific clinical measures to address the risk: • *Advice that Pyrukynd is not recommended during pregnancy and in women of childbearing potential not using contraception in SmPC Section 4.6* • *Advice that contraception should be used by women of childbearing potential during treatment and for at least 1 month after the last dose in SmPC Section 4.6* • *Advice that mitapivat may decrease systemic exposure of hormonal contraceptives that are sensitive substrates of CYP3A4 in SmPC Sections 4.4, 4.5 and 4.6* • *Advice that women of childbearing potential should be counselled regarding the use of additional or alternative contraception methods in SmPC Section 4.4* • *Advice that Pyrukynd should be avoided during pregnancy and women of childbearing potential must use reliable contraception and for at least 1 month after the last dose in PL Section 2* • *Advice that birth control medicines containing hormones may not work as well as expected and pregnancy may occur so a patient should discuss contraception methods with their doctor in PL Section 2* Other routine risk minimization measures beyond the Product Information: • *None*
Use in patients with hepatic impairment (Missing information)	Routine risk communication: • *Information that the pharmacokinetics of mitapivat in patients with mild, moderate, or severe hepatic impairment have not been studied in SmPC Section 5.2* Routine risk minimization activities recommending specific clinical measures to address the risk: • *Advice that Pyrukynd has not been studied in patients with hepatic impairment and no dose recommendations can be made in SmPC Sections 4.2 and 5.2* Other routine risk minimization measures beyond the Product Information: • *None*

Table V.1.1: Description of Routine Risk Minimization Measures by Safety Concern

Safety Concern	Routine Risk Minimization Activities
Long-term use (Missing information)	Routine risk communication: • *Information that the median duration of treatment with Pyrukynd was 24.1 weeks in AG348-C-006 and median duration of treatment in AG348-C-007 was 40.3 weeks in SmPC Section 5.1* Routine risk minimization activities recommending specific clinical measures to address the risk: • *Advice that treatment with Pyrukynd is intended to be long-term and should be discontinued if there is no improvement of haemolytic anaemia at the maximum recommended dose, based on the totality of laboratory results and clinical status of the patient, unless there is another explanation for response failure in SmPC Section 4.2.* Other routine risk minimization measures beyond the Product Information: • *None*

V.2 Additional risk minimization measures

Routine risk minimization activities as described in Part V.1 are sufficient to manage the safety concerns of the medicinal product.

Removal of additional risk minimization activities:

Not applicable

V.3 Summary of risk minimization measures

Table V.3.1: Summary Table of Pharmacovigilance Activities and Risk Minimization Activities by Safety Concern

Safety Concern	Risk Minimization Measures	Pharmacovigilance Activities
Acute hemolysis	Routine risk minimization measures: • *Acute haemolysis is listed as a special warnings and precautions for use in the Summary of Product Characteristics (SmPC) Section 4.4* • *Acute haemolysis is described as a selected adverse reaction in the SmPC Section 4.8* • *Acute haemolysis is listed as a warning and precaution in Package Leaflet (PL) Section 2* • *Acute haemolysis, after abrupt interruption or discontinuation of Pyrukynd, is described in PL Section 4* • *Warning and precaution that acute haemolysis with subsequent anaemia has been observed following abrupt interruption or discontinuation of Pyrukynd in SmPC Section 4.4*	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: • *None* Additional pharmacovigilance activities: • *Long-term safety and tolerability study AG348-C-011; final study report available 30 November 2025.*

Table V.3.1: Summary Table of Pharmacovigilance Activities and Risk Minimization Activities by Safety Concern

Safety Concern	Risk Minimization Measures	Pharmacovigilance Activities
	• *Warning that to minimize the risk of acute haemolysis, avoid abrupt interruption or discontinuation of Pyrukynd in SmPC Sections 4.2 and 4.4* • *Advice on the dose taper schedule to be followed when discontinuing Pyrukynd in SmPC Section 4.2* • *Warning to monitor patients for signs of acute haemolysis with worsening of anaemia if discontinuing treatment in SmPC Sections 4.2 and 4.4* • *Warning and precaution for the patient to talk to their doctor if they develop symptoms of acute haemolysis in PL Section 4* • *Pack size: Dose taper blister packs, that follow the dose taper schedule, when discontinuing Pyrukynd* • *Description of the dose taper blister packs in SmPC Section 6.5 and PL Section 6* Additional risk minimization measures: • *None*	

