

**[00118]** In an embodiment, the mutant PKR is selected from the group consisting of A31V, A36G, G37Q, R40W, R40Q, L73P, S80P, P82H, R86P, I90N, T93I, G95R, M107T, G111R, A115P, S120F, H121Q, S130P, S130Y, V134D, R135D, A137T, G143S, I153T, A154T, L155P, G159V, R163C, R163L, T164N, G165V, L167M, G169G, E172Q, W201R, I219T, A221Y, D221N, G222A, I224T, G232C, N253D, G263R, G263W, E266K, V269F, L272V, L272P, G275R, G275R, E277K, V280G, D281N, F287V, F287L, V288L, D293N, D293V, A295I, A295V, I310N, I314T, E315K, N316K, V320L, V320M, S330R, D331N, D331G, D331E, G332S, V335M, A336S, R337W, R337P, R337Q, D339N, D339Q, G341A, G341D, I342F, K348N, A352D, I357T, G358R, G358E, R359C, R359H, C360Y, N361D, G364D, K365M, V368F, T371I, L374P, S376I, T384M, R385W, R385K, E387G, D390N, A392T, N393D, N393S, N393K, A394S, A394D, A394V, V395L, D397V, G398A, M403I, G406R, E407K, E407G, T408P, T408A, T408I, K410E, G411S, G411A, Q421K, A423A, A423A, R426W, R426Q, E427A, E427N, A431T, R449C, I457V, G458D, A459V, V460M, A468V, A468G, A470D, T477A, R479C, R479H, S485F, R486W, R486L, R488Q, R490W, I494T, A495T, A495V, R498C, R498H, A503V, R504L, Q505E, V506I, R510Q, G511R, G511E, R518S, R531C, R532W, R532Q, E538D, G540R, D550V, V552M, G557A, R559G, R559P, N566K, M568V, R569Q, R569L, Q58X, E174X, W201X, E241X, R270X, E440X, R486X, Q501X, L508X, R510X, E538X, R559X. These mutations are described in Canu et al., *Blood Cells, Molecules and Diseases* **2016**, 57, pp. 100-109. In an embodiment, the mutant PKR is selected from G332S, G364D, T384M, K410E, R479H, R479K, R486W, R532W, R510Q, and R490W. In certain embodiments, the mutant PKR is selected from A468V, A495V, I90N, T408I, and Q421K, and R498H. In certain embodiments, the mutant PKR is R532W, K410E, or R510Q. In certain embodiments, the mutant PKR is R510Q, R486W, or R479H.

**[00119]** In other aspects, provided are methods of treating a disease selected from hemolytic anemia, hereditary spherocytosis, hereditary elliptocytosis, abetalipoproteinemia, Bassen-Kornzweig syndrome, and paroxysmal nocturnal hemoglobinuria in a subject in need thereof, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor). Also provided is mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor), for use in treating disease selected from hemolytic anemia, hereditary

spherocytosis, hereditary elliptocytosis, abetalipoproteinemia, Bassen-Kornzweig syndrome, and paroxysmal nocturnal hemoglobinuria in a subject. Further provided is the use of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor), in the manufacture of a medicament for treating a disease selected from hemolytic anemia, hereditary spherocytosis, hereditary elliptocytosis, abetalipoproteinemia, Bassen-Kornzweig syndrome, and paroxysmal nocturnal hemoglobinuria in a subject in need thereof. In one aspect, the disease to be treated is hemolytic anemia.

**[00120]** Thalassemia is an inherited blood disorder in which the normal ratio of  $\alpha$ - to  $\beta$ -globin production is disrupted due to a disease-causing variant in 1 or more of the globin genes. In certain embodiments, Alpha-globin aggregates (as found in  $\beta$ -thalassemia) readily precipitate, which disrupts the red blood cell (RBC) membrane and results in oxidative stress. In certain embodiments, Beta-globin tetramers (Hb H, found in  $\alpha$ -thalassemia) are generally more soluble, but are still unstable and can form precipitates. The imbalance of the globin chain synthesis can lead to a net reduction in Hb concentrations and has dramatic effects on the survival of RBC precursors, ultimately resulting in their premature destruction in the bone marrow and in extramedullary sites (Cappellini et al., 2014). In certain embodiments, the disorder results in large numbers of red blood cells being destroyed, which leads to anemia. In certain embodiments, the thalassemia is alpha thalassemia. In certain embodiments, the thalassemia is beta thalassemia. In other embodiments, the thalassemia is non-transfusion-dependent thalassemia. In other embodiments, the thalassemia is beta thalassemia intermedia. In other embodiments, the thalassemia is Hb E beta thalassemia. In other embodiments, the thalassemia is beta thalassemia with mutations of 1 or more alpha genes.

**[00121]** In certain embodiments, the subject is an adult subject with thalassemia. In certain embodiments, the subject has thalassemia such as  $\beta$ -thalassemia intermedia, Hb E  $\beta$ -thalassemia,  $\alpha$ -thalassemia (Hb H disease), or  $\beta$ -thalassemia with mutations of 1 or more  $\alpha$  genes. In certain embodiments, the subject has non-transfusion-dependent thalassemia. In certain embodiments, the subject has non-transfusion-dependent alpha thalassemia. In certain embodiments, the subject has beta-thalassemia or non-transfusion-dependent beta-thalassemia. In certain embodiments, the subject is an adult male subject with thalassemia such as beta-thalassemia or non-transfusion-dependent thalassemia. In certain embodiments, the subject is a female subject with thalassemia such as beta-thalassemia or non-transfusion-

dependent thalassemia. In certain embodiments, the subject is an adult female subject with thalassemia such as beta-thalassemia or non-transfusion-dependent thalassemia. In certain embodiments, the subject has a hemoglobin concentration of less than or equal to 6.0 g/dL. In certain embodiments, the subject has a hemoglobin concentration of less than or equal to 7.0 g/dL. In certain embodiments, the subject has a hemoglobin concentration of less than or equal to 8.0 g/dL. In certain embodiments, the subject has a hemoglobin concentration of less than or equal to 9.0 g/dL. In certain aspects, the subject having non-transfusion-dependent thalassemia does not have a known history (*e.g.*, has been diagnosed in the past) of Hb S or Hb C forms of thalassemia. In certain embodiments, the term “non-transfusion dependent” thalassemia refers to subjects with thalassemia having no more than 4 (*e.g.*, five) units of RBCs transfused during a 24-week period up to the first day of administration of mitapivat and/or no RBC transfusions in the 8 weeks prior to the first day of administration of mitapivat. In certain embodiment, the subject is an adult over 18 years old. In certain embodiment, the subject is a child 18 years old or younger.

**[00122]** As used herein, sickle cell disease (SCD), Hemoglobin SS disease, and sickle cell anemia are used interchangeably. Sickle cell disease (SCD) is an inherited blood disorder caused by the presence of sickle hemoglobin (HbS). In certain embodiments, subjects with SCD have abnormal hemoglobin, called hemoglobin S or sickle hemoglobin, in their red blood cells. In certain embodiments, people having SCD have at least one abnormal gene causing the body to make hemoglobin S. In certain embodiments, people having SCD have two hemoglobin S genes, Hemoglobin SS.

**[00123]** In other aspects, provided herein are methods for treating anemia in a subject in need thereof comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor). Also provided is mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor), for use in treating anemia in a subject in need thereof. Further provided is the use of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor), in the manufacture of a medicament for treating anemia. In one aspect, the anemia to be treated is dyserythropoietic anemia.

**[00124]** In certain embodiments, the anemia is a dyserythropoietic anemia such as congenital dyserythropoietic anemia type I, II, III, or IV. In certain embodiments, the anemia is hemolytic anemia. In certain embodiments, the hemolytic anemia is a congenital and/or hereditary form of hemolytic anemia such as PKD, sickle cell disease, thalassemias (*e.g.*, alpha or beta or non-transfusion-dependent thalassemia), hereditary spherocytosis, hereditary elliptocytosis), paroxysmal nocturnal hemoglobinuria, abeta-lipoproteinemia (Bassen-Kornzweig syndrome). In certain embodiments, the hemolytic anemia is acquired hemolytic anemia such as autoimmune hemolytic anemia, drug-induced hemolytic anemia. In certain embodiments, the hemolytic anemia is anemia as part of a multi-system disease, such as the anemia of Congenital Erythropoietic Purpura, Fanconi, Diamond-Blackfan.

**[00125]** As used herein, the term “anemia” refers to a deficiency of red blood cells (RBCs) and/or hemoglobin. As used herein, anemia includes all types of clinical anemia, for example (but not limited to): microcytic anemia, iron deficiency anemia, hemoglobinopathies, heme synthesis defect, globin synthesis defect, sideroblastic defect, normocytic anemia, anemia of chronic disease, aplastic anemia, hemolytic anemia, macrocytic anemia, megaloblastic anemia, pernicious anemia, dimorphic anemia, anemia of prematurity, Fanconi anemia, hereditary spherocytosis, sickle cell disease, warm autoimmune hemolytic anemia, cold agglutinin hemolytic anemia, osteopetrosis, thalassemia, and myelodysplastic syndrome.

**[00126]** In certain embodiments, anemia can be diagnosed on a complete blood count. In certain embodiments, anemia can be diagnosed based on the measurement of one or more markers of hemolysis (*e.g.*, RBC count, hemoglobin, reticulocytes, schistocytes, lactate Dehydrogenase (LDH), haptoglobin, bilirubin, and ferritin) and/or hemosiderinuria mean corpuscular volume (MCV) and/or red cell distribution width (RDW). In the context of the present invention, anemia is present if an individual has a hemoglobin (Hb) less than the desired level, for example, the Hb concentration of less than 14 g/dL, more preferably of less than 13 g/dL, more preferably of less than 12 g/dL, more preferably of less than 11 g/dL, or most preferably of less than 10 g/dL.

**[00127]** In certain embodiments, provided herein is a method of increasing the amount of hemoglobin in a subject by administering an effective amount of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor). In certain embodiments, also provided herein is a method of increasing the amount of

hemoglobin in a subject having thalassemia comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor). Further provided is a method of increasing the amount of hemoglobin in subjects having non-transfusion-dependent thalassemia comprising administering an effective amount of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor) to the subject. In certain embodiments, the provided methods increase hemoglobin concentration in the subject. In certain embodiments, the provided methods increase Hb concentration to a desired level, for example, above 10 g/dL, more preferably above 11 g/dL, more preferably above 12 g/dL, more preferably above 13 g/dL, or most preferably above 14 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 0.5 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 1.0 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 1.5 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 2.0 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 2.5 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 3.0 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 3.5 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 4.0 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 4.5 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 5.0 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 5.5 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 6.0 g/dL. In certain embodiments, the increase in Hb concentration is determined from baseline at one or more assessment between week 1 and week 20 (*e.g.*, between week 2 and week 15, between week 3 and week 15, and between week 4 and week 12) of treatment with an effective amount of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor). In certain embodiments, the provided methods increase Hb concentration as described above in female subjects having thalassemia (*e.g.*, beta-thalassemia or non-transfusion-dependent thalassemia). In certain embodiments, the provided methods increase Hb concentration from baseline to about 12 g/dL in female subjects having

thalassemia (*e.g.*, beta-thalassemia or non-transfusion-dependent thalassemia). In certain embodiments, the provided methods increase Hb concentration as described above in male subjects having thalassemia (*e.g.*, beta-thalassemia or non-transfusion-dependent thalassemia). In certain embodiments, the provided methods increase Hb concentration from baseline to about 13 g/dL in male subjects having thalassemia (*e.g.*, beta-thalassemia or non-transfusion-dependent thalassemia).

**[00128]** In some aspects, provided herein are methods for treating hemolytic anemia in a subject in need thereof comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor). Also provided is mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor), for use in treating hemolytic anemia in a subject in need thereof. Further provided is the use of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor), in the manufacture of a medicament for treating hemolytic anemia. In one aspect, the hemolytic anemia to be treated is hereditary and/or congenital hemolytic anemia, acquired hemolytic anemia, or anemia as part of a multi-system disease.

**[00129]** In some aspects, provided herein are methods for activating wild-type or mutant PKR in red blood cells in a subject in need thereof comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor). Also provided is mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor), for use in activating wild-type or mutant PKR in red blood cells in a subject in need thereof. Further provided is the use of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor), in the manufacture of a medicament for activating wild-type or mutant PKR in red blood cells.

**[00130]** Mitapivat is an activator of PKR mutants having lower activities compared to the wild type, thus are useful for methods of the present disclosure. Such mutations in PKR can affect enzyme activity (catalytic efficiency), regulatory properties (modulation by fructose biphosphate (FBP)/ATP), and/or thermostability of the enzyme. Examples of such mutations are described in Valentini et al., JBC 2002. Some examples of the mutants that are activated by the compounds described herein include G332S, G364D, T384M, R479H, R479K, R486W, R532W, R510Q, and R490W. Without being bound by theory, in certain embodiments, mitapivat affects the activities of PKR mutants by activating FBP non-responsive PKR mutants, restoring thermostability to mutants with decreased stability, or restoring catalytic efficiency to impaired mutants.

**[00131]** In certain embodiments, mitapivat increases the affinity of PKR to phosphoenolpyruvate (PEP). In certain embodiments, mitapivat restores the ability of RBCs to cover PEP and ADP to pyruvate and ATP.

**[00132]** In certain embodiments, provided herein are methods of reducing transfusion frequency of a subject with PKD comprising administering to the subject mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor). In certain embodiments, the transfusion frequency is reduced by at least 5% in the number of RBC units transfused over at least 15 weeks. In certain embodiments, the transfusion frequency is reduced by at least 10% in the number of RBC units transfused over at least 15 weeks. In certain embodiments, the transfusion frequency is reduced by at least 15% in the number of RBC units transfused over at least 15 weeks. In certain embodiments, the transfusion frequency is reduced by at least 20% in the number of RBC units transfused over at least 15 weeks. In certain embodiments, the transfusion frequency is reduced by at least 25% in the number of RBC units transfused over at least 15 weeks. In certain embodiments, the transfusion frequency is reduced by at least 30% in the number of RBC units transfused over at least 15 weeks. In certain embodiments, the transfusion frequency is reduced by at least 35% in the number of RBC units transfused over at least 15 weeks. In certain embodiments, the transfusion frequency is reduced by at least 40% in the number of RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 5% in the number of RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 10% in the number of

RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 15% in the number of RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 20% in the number of RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 25% in the number of RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 30% in the number of RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 35% in the number of RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 40% in the number of RBC units transfused over at least 20 weeks.

**[00133]** In some aspects, provided herein are methods of evaluating the efficacy of mitavipat therapy (monotherapy or concomitant medications with a CYP3A4/5 inducer or a CYP3A4/5 inhibitor) in a subject, the method comprising: administering to the subject mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor); and acquiring a value for the level of mitapivat, the level of 2,3-diphosphoglycerate (2,3-DPG), the level of adenosine triphosphate (ATP), or the activity of PKR in the subject, to thereby evaluate the efficacy of the mitapivat therapy. In some aspects, the value for the level is acquired by analyzing the plasma concentration of mitapivat and optionally, the metabolite known to be formed by CYP3A4/5. In some aspects, the level of 2,3-DPG is acquired by analyzing the blood concentration of 2,3-DPG. In some aspects, the level of ATP is acquired by analyzing the blood concentration of ATP. In some aspects, the activity of PKR is acquired by analyzing the blood concentration of a <sup>13</sup>C-label in the blood. In some aspects, the analysis is performed by sample analysis of bodily fluid. In some aspects, the bodily fluid is blood. In some aspects, the analysis is performed by mass spectroscopy. In some aspects, the analysis is performed by LC-MS.

**[00134]** In some aspects, provided herein are methods of treating a subject, the method comprising: administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor); and acquiring a value for the level of mitapivat and optionally its metabolite known to be

formed by CYP3A4/5, the level of 2,3-diphosphoglycerate (2,3-DPG), the level of adenosine triphosphate (ATP), or the activity of PKR in the subject, to thereby treat the subject.

### EXEMPLIFICATIONS

**[00135]** As depicted in the Examples below, the drug-drug interactions between mitapivat sulfate and a CYP3A4/5 inducer (*e.g.*, rifampin) or a CYP3A4/5 inhibitor (*e.g.*, itraconazole) are studied. Mitapivat can be synthesized using the procedures described in International Patent Application Publication Nos. WO 2011/002817 and WO 2016/201227.

#### **Example 1: Mitapivat sulfate is primarily metabolized by CYP3A4**

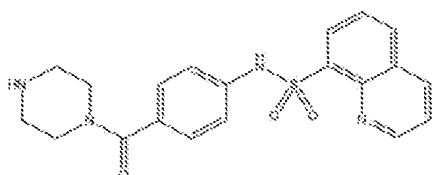
**[00136]** CYP-enzyme phenotyping using human liver microsomes and recombinant CYP enzymes revealed that mitapivat sulfate was primarily metabolized by CYP3A4/5 (>90%), with minor contributions from CYP2C9, CYP2C8, and CYP1A2. There was evidence of metabolism-dependent inhibition of CYP2C19 (largely reversible) and CYP3A4 (largely irreversible). Mitapivat sulfate was found to be a substrate and inhibitor for P-gp but not for breast cancer resistance protein (BCRP) and was found to be a potential inducer of human CYP2B6 and CYP3A4. Mitapivat sulfate appeared to be a mild inhibitor of CYP2C8, CYP2C9, xYP2C19, CYP2D6, and CYP3A4/5 enzymes (testosterone 6 $\beta$ -hydroxylation), bile salts export pump (BSEP), organic anion transporting polypeptide (OATP)1B1, organic anion transporter (OAT)3, and organic cation transporter (OCT)2, and of uridine-5'-diphospho-glucuronosyltransferase (UGT) 1A3, 1A4, and 1A9. Mitapivat sulfate does not appear to be an inhibitor of multidrug resistance-associated protein (MRP)2, MRP3, OATP1B3, and OAT1. The metabolite was found to be a mild inhibitor of CYP2C9 and CYP2C19 and was not an inhibitor of P-gp or BCRP under tested concentrations.

#### **Example 2: Drug-drug Interaction (DDI) Studies of Mitapivat Sulfate and a CYP3A4 Inducer or a CYP3A4 Inhibitor**

**[00117]** Twenty-eight (28) healthy, adult male and female (non-childbearing potential) subjects were enrolled in the study in total; 14 subjects in each study part (Parts 1 and 2). A minimum of 8 female subjects were enrolled in the study (*i.e.*, a minimum of 4 female subjects per study part). Each subject participated in either Part 1 or Part 2, but not both.

**Part 1**

**[00118]** On Day 1 of Treatment Period 1, a single oral dose of 20 mg mitapivat sulfate was administered. Serial blood samples for plasma assay of mitapivat concentrations and its CYP3A4 metabolite, referred to herein as the “Metabolite” (structure below),



were collected from predose to 120 hours following administration of mitapivat sulfate. In Treatment Period 2, an oral dose of 200 mg itraconazole was administered once daily (QD) for 9 consecutive days (Day 1 through Day 9 of Treatment Period 2) with a single oral dose of 20 mg mitapivat sulfate coadministered on Day 5. Serial blood samples for plasma assay of mitapivat and the Metabolite concentrations were collected from predose to 120 hours following coadministration of mitapivat sulfate and itraconazole on Day 5.

**[00119]** In Treatment Period 1, mitapivat sulfate was administered orally with approximately 240 mL of water. In Treatment Period 2, on Days 1 to 4, itraconazole was administered alone immediately followed by approximately 220 mL of water, and on Day 5, itraconazole was administered first (no water) and was immediately followed by mitapivat sulfate administration with approximately 220 mL of water. Study drugs (mitapivat sulfate and itraconazole) were administered following an overnight fast of at least 10 hours on Day 1 of Treatment Period 1 (mitapivat sulfate only) and Day 5 of Treatment Period 2 (mitapivat sulfate and itraconazole), and subjects remained fasted for 4 hours after dosing. On all other dosing days, itraconazole was administered following a predose fast of at least 4 hours and subjects remained fasted for at least 2 hours after dosing.

**Part 2**

**[00120]** On Day 1 of Treatment Period 1, a single oral dose of 50 mg mitapivat sulfate was administered. Serial blood samples for plasma assay of mitapivat and the Metabolite concentrations were collected from predose to 120 hours following administration of mitapivat sulfate. In Treatment Period 2, an oral dose of 600 mg rifampin was administered QD for 12 consecutive days (Day 1 through Day 12 of Treatment Period 2) with a single oral dose of 50 mg mitapivat sulfate coadministered on Day 8. Serial blood samples for plasma

assay of mitapivat sulfate and the Metabolite concentrations were collected from predose to 120 hours following coadministration of mitapivat and rifampin on Day 8.

**[00121]** In Part 2, study drugs were administered with approximately 240 mL of water on all dosing days including the coadministration of mitapivat sulfate and rifampin on Day 8 of Treatment Period 2. Mitapivat sulfate and rifampin was administered following an overnight fast of at least 10 hours on Day 1 of Treatment Period 1 (mitapivat sulfate only) and Day 8 of Treatment Period 2 (both mitapivat sulfate and rifampin) and subjects remained fasted for 4 hours after dosing. On all other dosing days, rifampin was administered following a predose fast of at least 4 hours and subjects remained fasted for at least 2 hours after dosing. There was a washout period of 7 days between the mitapivat sulfate dose in Treatment Period 1 and the first itraconazole (Part 1) or rifampin (Part 2) dose in Treatment Period 2. All study drugs were consumed within 5 minutes for both Part 1 and Part 2.

### **Test and Reference Products, Dose, Duration and Mode of Administration**

#### **Part 1**

**[00122]** Mitapivat sulfate (20 mg tablets) administered along with Itraconazole (also referred to as Sporanox®, 10 mg/mL oral solution, is considered as the test arm in this study. Mitapivat sulfate administered alone is considered as the reference arm in this study.

In Period 1, a single oral dose of 20 mg mitapivat sulfate was administered orally with approximately 240 mL of water. In Period 2, on Day 5 (coadministration of itraconazole and mitapivat sulfate), 200 mg itraconazole (20 mL of 10 mg/mL oral solution) was administered first (no water) and was immediately followed by 20 mg mitapivat sulfate (1 x 20 mg tablet) administration with approximately 220 mL of water. On all other dosing days, itraconazole was administered alone immediately followed by approximately 220 mL of water.