Table V.3.1: Summary Table of Pharmacovigilance Activities and Risk Minimization Activities by Safety Concern

Safety Concern	Risk Minimization Measures	Pharmacovigilance Activities
Embryo-fetal toxicity	Routine risk minimization measures: • *Information on nonclinical findings in SmPC Section 5.3* • *Advice that Pyrukynd is not recommended during pregnancy and in women of childbearing potential not using contraception in SmPC Section 4.6* • *Advice that contraception should be used by women of childbearing potential during treatment and for at least 1 month after the last dose in SmPC Section 4.6* • *Advice that mitapivat may decrease systemic exposure of hormonal contraceptives that are sensitive substrates of CYP3A4 in SmPC Sections 4.4, 4.5 and 4.6* • *Advice that women of childbearing potential should be counselled regarding the use of additional or alternative contraception methods in SmPC Section 4.4* • *Advice that Pyrukynd should be avoided during pregnancy and women of childbearing potential must use reliable contraception and for at least 1 month after the last dose in PL Section 2* • *Advice that birth control medicines containing hormones may not work as well as expected and pregnancy may occur so a patient should discuss contraception methods with their doctor in PL Section 2* Additional risk minimization measures: • *None*	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: • *Pregnancy-, lactation-, embryo-fetal toxicity-follow up form* Additional pharmacovigilance activities: • *None*

Table V.3.1: Summary Table of Pharmacovigilance Activities and Risk Minimization Activities by Safety Concern

Safety Concern	Risk Minimization Measures	Pharmacovigilance Activities
Use in patients with hepatic impairment	Routine risk minimization measures: • *Information that the pharmacokinetics of mitapivat in patients with mild, moderate, or severe hepatic impairment have not been studied in SmPC Section 5.2* • *Advice that Pyrukynd has not been studied in patients with hepatic impairment and no dose recommendations can be made in SmPC Sections 4.2 and 5.2* Additional risk minimization measures: • *None*	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: • *None* Additional pharmacovigilance activities: • *Hepatic impairment Study AG348-C-0HEP; final study report available: 31 March 2024*
Long-term Use	Routine risk minimization measures: • *Information that the median duration of treatment with Pyrukynd was 24.1 weeks in AG348-C-006 and median duration of treatment in AG348-C-007 was 40.3 weeks in SmPC Section 5.1* • *Advice that treatment with Pyrukynd is intended to be long-term and should be discontinued if there is no improvement of haemolytic anaemia at the maximum recommended dose, based on the totality of laboratory results and clinical status of the patient, unless there is another explanation for response failure in SmPC Section 4.2.* Additional risk minimization measures: • *None*	Routine pharmacovigilance activities beyond adverse reactions reporting and signal detection: • *None* Additional pharmacovigilance activities: • *Long-term safety and tolerability study AG348-C-011; final study report available 30 November 2025.* • *Longitudinal observational study AG348-C-008 (Peak Registry); final study report for patients that received mitapivat available 30 September 2028.*

PART VI: SUMMARY OF THE RISK MANAGEMENT PLAN

Summary of risk management plan for Pyrukynd (mitapivat)

This is a summary of the RMP for Pyrukynd. The RMP details important risks of Pyrukynd, how these risks can be minimized, and how more information will be obtained about Pyrukynd's risks and uncertainties (missing information).

Pyrukynd's summary of product characteristics (SmPC) and its package leaflet (PL) give essential information to health-care professionals and patients on how Pyrukynd should be used.

This summary of the RMP for Pyrukynd should be read in the context of all of this information including the assessment report of the evaluation and its plain-language summary, all which is part of the European Public Assessment Report (EPAR).

Important new concerns or changes to the current ones will be included in updates of Pyrukynd's RMP.

1. The Medicine and What It Is Used for

Pyrukynd is authorized for treatment of pyruvate kinase deficiency (PK deficiency) in adult patients (see SmPC for the full indication). It contains mitapivat as the active substance and it is taken orally.

Further information about the evaluation of Pyrukynd's benefits can be found in Pyrukynd's EPAR, including in its plain-language summary, available on the European Medicines Agency (EMA) website, under the medicine's webpage <link to the EPAR summary landing page>.

2. Risks Associated With the Medicine and Activities to Minimize or Further Characterize the Risks

Important risks of Pyrukynd, together with measures to minimize such risks and the proposed studies for learning more about Pyrukynd's risks, are outlined below.

Measures to minimize the risks identified for medicinal products can be:

- Specific information, such as warnings, precautions, and advice on correct use, in the package leaflet and SmPC addressed to patients and healthcare professionals;
- Important advice on the medicine's packaging;
- The authorized pack size – the amount of medicine in a pack is chosen to ensure that the medicine is used correctly;
- The medicine's legal status – the way a medicine is supplied to the patient (eg, with or without prescription) can help to minimize its risks

Together, these measures constitute *routine risk minimization measures*.

In addition to these measures, information about adverse reactions is collected continuously and regularly analyzed, including periodic safety update report (PSUR) assessment so that immediate action can be taken as necessary. These measures constitute *routine pharmacovigilance activities*.

If important information that may affect the safe use of Pyrukynd is not yet available, it is listed under "missing information" below.

II.A List of important risks and missing information

Important risks of Pyrukynd are risks that need special risk management activities to further investigate or minimize the risk, so that the medicinal product can be safely taken. Important risks can be regarded as identified or potential. Identified risks are concerns for which there is sufficient proof of a link with the use of Pyrukynd. Potential risks are concerns for which an association with the use of this medicine is possible based on available data, but this association has not been established yet and needs further evaluation. Missing information refers to information on the safety of the medicinal product that is currently missing and needs to be collected (eg, on the long-term use of the medicine).

List of Important Risks and Missing Information	
Important identified risks	Acute hemolysis
Important potential risks	Embryo-fetal toxicity
Missing information	Use in patients with hepatic impairment Long-term use

II.B Summary of important risks

Important Identified Risk: Acute Hemolysis	
Evidence for linking the risk to the medicine	Acute hemolysis is an important identified risk as it is a severe condition that may lead to acute exacerbation of the patient's underlying health condition if left untreated. In clinical trials with Pyrukynd, 2 subjects with PK deficiency dosed at 300 mg BID (2 of 155; 1.3%) experienced acute hemolysis upon sudden withdrawal of Pyrukynd.
Risk factors and risk groups	All patients treated with Pyrukynd for PK deficiency who experience sustained increases in hemoglobin are at risk of acute hemolysis upon subsequent interruption or withdrawal of Pyrukynd without a gradual reduction in dosing.
Risk minimization measures	Routine risk minimization measures: *To communicate and reduce the risk of acute hemolysis* • *SmPC: Sections 4.2, 4.4, 4.8, and 6.5* • *PL: Sections 2, 4 and 6* Additional risk minimization measures: • *None*
Additional pharmacovigilance activities	Additional pharmacovigilance activities: • *Long-term safety and tolerability study AG348-C-011; final study report available 30 November 2025.* See Section II.C of this summary for an overview of the postauthorization development plan.

Important Potential Risk: Embryo-fetal toxicity	
Evidence for linking the risk to the medicine	Although human data on pregnancies are limited, there is no evidence of human teratogenicity based on genotoxic potential, and nonclinical reproductive toxicity studies did not demonstrate teratogenicity/fetotoxicity at maternal exposures comparable to those in humans administered mitapivat at

Important Potential Risk: Embryo-fetal toxicity	
	50 mg BID (proposed clinical dose). In addition, mitapivat-induced changes in aromatase-dependent hormone levels are not associated with development of adverse events. However, with regard to the use of mitapivat during pregnancy, sex hormone changes due to off-target aromatase inhibition will be further monitored.
Risk factors and risk groups	Embryo/fetus following a pregnancy when a female treated with mitapivat becomes pregnant, or a male treated with mitapivat who's partner becomes pregnant.
Risk minimization measures	Routine risk minimization measures: *To inform that the safety of Pyrukynd in pregnancy is not known.* • *SmPC: Sections 4.4, 4.5, 4.6, and 5.3* • *PL: Section 2* Additional risk minimization measures: *None*

Missing Information: Use in Patients With Hepatic Impairment	
Risk minimization measures	Routine risk minimization measures: *To inform that the safety and efficacy of Pyrukynd in hepatic impaired patients is not known* • *SmPC: Sections 4.2 and 5.2* Additional risk minimization measures: • *None*
Additional pharmacovigilance activities	Additional pharmacovigilance activities: • *Hepatic impairment Study AG348-C-0HEP; final study report available 31 March 2024.* See Section II.C of this summary for an overview of the postauthorization development plan.