Each subject received 2 doses of mitapivat sulfate and 9 doses of itraconazole in Part 1.

#### **Part 2**

**[00123]** Mitapivat sulfate (50 mg tablets) administered along with Rifampin (also referred to as Rifadin®, 300 mg capsules) is considered as the test arm in this study. Mitapivat sulfate (50 mg tablets) administered alone is considered as the reference arm in this study.

**[00124]** In Period 1, a single oral dose of 50 mg mitapivat sulfate was administered orally. In Period 2, subjects were administered 600 mg rifampin (2 x 300 mg capsules) QD on

Day 1 to Day 12 inclusive, with 50 mg mitapivat sulfate (1 x 50 mg tablet) coadministered on Day 8. Study drugs were administered with approximately 240 mL of water on all dosing days including the coadministration of mitapivat sulfate and rifampin on Day 8 of Period 2.

**[00125]** Each subject received 2 doses of mitapivat sulfate and 12 doses of rifampin in Part 2.

#### **Duration of Treatment:**

**[00126]** The total duration of participation including the screening period for each subject was approximately 49 to 62 days for Part 1 and approximately 61 or 63 days in Part 2 of the study.

**[00127]** In each study part, Treatment Period 1 was approximately 6 days and Treatment Period 2 was approximately 12 days; a washout phase was 7 days between both treatment periods in each study part.

#### **Criteria for Evaluation of Pharmacokinetics**

**[00128]** In Parts 1 and 2, for mitapivat and the Metabolite, blood samples were obtained for all subjects to determine the plasma concentrations of mitapivat, and the Metabolite, before mitapivat sulfate dosing (0 hour) on Day 1 of Period 1 (Parts 1 and 2) and Day 5 (Part 1) or Day 8 (Part 2) of Period 2 at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 16, 24, 48, 72, 96, and 120 hours postdose. Plasma concentrations of mitapivat and the Metabolite were determined using methods validated with respect to accuracy, precision, linearity, sensitivity, and specificity, with a lower limit of quantitation (LLOQ) of 0.5 ng/mL for both analytes. A noncompartmental PK approach was used to analyze individual plasma mitapivat and the Metabolite concentration time data (using Phoenix® WinNonlin® Version 7.0). The following PK parameters were calculated:  $AUC_{0-t}$ ,  $AUC_{0-inf}$ ,  $AUC_{0-24}$ ,  $AUC_{\%extrap}$ ,  $C_{max}$ ,  $CL/F$  (mitapivat only),  $T_{last}$ ,  $T_{max}$ ,  $t_{1/2}$ , and  $V_z/F$  (mitapivat only).

#### **Statistical Methods for Pharmacokinetics**

**[00129]** The plasma concentrations of mitapivat and the Metabolite were listed and summarized by treatment and collection time for all subjects in the PK Population. Plasma concentrations were presented with the same level of precision as received from the bioanalytical laboratory including sample size (n), arithmetic mean (Mean), standard

deviation (SD), coefficient of variation (CV%), standard error of the mean (SEM), minimum, maximum, and median. Concentrations from excluded subjects were included in the plasma concentration tables, but were excluded from the summary statistics and noted as such in the tables. All BLQ values were presented as “BLQ” in the plasma concentration table listings and footnoted accordingly.

**[00130]** PK parameters were presented with a minimum of 3 significant figures. PK parameters were listed by subject, tabulated by treatment, and summarized using descriptive statistics (n, mean, SD, CV%, SEM, minimum, median, maximum, geometric mean (Geom. Mean) and geometric mean coefficient of variation (Geom. CV%). Time-based parameters (*i.e.*, Tmax and  $t_{1/2}$ ) and treatment:reference AUC and Cmax ratios were presented to 2 decimals. Excluded subjects were included in the PK parameter table listings, but were excluded from the summary statistics and noted as such in the tables.

**[00131]** To evaluate the effect of itraconazole and rifampin on mitapivat and the Metabolite (primary objective), plasma mitapivat and the Metabolite PK parameters (Cmax, AUC<sub>0-t</sub>, and AUC<sub>0-inf</sub>) were natural log (ln) transformed before analysis. The ln-transformed value of the PK parameters was analyzed using a linear mixed effect model for each study part separately with treatment as a fixed effect and subject as a random effect. The linear mixed effect model included treatment as a fixed effect and subject as a random effect. The inferential results (least-squares means [LSMs], difference between LSMs, and 90% confidence intervals [CIs] of the difference) were back transformed to the original scale. Geometric LSMs, geometric mean ratios (GMRs), and 90% CIs were presented. The GMR of the LSMs were calculated from the back-transformed difference between the treatment LSMs. The GMRs were expressed as a percentage relative to mitapivat sulfate given alone for both Part 1 and Part 2. The 90% CIs for the ratios were derived by back-transformation of the CIs obtained for the difference between the treatment LSMs. The CIs were expressed as a percentage relative to mitapivat sulfate given alone for both Part 1 and Part 2.

**[00132]** The median differences in Tmax for evaluation of the effect of itraconazole and rifampin on mitapivat and the Metabolite along with the range of the difference were calculated using the non parametric Wilcoxon Signed Rank test, and the p-value was presented. Note that Tmax was not ln-transformed for these analyses.

## Pharmacokinetic Results

## **Part 1**

**[00133]** Mean plasma mitapivat and the Metabolite concentration-time profiles following 20 mg mitapivat sulfate administered alone (Period 1) or coadministered with multiple doses of 200 mg itraconazole (Period 2) on linear and semi-log scales are presented in Figures 1 and 2.

### **Mitapivat**

**[00134]** Mitapivat plasma concentrations were detectable in all subjects at 0.5 hour (the first scheduled postdose sample) in both Periods 1 and 2. Mitapivat was still detectable up to the last sample time at 120 hours postdose in the majority of subjects in Period 1 and in all subjects in Period 2.

**[00135]** Measurable predose mitapivat concentrations were present in all but 2 subjects in Period 2 (two subjects were BLQ). As used herein, BLQ refers to below the limit of quantification. These predose concentrations were < 5% of the C<sub>max</sub>, so PK calculations were performed.

**[00136]** Mitapivat concentrations exhibited a characteristic multi-compartmental pharmacokinetic profile. When coadministered with itraconazole, mitapivat concentrations rose to a higher mean peak with a delay in time to peak by approximately 2 hours compared to when the drug was administered alone. The post-peak decline of the multi-compartmental behavior was slower after coadministration with itraconazole when compared to mitapivat administered alone. Overall, higher mitapivat concentrations were experienced for a longer time period when mitapivat was coadministered with itraconazole.

### **The Metabolite**

**[00137]** When mitapivat was administered alone, the Metabolite plasma concentrations were detectable in all but 1 subject at 0.5 hour. The Metabolite remained detectable all but 1 subject up to 48 hours postdose, with quantifiable the Metabolite out to 72 hours in 3 subjects and 96 hours in 1 subject. In Period 2 (coadministration with itraconazole), the Metabolite concentrations were delayed relative to administration of mitapivat alone. Concentrations were not measurable in all subjects until 1.5 hours postdose. Maximum concentrations were also reduced. However, the Metabolite residence was prolonged in the presence of itraconazole, with concentrations still detectable in all but 1 subject throughout the 120-hour sampling interval (in 1 subject the last measurable concentration was at 96 hours postdose).

[00138] The statistical comparisons of mitapivat PK parameters following 20 mg mitapivat sulfate administered alone (Period 1) or coadministered with multiple doses of 200 mg itraconazole (Period 2) PK parameters are summarized below in Table 1.

**Table 1**  
**Summary of Statistical Comparisons of Plasma Mitapivat Pharmacokinetic Parameters Following 20 mg Mitapivat Sulfate Co-Administered With Multiple Doses of 200 mg Itraconazole Versus Administered Alone (Part 1)**

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Mitapivat Sulfate + Itraconazole (Test) |    | Mitapivat sulfate alone (Reference) |    |        |                         |                   |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------|----|-------------------------------------|----|--------|-------------------------|-------------------|
| Parameter (unit)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Geometric LSM                           | n  | Geometric LSM                       | n  | GMR    | 90% Confidence Interval | Intra-subject CV% |
| AUC <sub>0-t</sub> (ng*hr/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | 8860                                    | 14 | 1885                                | 14 | 4.6998 | 4.2519 - 5.1949         | 15.05             |
| AUC <sub>0-inf</sub> (ng*hr/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 9475                                    | 14 | 1942                                | 14 | 4.8780 | 4.4154 - 5.3890         | 14.97             |
| C <sub>max</sub> (ng/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | 704.3                                   | 14 | 412.5                               | 14 | 1.7074 | 1.5815 - 1.8433         | 11.48             |
| Mitapivat sulfate alone: 20 mg mitapivat sulfate (1 x 20 mg tablet) at Hour 0 on Day 1 (Period 1)<br>Mitapivat sulfate + itraconazole: 200 mg itraconazole (20 mL of 10 mg/mL oral solution) QD on Days 1 to Day 9 inclusive (within ± 1 hour of dosing time on Day 1) with 20 mg mitapivat sulfate (1 x 20 mg tablet) coadministered at Hour 0 on Day 5 (Period 2)<br>Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the linear mixed effect model.<br>Geometric Mean Ratio (GMR) = test / reference<br>Intra-subject CV% = 100 x (square root (exp[MSE]-1), where MSE = Residual variance from linear mixed effect model. |                                         |    |                                     |    |        |                         |                   |

[00139] The coadministration of 200 mg itraconazole with 20 mg mitapivat sulfate increased mitapivat exposure relative to when mitapivat sulfate was administered alone. The geometric mean AUC<sub>0-t</sub> and AUC<sub>0-inf</sub> ratios of mitapivat in the presence and absence of itraconazole were 4.7 and 4.9, respectively. The geometric mean Cmax ratio of mitapivat in the presence and absence of itraconazole was 1.7. The intra-subject variability was low at approximately 15% for AUCs and 11% for Cmax.

[00140] The statistical comparisons of the Metabolite PK parameters following 20 mg mitapivat sulfate administered alone (Period 1) or coadministered with multiple doses of 200 mg itraconazole (Period 2) PK parameters are summarized below in Table 2.

**Table 2**  
**Summary of Statistical Comparisons of Plasma Metabolite Pharmacokinetic Parameters Following 20 mg Mitapivat Sulfate Co-Administered With Multiple Doses of 200 mg Itraconazole Versus Administered Alone (Part 1)**

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Mitapivat Sulfate + Itraconazole (Test) |    | Mitapivat sulfate alone (Reference) |    |        |                         |                   |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------|----|-------------------------------------|----|--------|-------------------------|-------------------|
| Parameter (unit)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Geometric LSM                           | n  | Geometric LSM                       | n  | GMR    | 90% Confidence Interval | Intra-subject CV% |
| AUC <sub>0-t</sub> (ng*hr/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | 300.0                                   | 14 | 197.1                               | 14 | 1.5218 | 1.4268 - 1.6231         | 9.65              |
| AUC <sub>0-inf</sub> (ng*hr/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 338.6                                   | 14 | 216.2                               | 14 | 1.5661 | 1.4784 - 1.6590         | 8.63              |
| C <sub>max</sub> (ng/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | 9.110                                   | 14 | 18.79                               | 14 | 0.4849 | 0.4441 - 0.5295         | 13.20             |
| Mitapivat sulfate alone: 20 mg mitapivat sulfate (1 x 20 mg tablet) at Hour 0 on Day 1 (Period 1)<br>Mitapivat sulfate + itraconazole: 200 mg itraconazole (20 mL of 10 mg/mL oral solution) QD on Days 1 to Day 9 inclusive (within ± 1 hour of dosing time on Day 1) with 20 mg mitapivat sulfate (1 x 20 mg tablet) coadministered at Hour 0 on Day 5 (Period 2)<br>Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the linear mixed effect model.<br>Geometric Mean Ratio (GMR) = test / reference<br>Intra-subject CV% = 100 x (square root (exp[MSE]-1), where MSE = Residual variance from linear mixed effect model. |                                         |    |                                     |    |        |                         |                   |

[00141]        The coadministration of 200 mg itraconazole with 20 mg mitapivat sulfate increased total Metabolite exposure relative to when mitapivat sulfate was administered alone. The geometric mean AUC<sub>0-t</sub> and AUC<sub>0-inf</sub> ratios of the Metabolite in the presence and absence of itraconazole were 1.5 and 1.6, respectively. The geometric mean C<sub>max</sub> ratio of the Metabolite in the presence and absence of itraconazole was 0.5. The intra-subject variability was low at approximately 9% to 10% for AUCs and 13% for C<sub>max</sub>.

Part 2

[00142]        Mean plasma mitapivat and metabolite the Metabolite concentration-time profiles following 50 mg mitapivat sulfate administered alone (Period 1) or coadministered with multiple doses of 600 mg rifampin (Period 2) on linear and semi-log scales are presented in Figures 3 and 4.

Mitapivat

[00143]        Mitapivat plasma concentrations were detectable in all subjects at 0.5 hour (the 1st scheduled postdose sample) when given alone or with rifampin. Mitapivat was still detectable up to the last sample time at 120 hours postdose in all but 1 subject when given alone had a BLQ concentration at 120 hours). When coadministered with rifampin, detectable mitapivat concentrations were not present in any sample collections from any subject beyond 24 hours postdose (except 1 subject with detectable mitapivat at 48 hours).

[00144]        Mitapivat concentrations increased to a lower and slightly earlier peak when coadministered with rifampin compared to when administered alone. The peak arithmetic

mean mitapivat concentrations were observed at a nominal time of approximately 1 hour postdose for Period 1 and 0.5 hour postdose for Period 2. The mean concentrations of mitapivat then declined in a multi exponential manner for both periods; however, the post-peak initial decline in mitapivat concentrations was more rapid and to lower levels when coadministered with rifampin compared to dosing alone.

**The Metabolite**

**[00145]** The Metabolite plasma concentrations were detectable at 0.5 hour postdose in all subjects after mitapivat sulfate was given alone and in all but 1 subject (1 subject was reported as BLQ) when mitapivat sulfate was coadministered with rifampin. The Metabolite was still detectable in all but 4 subjects up to the last scheduled sampling time of 120 hours postdose when mitapivat sulfate was given alone. When mitapivat sulfate was coadministered with rifampin, The Metabolite remained detectable in all subjects up to 24 hours postdose, with quantifiable The Metabolite out to 48 hours in 6 subjects and 72 hours in 1 subject. No Metabolite concentrations were detectable in samples collected beyond 72 hours.

**[00146]** The peak arithmetic mean Metabolite concentrations were observed at a nominal time of approximately 2 hours postdose with mitapivat sulfate given alone and 1 hour when mitapivat sulfate was coadministered with rifampin. When mitapivat sulfate was given alone, higher mean concentrations The Metabolite were observed over the initial 1 hour postdose. Metabolite concentrations were higher when mitapivat sulfate was coadministered with rifampin beyond 1 hour. The mean concentrations of The Metabolite then declined in a multi exponential fashion for both treatments. Decline in Metabolite concentrations was more rapid when mitapivat sulfate was coadministered with rifampin.

**[00147]** The statistical comparisons of mitapivat PK parameters following 50 mg mitapivat sulfate administered alone (Period 1) or coadministered with multiple doses of 600 mg rifampin (Period 2) PK parameters are summarized below in Table 3.

**Table 3**  
**Summary of Statistical Comparisons of Plasma Mitapivat Pharmacokinetic Parameters Following 50 mg Mitapivat Sulfate Co-Administered With Multiple Doses of 600 mg Rifampin Versus Administered Alone (Part 2)**

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | Mitapivat sulfate + rifampin (Test) |    | Mitapivat sulfate alone (Reference) |    |        |                         |                   |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|----|-------------------------------------|----|--------|-------------------------|-------------------|
| Parameter (unit)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Geometric LSM                       | n  | Geometric LSM                       | n  | GMR    | 90% Confidence Interval | Intra-subject CV% |
| AUC <sub>0-t</sub> (ng*hr/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 486.4                               | 14 | 5565                                | 14 | 0.0874 | 0.0719 - 0.1062         | 29.73             |
| AUC <sub>0-inf</sub> (ng*hr/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 496.4                               | 14 | 5681                                | 14 | 0.0874 | 0.0719 - 0.1062         | 29.84             |
| C <sub>max</sub> (ng/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 292.4                               | 14 | 1263                                | 14 | 0.2316 | 0.1933 - 0.2773         | 27.45             |
| Mitapivat sulfate alone: 50 mg mitapivat sulfate (1 x 50 mg tablet) at Hour 0 on Day 1 (Period 1)<br>Mitapivat sulfate + rifampin: 600 mg rifampin (2 x 300 mg capsules) QD on Day 1 to Day 12 inclusive (within ± 1 hour of dosing time on Day 1) with 50 mg mitapivat sulfate (1 x 50 mg tablet) coadministered at Hour 0 on Day 8 (Period 2)<br>Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the linear mixed effect model.<br>Geometric Mean Ratio (GMR) = test / reference<br>Intra-subject CV% = 100 x (square root (exp[MSE]-1), where MSE = Residual variance from linear mixed effect model. |                                     |    |                                     |    |        |                         |                   |

[00148]           The coadministration of 600 mg rifampin with 50 mg mitapivat sulfate decreased mitapivat exposure relative to when mitapivat sulfate was administered alone. The geometric mean AUC<sub>0-t</sub> and AUC<sub>0-inf</sub> ratios of mitapivat in the presence and absence of rifampin were 0.09. The geometric mean C<sub>max</sub> ratio of mitapivat in the presence and absence of rifampin was 0.23. The intra-subject variability was low at approximately 30% for AUCs and 27% for C<sub>max</sub>.

[00149]           The statistical comparisons of Metabolite PK parameters following 50 mg mitapivat sulfate administered alone (Period 1) or co-administered with multiple doses of 600 mg rifampin (Period 2) PK parameters are summarized below in Table 4.

**Table 4**  
**Summary of Statistical Comparisons of Plasma Metabolite Pharmacokinetic Parameters Following 50 mg Mitapivat Sulfate Co-Administered With Multiple Doses of 600 mg Rifampin Versus Administered Alone (Part 2)**

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | Mitapivat sulfate + rifampin (Test) |    | Mitapivat sulfate alone (Reference) |    |        |                         |                   |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|----|-------------------------------------|----|--------|-------------------------|-------------------|
| Parameter (unit)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Geometric LSM                       | n  | Geometric LSM                       | n  | GMR    | 90% Confidence Interval | Intra-subject CV% |
| AUC <sub>0-t</sub> (ng*hr/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 230.5                               | 14 | 623.0                               | 14 | 0.3700 | 0.3316 - 0.4129         | 16.50             |
| AUC <sub>0-inf</sub> (ng*hr/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 244.9                               | 14 | 662.6                               | 14 | 0.3696 | 0.3321 - 0.4113         | 16.08             |
| C <sub>max</sub> (ng/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 64.47                               | 14 | 53.80                               | 14 | 1.1983 | 1.0642 - 1.3492         | 17.86             |
| Mitapivat sulfate alone: 50 mg mitapivat sulfate (1 x 50 mg tablet) at Hour 0 on Day 1 (Period 1)<br>Mitapivat sulfate + rifampin: 600 mg rifampin (2 x 300 mg capsules) QD on Day 1 to Day 12 inclusive (within ± 1 hour of dosing time on Day 1) with 50 mg mitapivat sulfate (1 x 50 mg tablet) coadministered at Hour 0 on Day 8 (Period 2)<br>Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the linear mixed effect model.<br>Geometric Mean Ratio (GMR) = test / reference<br>Intra-subject CV% = 100 x (square root (exp[MSE]-1), where MSE = Residual variance from linear mixed effect model. |                                     |    |                                     |    |        |                         |                   |

**[00150]** The coadministration of 600 mg rifampin with 50 mg mitapivat sulfate decreased the Metabolite exposure relative to when mitapivat sulfate was administered alone. The geometric mean AUC<sub>0-t</sub> and AUC<sub>0-inf</sub> ratios of the Metabolite in the presence versus absence of rifampin were 0.37. The geometric mean C<sub>max</sub> ratio of the Metabolite in the presence versus absence of rifampin was 1.2. The intra-subject variability was low at approximately 16% to 18% for AUCs and C<sub>max</sub>.

### **Conclusions**

**[00151]** Total exposure of mitapivat increased in the presence of itraconazole compared to dosing of mitapivat sulfate alone.

**[00152]** Total exposure of mitapivat decreased in the presence of rifampin compared to dosing of mitapivat sulfate alone.

### **Example 3. Development of a PBPK Model for Mitapivat Sulfate to Evaluate the Potential for Drug-Drug Interactions**

#### **Part I**

**[00153]** A physiologically based pharmacokinetic model (hereinafter “PBPK” model) for mitapivat sulfate based on the currently available in-vitro and clinical PK data is being developed. The developed PBPK model will be used to assess DDI liability with mitapivat sulfate acting as a victim of CYP3A4-mediated metabolism as well as a perpetrator of CYP and transporter mediated DDI.

**[00154]** Specifically, a PBPK model that includes a simple first order absorption model will be developed. Full PBPK and minimal PBPK distribution models will be evaluated, both of which consider liver and intestinal metabolism. The fraction of mitapivat sulfate absorbed will be estimated from mass balance data from the [14C] human ADME study (Clinical Study AG348-C-009). In-vitro data will be used to assign the relative contribution of CYP3A4 to the clearance of mitapivat sulfate.  $K_I$  and  $k_{inact}$  values for mechanism based inhibition of CYP3A4 and CYP3A4 induction ( $E_{max}$  and  $EC_{50}$ ) data by mitapivat sulfate will be incorporated within the PBPK model to assess the net DDI effects at steady state. Once an mitapivat sulfate PBPK model has been verified against the available clinical data, a series of

DDI simulations assessing mitapivat sulfate as a victim or perpetrator of DDI will be performed in healthy volunteers.