Missing Information: Long-term use	
Risk minimization measures	Routine risk minimization measures: *To inform that information on the long-term use of Pyrukynd in pyruvate kinase deficiency patients is not known.* *SmPC: Section 4.2, 5.1* Additional risk minimization measures: • *None*
Additional pharmacovigilance activities	Additional pharmacovigilance activities: • *Long-term safety and tolerability study AG348-C-011; final study report available 30 November 2025.* • *Longitudinal observational study AG348-C-008(Peak Registry); final study report for patients that received mitapivat available 30 September 2028.*

Missing Information: Long-term use	
	See Section II.C of this summary for an overview of the postauthorization development plan.

II.C Post-Authorization Development Plan

II.C.1 Studies which are conditions of the Marketing Authorization

There are no studies that are conditions of the marketing authorization or specific obligation of Pyrukynd.

II.C.2 Other studies in the postauthorization development plan

Hepatic impairment Study AG348-C-0HEP

A Phase 1, Single-Dose, Open-label Study to Evaluate the Pharmacokinetics, Safety, and Tolerability of Pyrukynd in Subjects With Moderate Hepatic Impairment or Normal Hepatic Function

Purpose of the study:

To evaluate the pharmacokinetics, safety, and tolerability of Pyrukynd in subjects with PK deficiency with moderate hepatic impairment or normal hepatic function

Long-term safety and tolerability Study AG348-C-011

A Phase 3, Multicenter, Open-label, Long-term, Extension Study of Pyrukynd in Adults with PK Deficiency Previously Treated in Studies AG348-C-006 or AG348-C-007.

Purpose of the study:

To evaluate the long-term safety and tolerability of Pyrukynd.

Longitudinal observational study (Peak Registry)

An ongoing Agios-sponsored, global, retrospective and prospective, longitudinal observational study of pediatric and adult patients with PK deficiency.

Purpose of the study:

To understand better the natural history of PK deficiency, including diagnosis, demographic and clinical characteristics, burden of disease, treatment patterns, and clinical outcomes in a real-world setting.

PART VII: ANNEXES

LIST OF ANNEXES

ANNEX 4 SPECIFIC ADVERSE DRUG REACTION FOLLOW-UP FORMS

Follow-up questionnaires are being developed to collect and evaluate specific data related to the following safety concerns in the postmarketing period:

Pregnancy, lactation, and embryo-fetal toxicity specific follow-up form.

PYRUKYND EXPOSURE DURING PREGNANCY AND LACTATION
POST MARKETING QUESTIONNAIRE

Please complete this form and return with any supporting documentation to Agios Medical Safety Risk Management

Email: << >> If you have questions, please call us at: <<number>>

Reporter's Name:	**Manufacturer Case Number:**

PYRUKYND (mitapivat) INFORMATION							
PYRUKYND ***Lot # & Exp. date:***	**Dose:**	**Route:**	**Frequency:**	**Start Date:** **(dd/mmm/yyyy)**	**Stop Date:** **(dd/mmm/yyyy)**	**Ongoing?** □Yes □No	**Indication:**

MATERNAL INFORMATION: Patient or Female Partner of Patient

(Privacy laws may prohibit the sharing of protected information by healthcare providers. If information is not available, please indicate "n/a")

Patient / Female Partner of Patient Initials: ☐**Patient Pregnancy** ☐**Partner Pregnancy**	**Age or DOB:**	**Height:** _____**cm**/_____**in**	**Weight:** _____**kg**/_____**lb**

Ethnicity: ☐**Hispanic or Latino** ☐**Not Hispanic or Latino** ☐**Not reported**

Race: ☐**American Indian or Alaska Native** ☐**Asian** ☐**Black or African-American** ☐ **White** ☐**Native Hawaiian or Other Pacific Islander** ☐ **Not reported** ☐**Other, (specify):**

Pregnancy Information

Date of last menstrual period: **dd/mmm/yyyy**

Date Pregnancy Confirmed: □**Urine βhCG** □ **Serum βhCG** **dd/mmm/yyyy**

Status of Pregnancy:

□ Continuing If continuing, estimated delivery date: dd/mmm/yyyy

Clinical condition of fetus if pregnancy is continuing:

□ Normal □ Multiple fetuses (provide # ____) □Unknown □Abnormal (explain):

□ Completed If completed, date of delivery: dd/mmm/yyyy Method of delivery: □ **Vaginal** □ **C-section**

□ Spontaneous abortion / miscarriage **Gestational Age**: _____weeks

□ Elective termination* **Gestational Age**: _____weeks

*Was elective termination for a medical reason? □ No □ Yes (If yes, specify):

Did the patient experience complications during this pregnancy? □ No □ Yes (If yes, specify):

Pre-natal tests:

Test	**Date**	**Result**
Ultrasound		
Ultrasound		
Ultrasound		
Amniocentesis		
Maternal Serum AFP		
Chorionic villi biopsy (CVS)		
Serology test results e.g. rubella, toxoplasmosis		
Other (specify):		

Pregnancy Medical History

Number of prior pregnancies: **Date of last pregnancy**: dd/mmm/yyyy

Outcomes: Live births:_____ Full term:_____ Pre-term:_____ Abortions:_____ Elective _____ Spontaneous

Miscarriage(s):_____ Intrauterine death(s):_____

If miscarriage(s) and/or intrauterine death(s) occurred, specify in what week of pregnancy: _____

Did a birth defect occur in any previous pregnancy? □ No □ Yes (If yes, specify): _____

Has the patient experienced complications during previous pregnancies? □No □ Yes (If yes, specify): _____

PYRUKYND EXPOSURE DURING PREGNANCY AND LACTATION
POST MARKETING QUESTIONNAIRE

Please complete this form and return with any supporting documentation to Agios Medical Safety Risk Management

Email: << >> If you have questions, please call us at: <<number>>

Reporter's Name:	**Manufacturer Case Number:**

Does the Mother or Father have any of the following pregnancy risk factors:

Pregnancy risk factors	**Mother**	**Father**
Congenital malformation (immediate relatives)		
Chromosomal abnormality (immediate relatives)		
Developmental delay (immediate relatives)		
Transfusion dependent? If yes, number?		
Alcohol consumption		
Smoking		
Diabetes		
Hypertension		
Asthma		
Heart disease		
Thyroid disease		
Radiation exposure or other environmental exposure (specify)		
Seizure disorder		
Sexually transmitted disorders		
Hepatitis		
HIV (specify viral load, CD4 count)		
Depression		
Psychiatric disorders		
IV or recreational drug use (specify)		
Consanguinity between parents (specify degree)		
Other (specify)		

Age or DOB of Father:

Concomitant Medications (Include herbal remedies, chelators, OTC medications and/or dietary supplements)

Medication Name	**Dose**	**Route**	**Frequency**	**Start Date** dd/mmm/yyyy	**Stop Date** dd/mmm/yyyy	**Ongoing?**	**Indication**
						□Yes □No	
						□Yes □No	
						□Yes □No	

Birth Control: Were the mother and/or father using birth control during PYRUKYND treatment? □No □Yes If yes, specify:

□ **No concomitant medications**

PYRUKYND EXPOSURE DURING PREGNANCY AND LACTATION
POST MARKETING QUESTIONNAIRE

Please complete this form and return with any supporting documentation to Agios Medical Safety Risk Management

Email: << >> If you have questions, please call us at: <<number>>

Reporter's Name:	**Manufacturer Case Number:**

NEWBORN INFORMATION	
Outcome	□ Full term □ Premature birth **Gestational Age**: _____weeks □ Neonatal death (20 - <28 weeks)
Newborn Information	□ Male □ Female Length: _____ □ cm _____ □ in Weight: _____ □ kg _____ □ lb **Apgar Score** at 1 minute: _____ at 5 minutes _____ at 10 minutes _____ □ Unknown Did a birth defect occur? □ No □ Yes If yes, provide description of findings:
Complications of Labor/Delivery	□ No □ Yes If yes, provide description of complications:
Lactation Information	Did the mother breast feed? □ No □ Yes If yes, was the mother receiving mitapivat while breast feeding? □ No □ Yes If yes, provide age at first exposure: □ Neonate □ Other Please specify: If yes, provide length of exposure: If yes, did the infant experience any adverse events? □ No □ Yes If yes, provide description of findings:

ANNEX 6 DETAILS OF PROPOSED ADDITIONAL RISK MINIMIZATION ACTIVITIES

Not applicable