**Experiments with mitapivat sulfate as a victim**

**[00155]** The impact of the co-administration of the strong CYP3A4 inhibitor, itraconazole, on the PK of mitapivat sulfate will be determined based on the following single and multiple-dose administration of 5, 20 and 50 mg BID.

**[00156]** The impact of the co-administration of the strong CYP3A4 inhibitor, ketoconazole, on the PK of mitapivat sulfate will be determined based following single and multiple-dose administration of 5, 20 and 50 mg BID.

**[00157]** The impact of the co-administration of the moderate CYP3A4 inhibitor fluconazole on the PK of mitapivat sulfate will be determined based on the following single- and multiple dose administration of 5, 20 and 50 mg BID.

**[00158]** The impact of the co-administration of the mild CYP3A4 inhibitor cimetidine on the PK of mitapivat sulfate will be determined based on the following single- and multiple dose administration of 5, 20 and 50 mg BID.

**[00159]** The impact of the co-administration of the strong CYP3A4 inducer, rifampin, on the PK of mitapivat sulfate will be determined based on the following single- and multiple dose administration of 5, 20 and 50 mg BID.

**[00160]** The impact of the co-administration of the moderate CYP3A4 inducer efavirenz on the PK of mitapivat sulfate will be determined following single- and multiple dose administration of 5, 20 and 50 mg BID.

**Experiments with mitapivat sulfate as perpetrator**

**[00161]** The extent to which 5 to 50 mg BID mitapivat sulfate affects the systemic exposure of CYP3A4 substrate midazolam when co-administered with mitapivat sulfate will be determined.

**[00162]** The effect of 5 to 50 mg mitapivat sulfate on the systemic exposures of CYP2B6 substrate bupropion when co-administered will be determined. CYP2B6 induction (Emax and EC50 ) data by mitapivat sulfate will be incorporated within the PBPK model. Sensitivity analysis will be conducted to gauge the impact of the uncertainty around Emax and EC50 on the predicted DDIs.

**[00163]** The effect of 5 to 50 mg AG mitapivat sulfate BID on the systemic exposures of CYP2C9 substrate warfarin when co-administered will be determined. CYP2C9 induction (Emax and EC50) data by mitapivat sulfate will be incorporated within the PBPK model. Sensitivity analysis will be conducted to gauge the impact of the uncertainty around Emax and EC50 on the predicted DDIs.

**[00164]** The effect of 5 to 50 mg mitapivat sulfate BID on the systemic exposures of CYP2C8/CYP3A4 substrate repaglinide when co-administered will be determined. CYP2C8 induction (Emax and EC50) data by mitapivat sulfate will be incorporated within the PBPK model. Sensitivity analysis will be conducted to gauge the impact of the uncertainty around Emax and EC50 on the predicted DDIs.

**[00165]** The effect of 5 to 50 mg mitapivat sulfate BID on the systemic exposures of CYP2C19 substrate omeprazole when co-administered will be determined. CYP2C19 induction (Emax and EC50) data by mitapivat sulfate will be incorporated within the PBPK model. Sensitivity analysis will be conducted to gauge the impact of the uncertainty around Emax and EC50 on the predicted DDIs.

**[00166]** The effect of mitapivat sulfate (5 to 50 mg BID) on the systemic exposures of OATP1B1/OATP1B3 (rosuvastatin), P-gp (digoxin), metformin (MATE1 and OCT2), OAT3 (methotrexate) substrate drugs when co-administered will be determined. The respective IC50 values for inhibition of OATP1B1, P-gp, MATE1, OCT2 and OAT3 by mitapivat sulfate will be incorporated within the PBPK model. Sensitivity analysis will be performed to assess the impact of these parameters on the DDI liability of mitapivat sulfate.

## **Part II**

**[00167]** The first order absorption component of the previously developed PBPK model will be extended using available pH solubility data to include the ADAM model. The recovery of mitapivat sulfate plasma concentration profiles after single and multiple dosing

(up to 50 mg BID) to healthy volunteer subjects will be demonstrated. Once verified against the available clinical data, the modified PBPK model will be used to assess the impact of an increase in gastric pH on the pharmacokinetics of mitapivat sulfate via manipulation of system parameters (gastric pH).

**[00168]** While a number of embodiments have been described, the scope of this disclosure is to be defined by the appended claims, and not by the specific embodiments that have been represented by way of example. The contents of all references (including literature references, issued patents, published patent applications, and co-pending patent applications) cited throughout this application are hereby expressly incorporated herein in their entireties by reference. Unless otherwise defined, all technical and scientific terms used herein are accorded the meaning commonly known to one with ordinary skill in the art.

**MITAPIVAT THERAPY AND MODULATORS OF CYTOCHROME P450**

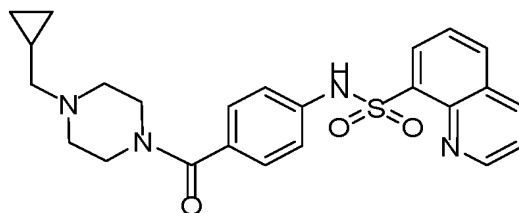
**RELATED APPLICATIONS**

The application claims the benefit of U.S. Provisional Application Serial No. 62/861,600, filed June 14, 2019, the entire teachings of which are incorporated herein by reference.

**BACKGROUND**

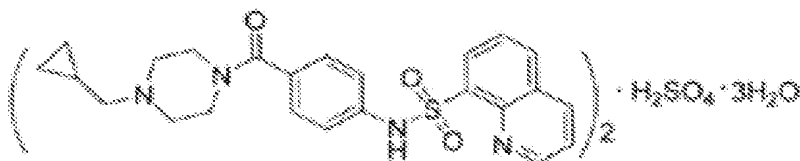
[0001] Pyruvate kinase deficiency (PKD) is a disease of the red blood cells caused by a deficiency of the pyruvate kinase R (PKR) enzyme due to recessive mutations of PKLR gene. PKR activators can be beneficial to treat PKD, thalassemia (*e.g.*, beta-thalassemia), abetalipoproteinemia or Bassen-Kornzweig syndrome, sickle cell disease, paroxysmal nocturnal hemoglobinuria, anemia (*e.g.*, congenital anemias) (*e.g.*, enzymopathies), hemolytic anemia (*e.g.*, hereditary and/or congenital hemolytic anemia, acquired hemolytic anemia, chronic hemolytic anemia caused by phosphoglycerate kinase deficiency, anemia of chronic diseases, non-spherocytic hemolytic anemia or hereditary spherocytosis). Treatment of PKD is supportive, including blood transfusions, splenectomy, chelation therapy to address iron overload, and/or interventions for other disease-related morbidity. Currently, however, there is no approved medicine that treats the underlying cause of PKD, and thus the etiology of life-long hemolytic anemia.

[0002] Mitapivat, also known as AG-348 or by its chemical name *N*-(4-(4-(cyclopropylmethyl)piperazine-1-carbonyl)phenyl)quinoline-8-sulfonamide, and is represented by the following structural formula:



is an allosteric activator of pyruvate kinase R (PKR). See, *e.g.*, International Patent Application Publication Nos. WO 2011/002817 and WO 2016/201227.

**[0003]** Mitapivat sulfate that is represented by the following structural formula:



is currently in Phase 2 and Phase 3 clinical trials as a disease-altering therapy in patients with PKD, hemolytic anemia and thalassemia. See, *e.g.*, U.S. Clinical Trials Identifier Nos. NCT03692052, NCT03559699, NCT03548220, NCT03853798, and NCT02476916.

### SUMMARY

**[0004]** Non-clinical studies described in Example 1 show that the PKR activator, mitapivat, is primarily metabolized by the cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5) enzymes (*i.e.*, >90%), with minor contributions from other detoxification enzymes, namely CYP2C8, CYP2C9, and CYP1A2. It has also been found that in a clinical trial, total exposure of mitapivat increased in the presence of itraconazole, a strong CYP3A4A and p-gp inhibitor, compared with dosing of mitapivat sulfate alone. It has further been found that total exposure of mitapivat decreased in the presence of rifampin, a strong CYP3A4A and p-gp inducer, compared with dosing of mitapivat sulfate alone.

**[0005]** Accordingly, the present disclosure provides methods of treating a disease, disorder or a condition with mitapivat or a pharmaceutically acceptable salt thereof and a CYP3A4/5 inducer or a CYP3A4/5 inhibitor. In another aspect, the present disclosure provides methods of treating a disease, disorder or a condition with mitapivat or a pharmaceutically acceptable salt thereof and a p-glycoprotein (p-gp) inhibitor. As used herein, “CYP3A4/5” means cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5). In some embodiments, the pharmaceutically acceptable salt is mitapivat sulfate.

**[0006]** Specifically, in one embodiment, the present disclosure relates to a method of treating pyruvate kinase deficiency (PKD) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inhibitor of cytochrome P450 3A4/5 (CYP3A4/5)[[ ]]. In another embodiment, the present disclosure relates to a method of treating sickle cell disease in a subject, comprising administering to the subject an effective amount of mitapivat or a

pharmaceutically acceptable salt thereof and an effective amount of an inhibitor of cytochrome P450 3A4/5 (CYP3A4/5). In another embodiment, the present disclosure relates to a method of treating thalassemia ([e.g.] e.g., alpha-thalassemia and beta-thalassemia) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inhibitor of cytochrome P450 3A4/5 (CYP3A4/5). In another embodiment, the present disclosure relates to a method of treating hemolytic anemia in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inhibitor of cytochrome P450 3A4/5 (CYP3A4/5). In some embodiments, the inhibitor of CYP3A4/5 is a moderate CYP3A4/5 inhibitor. In some embodiments, the inhibitor of CYP3A4/5 is a mild CYP3A4/5 inhibitor.

**[0007]** In another embodiment, the present disclosure relates to a method of treating pyruvate kinase deficiency (PKD) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inducer of cytochrome P450 3A4/5 (CYP3A4/5)[[ ]]. In another embodiment, the present disclosure relates to a method of treating sickle cell disease in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inducer of cytochrome P450 3A4/5 (CYP3A4/5). In another embodiment, the present disclosure relates to a method of treating thalassemia ([e.g.] e.g., alpha-thalassemia and/or beta-thalassemia) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inducer of cytochrome P450 3A4/5 (CYP3A4/5). In another embodiment, the present disclosure relates to a method of treating hemolytic anemia in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inducer of cytochrome P450 3A4/5 (CYP3A4/5). In some embodiments, the inducer of CYP3A4/5 is a moderate CYP3A4/5 inducer. In some embodiments, the inhibitor of CYP3A4/5 is a mild CYP3A4/5 inducer.

**[0008]** Specifically, in one embodiment, the present disclosure relates to a method of treating pyruvate kinase deficiency (PKD) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of a p-glycoprotein inhibitor. In another embodiment, the present disclosure

relates to a method of treating sickle cell disease in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of a p-glycoprotein inhibitor. In another embodiment, the present disclosure relates to a method of treating thalassemia ([e.g.] e.g., alpha-thalassemia and beta-thalassemia) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of a p-glycoprotein inhibitor. In another embodiment, the present disclosure relates to a method of treating hemolytic anemia in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of a p-glycoprotein inhibitor. In some embodiments, the p-glycoprotein inhibitor is a moderate p-glycoprotein inhibitor. In some embodiments, the p-glycoprotein inhibitor is a mild p-glycoprotein inhibitor.

**[0009]** The present disclosure further provides methods of treating a disease, disorder or a condition with [☐] mitapivat or a pharmaceutically acceptable salt thereof in the absence of a CYP3A4/5 inducer or a CYP3A4/5 inhibitor or p-glycoprotein inhibitor.

**[0010]** Specifically, in one embodiment, the present disclosure relates to a [☐] method of treating pyruvate kinase deficiency (PKD) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of an inducer or an inhibitor of cytochrome P450 3A4/5 (CYP3A4/5), respectively. In another embodiment, the present disclosure relates to a method of treating sickle cell disease in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of an inducer or an inhibitor of cytochrome P450 3A4/5 (CYP3A4/5), respectively. In yet another embodiment, the present disclosure relates to a [☐] method of treating thalassemia ([e.g.] e.g., alpha-thalassemia and/or beta-thalassemia) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of an inducer or an inhibitor of cytochrome P450 3A4/5 (CYP3A4/5), respectively. In yet another embodiment, the present disclosure relates to a method of treating hemolytic anemia in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of an inducer or an inhibitor of cytochrome P450 3A4/5 (CYP3A4/5), respectively. In some embodiments, the inducer of CYP3A4/5 is a strong inducer of CYP3A4/5. In some embodiments, the inducer of

CYP3A4/5 is a moderate inducer of CYP3A4/5. In some embodiments, the inhibitor of CYP3A4/5 is a strong inhibitor of CYP3A4/5. In some embodiments, the inhibitor of CYP3A4/5 is a moderate inhibitor of CYP3A4/5.

**[0011]** In another embodiment, the present disclosure relates to a method of treating pyruvate kinase deficiency (PKD) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of a p-glycoprotein inhibitor. In another embodiment, the present disclosure relates to a method of treating sickle cell disease in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of a p-glycoprotein inhibitor. In yet another embodiment, the present disclosure relates to a method of treating thalassemia ([e.g.], e.g., alpha-thalassemia and/or beta-thalassemia) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of a p-glycoprotein inhibitor. In yet another embodiment, the present disclosure relates to a method of treating hemolytic anemia in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of a p-glycoprotein inhibitor. In some embodiments, the p-glycoprotein inhibitor is a strong p-glycoprotein inhibitor. In some embodiments, ~~In some embodiments~~, the p-glycoprotein inhibitor is a mild p-glycoprotein inhibitor.

#### BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a graph showing Arithmetic Mean (SD) Plasma Mitapivat and Metabolite Concentration-Time Profiles following administration of 20 mg mitapivat sulfate alone (Treatment Period 1) and co-administered with multiple doses of 200 mg itraconazole (Treatment Period 2) on linear scale (Part 1). “Metabolite” refers to the CYP3A4 metabolite of mitapivat (see Example 2).

Figure 2 is a graph showing Arithmetic Mean Plasma Mitapivat and Metabolite Concentration-Time Profiles following administration of 20 mg mitapivat sulfate alone (Treatment Period 1) and co-administered with multiple doses of 200 mg itraconazole (Treatment Period 2) on semi-log scale (Part 1) (see Example 2).

Figure 3 is a graph showing Arithmetic Mean (SD) Plasma Mitapivat and Metabolite Concentration-Time Profiles following administration of 50 mg mitapivat sulfate alone

(Treatment Period 1) and co-administered with multiple doses of 600 mg rifampin on linear scale (Treatment Period 2, Part 2, see Example 2).

Figure 4 is a graph showing Arithmetic Mean Plasma Mitapivat and Metabolite Concentration-Time Profiles following administration of 50 mg mitapivat sulfate alone (Treatment Period 1) and co-administered with multiple doses of 600 mg rifampin on semi-log scale (Treatment Period 2, Part 2, see Example 2).

## DETAILED DESCRIPTION

### Definitions

#### [0012]

“CYP3A4/5 modulator” refers to any substance, including, but not limited to a small molecule (with a molecular weight of  $\leq 1000$  Da, or  $\leq 750$  Da, or  $\leq 500$  Da), a nucleoside, a nucleotide, a nucleobase, a sugar, a nucleic acid, an amino acid, a polysaccharide, a peptide, a polypeptide, a protein, an antibody, an aptamer, a natural or synthetic plant-based substance, or a complex or a composition comprising any combination of the aforementioned substances, that can modify or alter the activity and/or expression levels of the enzyme cytochrome P450 3A4 (CYP3A4; EC 1.14.13.97) or 3A5 (CYP3A5; EC1.14.14.1). Briefly, the cytochrome P450 (CYP) is a superfamily of detoxification enzymes that are responsible for the oxidative and reductive metabolic transformation of drug medications and the ultimate “clearance”, which is the elimination of the drug from the body as determined by the measured plasma levels of the drug and optionally, the drug metabolites, such as the metabolite known to be formed by CYP3A4/5. CYP3A4 and CYP3A5 are two of such enzymes and are largely prevalent in the liver.

**[0013]** A CYP3A4/5 modulator may be a CYP3A4/5 inducer or a CYP3A4/5 inhibitor. A “CYP3A4/5 inducer” or “inducer of CYP3A4/5” is a CYP3A4/5 modulator as defined above that can increase, enhance, stimulate, induce, accelerate or augment the CYP3A4/5 activity from its existing state or native state, such as by at least about 0.1%, 0.2%, 0.5%, 1%, 2%, 3%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% and 100% or more relative to a reference level, *e.g.*, the level of the CYP3A4/5 activity and/or expression prior to the exposure or contact with the CYP3A4/5 inducer, including all ranges and values therebetween not expressly mentioned above. The CYP3A4/5 inducer activity may cause mitapivat or a pharmaceutically acceptable

salt thereof that is co-administered with the CYP3A4/5 inducer to be metabolized and eliminated more quickly from the body, as determined by the measured plasma levels of the compound and its metabolites.

**[0014]** A “CYP3A4/5 inhibitor” or “inhibitor of CYP3A4/5” is a CYP3A4/5 modulator as defined above that can decrease, reduce, inhibit, decelerate, attenuate, or suppress the CYP3A4/5 activity and/or expression from its existing state or native state, such as by at least about 0.1%, 0.2%, 0.5%, 1%, 2%, 3%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% and 100% or more relative to a reference level, *e.g.*, the level of the CYP3A4/5 activity and/or expression prior to the exposure or contact with the CYP3A4/5 inhibitor, including all ranges and values therebetween not expressly mentioned above. The CYP3A4/5 inhibitor may cause mitapivat or a pharmaceutically acceptable salt thereof that is co-administered with the CYP3A4/5 inhibitor to be metabolized and eliminated more slowly from the body, as determined by the measured plasma levels of the compound and its metabolites.

**[0015]** If an investigational drug is a CYP inducer, it can be classified as a strong, moderate, or mild inducer based on its effect on an index CYP substrate. The convention is to categorize CYP induction in the following ways:

**[0016]** A strong inducer decreases the AUC of a sensitive index CYP by  $\geq 80$  percent.

**[0017]** A moderate inducer decreases the AUC of a sensitive index CYP substrate by  $\geq 50$  to  $< 80$  percent.

**[0018]** A mild inducer decreases the AUC of a sensitive index CYP substrate by  $\geq 20$  to  $< 50$  percent.[""]

**[0019]** A CYP inhibitor can be classified as a strong, moderate, or mild inhibitor based on its effect on an index CYP substrate. A strong inhibitor increases the AUC of a sensitive index CYP substrate  $\geq 5$ -fold. A moderate inhibitor increases the AUC of a sensitive index CYP substrate by  $\geq 2$ - to  $< 5$ -fold. A mild inhibitor increases the AUC of a sensitive index CYP substrate by  $\geq 1.25$ - to  $< 2$ - fold.[""]

**[0020]** A p-glycoprotein inhibitor refers to any substance, including, but not limited to a small molecule (with a molecular weight of  $\leq 1000$  Da, or  $\leq 750$  Da, or  $\leq 500$  Da), a

nucleoside, a nucleotide, a nucleobase, a sugar, a nucleic acid, an amino acid, a polysaccharide, a peptide, a polypeptide, a protein, an antibody, an aptamer, a natural or synthetic plant-based substance, or a complex or a composition comprising any combination of the aforementioned substances, that can decrease, reduce, inhibit, decelerate, attenuate, or suppress the p-glycoprotein activity and/or expression from its existing state or native state, such as by at least about 0.1%, 0.2%, 0.5%, 1%, 2%, 3%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% and 100% or more relative to a reference level, *e.g.*, the level of the p-glycoprotein. In some embodiments, the p-glycoprotein inhibitor is a strong p-glycoprotein inhibitor, selected from the group consisting of amiodarone, azithromycin, captopril, carvedilol, clarithromycin, conivaptan, cyclosporine, diltiazem, dronedarone, erythromycin, felodipine, itraconazole, ketoconazole, lopinavir, ritonavir, quercetin, quinidine, ranolazine, ticagrelor, and erapamil.

**[0021]** The terms “effective amount” and “therapeutically effective amount” are used interchangeably throughout the present disclosure, whether referring to mitavipat or a pharmaceutically acceptable salt thereof, a CYP3A4/5 inducer, or a CYP3A4/5 inhibitor.

**[0022]** An “effective amount” of mitavipat or a pharmaceutically acceptable salt thereof is an amount sufficient to provide a therapeutic benefit in the treatment of pyruvate kinase deficiency (PKD), thalassemia (*e.g.*, ☐ ☐ alfa-thalassemia, beta-thalassemia or non-transfusion-dependent thalassemia), abetalipoproteinemia or Bassen-Kornzweig syndrome, sickle cell disease, paroxysmal nocturnal hemoglobinuria, anemia (*e.g.*, congenital anemias (*e.g.*, enzymopathies), hemolytic anemia (*e.g.*, hereditary and/or congenital hemolytic anemia, acquired hemolytic anemia, chronic hemolytic anemia, chronic hemolytic anemia caused by phosphoglycerate kinase deficiency, anemia of chronic diseases, non-spherocytic hemolytic anemia or hereditary spherocytosis), as well as all other conditions disclosed in the section “Diseases, Disorders and Conditions” in the present disclosure, *i.e.*, conditions that are ☐ ☐ responsive to treatment with a PKR activator such as mitavipat or a pharmaceutically acceptable salt thereof. Such diseases, disorders and conditions are hereinafter referred as “a PKR activator-responsive condition” when referring to any one of these conditions or “PKR activator-responsive conditions” when referring to these conditions collectively. Additionally or alternatively, an “effective amount” of mitavipat or a pharmaceutically acceptable salt thereof is an amount sufficient to delay or minimize one or more effects or symptoms associated with these conditions. In one aspect, an “effective amount” of mitavipat or a

pharmaceutically acceptable salt thereof means an amount of mitavipat or a pharmaceutically acceptable salt thereof, alone or in combination with other therapies, which provides a therapeutic benefit in the treatment of the condition. The term “effective amount” can encompass an amount that improves overall therapy, reduces or avoids effects, symptoms, signs, or causes of the condition, and/or enhances the therapeutic efficacy of another therapeutic agent. In certain embodiments, an “effective amount” of mitavipat or a pharmaceutically acceptable salt thereof is an amount sufficient for eliciting measurable activation of wild-type or mutant PKR. In certain embodiments, an “effective amount” of mitavipat or a pharmaceutically acceptable salt thereof is an amount sufficient for regulating 2,3-diphosphoglycerate levels in blood in need thereof or for treating PKR activator-responsive conditions or treating diseases or conditions that are associated with increased 2,3-diphosphoglycerate levels (*e.g.*, liver diseases). In certain embodiments, an effective amount of mitavipat or a pharmaceutically acceptable salt thereof is an amount sufficient for eliciting measurable activation of wild-type or mutant PKR and for regulating 2,3-diphosphoglycerate levels in blood in need thereof or for treating PKR activator-responsive conditions or treating diseases or conditions that are associated with increased 2,3-diphosphoglycerate levels ([*e.g.*,]*e.g.*, liver diseases). In one aspect, the effective amount is the amount required to generate a subject’s hemoglobin response of  $\geq 1.0$  g/dL (such as  $\geq 1.5$  g/dL or  $\geq 2.0$  g/dL) increase in Hb concentration from baseline. In one aspect, the subject’s baseline Hb concentration is the average of all available Hb concentrations before treatment with a compound described herein. In certain aspects, the effective amount is the amount required to reduce the patient’s transfusion burden. In one aspect, the effective amount of mitavipat or a pharmaceutically acceptable salt thereof (*e.g.*, mitavipat sulfate) is between about 0.01-100 mg/kg body weight/day of mitavipat or a pharmaceutically acceptable salt thereof (*e.g.*, mitavipat sulfate), *e.g.*, about 0.1-100 mg/kg body weight/day.

**[0023]** Concomitant medications will be commonly used with mitavipat or a pharmaceutically acceptable salt thereof (*e.g.*, mitavipat sulfate). An “effective amount” of the drug (*e.g.*, an effective amount of a CYP3A4/5 inducer or an effective amount of a CYP3A4/5 inhibitor) being co-administered with mitavipat or a pharmaceutically acceptable salt thereof (*e.g.*, mitavipat sulfate) is an amount sufficient to provide a desired therapeutic benefit. Drugs that are being co-administered with mitavipat or a pharmaceutically acceptable salt thereof (*e.g.*, mitavipat sulfate), including some of the current antiarrhythmics, antibiotics, antidepressants, antifungals, calcium channel blockers, H<sub>2</sub> receptor antagonists,

non-nucleoside reverse transcriptase inhibitors (NNRTI), protease inhibitors, etc., will sometimes be CYP3A4/5 inducers or CYP3A4/5 inhibitors. In such instances, the amount of mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) or the amount of the drug being co-administered should be adjusted to reduce effects of a drug-drug interaction between mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) and the co-administered drug.

**[0024]** In one aspect, an effective amount of a CYP3A4/5 inducer is between about 0.1-1000 mg/kg/day of a CYP3A4/5 inducer, *e.g.*, about 1.0-1000 mg/kg/day, depending on the drug that is co-administered with.

**[0025]** In one aspect, an effective amount of a CYP3A4/5 inhibitor is between about 0.1-1000 mg/kg/day of a CYP3A4/5 inhibitor, *e.g.*, about 1.0-1000 mg/kg/day, depending on the drug that is co-administered with.

**[0026]** In one aspect, an effective amount of a p-gp inhibitor is between about 0.1-1000 mg/kg/day of a p-gp inhibitor, *e.g.*, about 1.0-1000 mg/kg/day, depending on the drug that is co-administered with.

**[0027]** As used herein, reduction in transfusion burden means at least 20% reduction in the number of RBC units transfused within at least 5 weeks of treatment. In certain embodiments, the reduction in transfusion burden is  $\geq 33\%$  reduction in the number of RBC units transfused within at least 5 weeks of treatment. In certain embodiments, reduction of transfusion burden is  $\geq 33\%$  reduction in the number of RBC units transfused within at least 10 weeks ([[e.g.,]]e.g., at least 20 weeks or at least 24 weeks) of treatment.

**[0028]** The term “activating” as used herein means an agent that (measurably) increases the activity of wild type pyruvate kinase R (wt PKR) or causes wild type pyruvate kinase R (wt PKR) activity to increase to a level that is greater than wt PKR’s basal levels of activity or an agent that (measurably) increases the activity of a mutant pyruvate kinase R (mPKR) or causes mutant pyruvate kinase R (mPKR) activity to increase to a level that is greater than that mutant PKR’s basal levels of activity, for examples, to a level that is 20%, 40%, 50%, 60%, 70%, 80%, 90% or 100% of the activity of wild type PKR.

**[0029]** The terms “treatment,” “treat,” and “treating” refer to reversing, alleviating, reducing the likelihood of developing, or inhibiting the progress of a disease or disorder, or

one or more effects or symptoms thereof, as described herein. In some embodiments, treatment may be administered after one or more symptoms have developed, *i.e.*, therapeutic treatment. In other embodiments, treatment may be administered in the absence of symptoms. For example, treatment may be administered to a susceptible individual prior to the onset of symptoms (*e.g.*, in light of a history of symptoms and/or in light of genetic or other susceptibility factors), *i.e.*, prophylactic treatment. Treatment may also be continued after symptoms have resolved, for example to reduce the likelihood of or delay their recurrence.

**[0030]** As used herein the terms “subject” and “patient” may be used interchangeably, and means a mammal in need of treatment with mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate).

**[0031]** The term “pharmaceutically acceptable salt” when referring to a pharmaceutically acceptable salt of mitapivat, refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response, and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art, for example, Berge et al. describe pharmaceutically acceptable salts in detail in *J. Pharmaceutical Sciences*, 1977, 66, 1-19. Pharmaceutically acceptable salts of mitapivat include those derived from suitable inorganic and organic acids. Examples of pharmaceutically acceptable acid addition salts are salts of an amino group formed with inorganic acids, such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid, and perchloric acid or with organic acids, such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid, or malonic acid or by using other methods known in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, besylate bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, gentisate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate,

sulfate, tartrate, thiocyanate, tosylate p-toluenesulfonate, undecanoate, valerate salts, and the like. Preferably, a pharmaceutically acceptable salt of mitapivat is a sulfate salt.

**[0032]** As used herein, the terms “about” and “approximately” are used herein to mean within the typical ranges of tolerance in the art. In one embodiment, “about” means within 2 standard deviations from the mean value. In one embodiment, “about” means  $\pm 10\%$ . In one embodiment, “about” means  $\pm 5\%$ . When “about” is present before a series of numbers or number ranges, it is understood that the term can apply ~~applies~~ to any and each of the numbers and ranges recited in the series.

### **Compositions and Administration**

**[0033]**

he effective amount of mitapivat, in accordance with a method of the invention, may be administered in the form of a pharmaceutical composition comprising mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate), together with a pharmaceutically acceptable carrier.

**[0034]** Pharmaceutical compositions of mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) can be prepared by any method known in the art of pharmacology. In general, such preparatory methods include the steps of bringing of mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) into association with a carrier and/or one or more other accessory ingredients, and then, if necessary and/or desirable, shaping and/or packaging the product into a desired single- or multi-dose unit.

**[0035]** Pharmaceutically acceptable carriers used in the manufacture of provided pharmaceutical compositions include inert diluents, dispersing and/or granulating agents, surface active agents and/or emulsifiers, disintegrating agents, binding agents, preservatives, buffering agents, lubricating agents, and/or oils. Exemplary carriers, diluents, granulating and/or dispersing agents, surface active agents and/or emulsifiers, binding agents, preservatives, antioxidants, chelating agent, antifungal preservatives, alcohol preservatives, acidic preservatives, other types of preservatives, buffering agents, lubricating agents, and natural oils that can be included in a pharmaceutical composition of [[ ]]mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) as disclosed in International Patent Application No. WO2019/104134.

**[0036]** Pharmaceutical compositions of mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, transmucosally, or in an ophthalmic preparation. The term “parenteral” as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection or infusion techniques. In one aspect, the pharmaceutical compositions provided herewith are orally administered in an orally acceptable dosage form including, but not limited to, capsules, tablets, emulsions and aqueous suspensions, dispersions and solutions. In the case of tablets for oral use, carriers which are commonly used include one or more substances selected from microcrystalline cellulose, mannitol, croscarmellose sodium, and sodium stearyl fumarate. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions and/or emulsions are administered orally, the active ingredient may be suspended or dissolved in an oily phase is combined with emulsifying and/or suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added.

**[0037]** A secondary drug can be co-administered with mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate). The effective amount of a secondary drug that is co-administered with mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate), such as a CYP3A4/5 inducer or a CYP3A4/5 inhibitor or a p-gp inhibitor, if used in a method of the invention, may also be administered in the form of a pharmaceutical composition comprising the CYP3A4/5 inducer or the CYP3A4/5 inhibitor or the p-gp inhibitor, together with a pharmaceutically acceptable carrier. Pharmaceutical compositions of the secondary drug may be prepared and administered as described above for pharmaceutical compositions of mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate).

**[0038]** In one aspect, mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) is formulated as a tablet composition together with a pharmaceutically acceptable carrier, in accordance with the disclosures of International Patent Application No. WO2019/104134.

**[0039]** In one embodiment, in therapies comprising mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) and a secondary drug (*e.g.*, a CYP3A4/5

inducer or a CYP3A4/5 inhibitor), mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) and the secondary drug are administered “concurrently” to the subject, which means that the subject is administered both drugs on the same day on all days of the treatment period. In one embodiment, mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) and the secondary drug are administered “sequentially” to the subject, which means that the subject is administered either of the two drugs on all days of the treatment period, but not both drugs. In another embodiment, mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) and the secondary drug are administered “sequentially and concurrently” to the subject, which means that the subject is administered either of the two drugs on certain days of the treatment period, but not both drugs; and the subject is administered both drugs on the same day on other days of the treatment period.

### **Doses and Dosing Regimens**

**[0040]** The amount of mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) that may be combined with carrier materials to produce a composition in a single dosage form will vary depending upon the subject to be treated and the particular mode of administration. For example, [ ] a specific dosage and treatment regimen for any particular subject will depend upon a variety of factors, including age, body weight, general health, sex, diet, time of administration, rate of excretion, drug combination, the judgment of the treating physician, and the severity of the particular disease being treated. The amount of mitapivat or a pharmaceutically acceptable salt thereof will also depend upon the particular form (*e.g.*, free base, salt form, crystalline form, etc.) in the composition. In one aspect, a provided composition may be formulated such that a dosage equivalent to about 0.001 to about 100 mg/kg body weight/day of mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, about 0.5 to about 100 mg/kg of mitapivat or a pharmaceutically acceptable salt thereof) can be administered to a subject receiving these compositions. Alternatively, dosages equivalent to 1 mg/kg and 1000 mg/kg of mitapivat or a pharmaceutically acceptable salt thereof every 4 to 120 hours is also acceptable.

**[0041]** In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose of equivalent to about 2 mg to about 3000 mg of mitapivat. In certain embodiments, the dose is an oral dose. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 2

[illegible]

[illegible]

**[0042]** In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 2 mg to about 3000 mg of [[ ]] mitapivat per day. In certain embodiments, [[ ]]mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 5 mg to about 500 mg of [[ ]]mitapivat of per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 5 mg to about 200 mg of [[ ]]mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 5 mg of [[ ]]mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 5 mg to about 10 mg of [[ ]]mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose of about 15 mg equivalent to mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 20 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 25 mg of mitapivat per day. In certain embodiments, [[ ]]mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 30 mg of [[ ]]mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 35 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 40 mg of [[ ]]mitapivat per day. In certain embodiments, [[ ]]mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 45 mg of [[ ]]mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 50 mg of [[ ]]mitapivat thereof per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 55 mg of [[ ]]mitapivat thereof per day. In certain embodiments, [[ ]]mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 60 mg of [[ ]]mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 70 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 80 mg of [[ ]]mitapivat per day. In certain

embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 90 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 100 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 110 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 120 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 130 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 140 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 150 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 160 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 170 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 180 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 190 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 200 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 210 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 220 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 230 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 240 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 250 mg of mitapivat per day. In certain

embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 260 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 270 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 280 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 290 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 300 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 300-360 mg of mitapivat per day. Dosing can be once, twice, or three times daily. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 5 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 10 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 15 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 20 mg of mitapivat twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 25 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 30 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 35 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 40 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 45 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 50 mg of mitapivat twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 55 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt

thereof is formulated for administration at a dose equivalent to about 60 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 65 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 70 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 75 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 80 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 85 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 90 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 95 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 100 mg of mitapivat twice per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 5 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 10 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 15 mg of mitapivat thereof once per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 20 mg of mitapivat once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 25 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 30 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 35 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 40 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for

administration at a dose equivalent to about 45 mg of mitapivat thereof once per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 50 mg of mitapivat once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 55 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 60 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 65 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 70 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 75 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 80 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 85 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 95 mg of mitapivat thereof once per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 100 mg of mitapivat once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 110 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 120 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 130 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 140 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 150 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 160 mg to about 200 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is

formulated for administration at a dose equivalent to about 200 mg to about 360 mg of mitapivat thereof once per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 5 mg of mitapivat once every other day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 20 mg of mitapivat once every other day. In one aspect, mitapivat or a pharmaceutically acceptable salt ~~thereof~~ thereof is formulated for administration at a dose equivalent to about 50 mg of mitapivat once every other day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 100 mg of mitapivat once every other day. In certain embodiment, the pharmaceutically acceptable salt of mitapivat is mitapivat sulfate.

**[0043]** In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof by about 70% to about 80%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof by about 60% to about 70%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof by about 50% to about 60%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof by about 40% to about 50%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof by about 30% to about 40%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof by about 20% to about 30%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof by about 10% to about 20%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof by about 50%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 400 mg of mitapivat to the dose equivalent to about 200 mg. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 300 mg of mitapivat to the dose equivalent to about 150 mg. In certain embodiments,

for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 200 mg of mitapivat to the dose equivalent to about 100 mg. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 100 mg of mitapivat to the dose equivalent to about 50 mg. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 50 mg of mitapivat to the dose equivalent to about 25 mg. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 20 mg of mitapivat to the dose equivalent to about 10 mg. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 10 mg of mitapivat to the dose equivalent to about 5 mg. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 5 mg of mitapivat to the dose equivalent to about 2.5 mg.

**[0044]** In certain embodiments, for any of the methods presented herein, further comprising reducing the dose frequency of mitapivat or a pharmaceutically acceptable salt thereof by about 70% to about 80%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose frequency of mitapivat or a pharmaceutically acceptable salt thereof by about 60% to about 70%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose frequency of mitapivat or a pharmaceutically acceptable salt thereof by about 50% to about 60%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose frequency of mitapivat or a pharmaceutically acceptable salt thereof by about 40% to about 50%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose frequency of mitapivat or a pharmaceutically acceptable salt thereof by about 30% to about 40%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose frequency of mitapivat or a pharmaceutically acceptable salt thereof by about 20% to about 30%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose

frequency of mitapivat or a pharmaceutically acceptable salt thereof by about 10% to about 20%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose frequency of mitapivat or a pharmaceutically acceptable salt thereof by about 50%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose frequency of mitapivat or a pharmaceutically acceptable salt thereof from twice daily to once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose frequency of mitapivat or a pharmaceutically acceptable salt thereof from once daily to once every other day.

**[0045]** In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 400 mg of mitapivat twice daily to the dose equivalent to about 200 mg twice daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 300 mg of mitapivat twice daily to the dose equivalent to about 150 mg twice daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 200 mg of mitapivat twice daily to the dose equivalent to about 100 mg twice daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 100 mg of mitapivat twice daily to the dose equivalent to about 50 mg twice daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 50 mg of mitapivat twice daily to the dose equivalent to about 25 mg twice daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 20 mg of mitapivat twice daily to the dose equivalent to about 10 mg twice daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 10 mg of mitapivat twice daily to the dose equivalent to about 5 mg twice daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 5 mg twice daily of mitapivat to the dose equivalent to about 2.5 mg twice daily.

**[0046]** In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 400 mg of mitapivat once daily to the dose equivalent to about 200 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 300 mg of mitapivat once daily to the dose equivalent to about 150 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 200 mg of mitapivat once daily to the dose equivalent to about 100 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 100 mg of mitapivat once daily to the dose equivalent to about 50 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 50 mg of mitapivat once daily to the dose equivalent to about 25 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 20 mg of mitapivat once daily to the dose equivalent to about 10 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 10 mg of mitapivat once daily to the dose equivalent to about 5 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 5 mg once daily of mitapivat to the dose equivalent to about 2.5 mg once daily.

**[0047]** In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 400 mg of mitapivat twice daily to the dose equivalent to about 200 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 300 mg of mitapivat twice daily to the dose equivalent to about 150 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from

the dose equivalent to about 200 mg of mitapivat twice daily to the dose equivalent to about 100 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 100 mg of mitapivat twice daily to the dose equivalent to about 50 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 50 mg of mitapivat twice daily to the dose equivalent to about 25 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 20 mg of mitapivat twice daily to the dose equivalent to about 10 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 10 mg of mitapivat twice daily to the dose equivalent to about 5 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 5 mg twice daily of mitapivat to the dose equivalent to about 2.5 mg once daily.

**[0048]** In some embodiments, mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) is present in the tablet composition in an amount equivalent to about 1 to about 200 mg of mitapivat. In some embodiments, mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) is present in the tablet composition in an amount equivalent to about 1 to about 150 mg of mitapivat. In some embodiments, mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) is present in the tablet composition in an amount equivalent to about 1 to about 100 mg of mitapivat. In some embodiments, mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) is present in the tablet composition in an amount equivalent to about 5 mg of mitapivat. In some embodiments, mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) is present in the tablet composition in an amount equivalent to about 20 mg of mitapivat. In some embodiments, mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) is present in the tablet composition in an amount equivalent to about 50 mg of mitapivat. In some embodiments, mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) is present in the tablet composition in an amount equivalent to about 75 mg of mitapivat. In some embodiments, mitapivat or a pharmaceutically acceptable

salt thereof (*e.g.*, mitapivat sulfate) is present in a tablet composition in an amount equivalent to about 100 mg of mitapivat. In certain embodiment, the pharmaceutically acceptable salt of mitapivat is mitapivat sulfate.

**[0049]** As used herein, the dose amount of mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) is based on the equivalence to the free-base form of mitapivat. For example, “mitapivat or pharmaceutically acceptable salt thereof present in the composition in an amount equivalent to about 2.0 mg of mitapivat” means about 2.0 mg of free-base mitapivat or about 2.33 mg of mitapivat sulfate having the structural formula as depicted in the background of the present disclosure.

**Methods of Treatment (~~Mitipivat~~ Mitapivat Monotherapy and Concomitant Medications)**

**[0050]** In one aspect, the present disclosure provides methods of treating a disease, disorder or condition with [[ ]]mitapivat or a pharmaceutically acceptable salt thereof and a CYP3A4/5 inducer or a CYP3A4/5 inhibitor or a p-gp inhibitor; use of mitapivat and a CYP3A4/5 inducer or a CYP3A4/5 inhibitor or a p-gp inhibitor for the manufacture of a medicament for treating a disease, disorder or condition; and mitapivat or a pharmaceutically acceptable salt thereof and a CYP3A4/5 inducer or a CYP3A4/5 inhibitor or a p-gp inhibitor for use in treating a disease, disorder or condition. The disease, disorder or condition treated is pyruvate kinase deficiency (PKD), sickle cell disease, or thalassemia as further discussed in the following embodiments, and any one disease, disorder or condition described in the section “Diseases, Disorders and Conditions” provided in the present disclosure.

**[0051]** In one embodiment, the present disclosure relates to a method of treating pyruvate kinase deficiency (PKD) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inducer of cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5).

**[0052]** In another embodiment, the present disclosure relates to a method of treating sickle cell disease in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inducer of cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5).

**[0053]** In yet another embodiment, the present disclosure relates to a method of treating thalassemia in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inducer of cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5). In certain embodiments, the thalassemia is alpha thalassemia. In certain embodiments, the thalassemia is beta thalassemia.

**[0054]** In one embodiment, the method of any one of the preceding embodiments further comprises:

- (a) monitoring the subject for an effect of a drug-drug interaction between mitapivat or a pharmaceutically acceptable salt thereof and the CYP3A4/5 inducer; and
- (b) in the event that an effect of the drug-drug interaction is present, adjusting the effective amount of mitapivat or a pharmaceutically acceptable salt thereof and/or the [[ ]]effective amount of the CYP3A4/5 inducer being administered to the subject to reduce the effect.

**[0055]** In some embodiments, the adjustment is to reduce the dose of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the adjustment is to reduce the dose frequency of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the adjustment is to reduce the dose and dose frequency of mitapivat or a pharmaceutically acceptable salt thereof. Details of the dose and/or dose frequency adjustment are shown in the dose and dose regime section.

**[0056]** In one embodiment, the method of the preceding embodiment further comprises:

- (c) repeating [[ ]](a) and (b) until there are no further detectable symptoms of the drug-drug interaction.

Steps (a) and (b) can be repeated as many times as necessary until there are no further detectable symptoms of the drug-drug interaction. In one embodiment, step (a) comprises measuring and analyzing the pharmacokinetic (PK) such as AUC (*i.e.*, area under the plasma concentration-time curve, such as AUC<sub>0-24</sub>, AUC<sub>0-t</sub>, AUC<sub>0-inf</sub>, AUC<sub>%estrap</sub>) and C<sub>max</sub> values (*i.e.*, the maximum (or peak) serum concentration that a drug achieves in a specified compartment or test area of the body after the drug has been administered and before the administration of a second dose). In one embodiment, step (a) comprises measuring and

analyzing other PK parameters such as but not limited to  $C_l/F$  (*i.e.*, apparent total body clearance of the drug from plasma),  $V_z/F$  (*i.e.*, apparent volume of distribution during terminal phase after non-intravenous administration)[[ ]],  $t_{1/2}$  (elimination half-life),  $t_{last}$ ,  $t_{max}$  (time to reach maximum (peak) plasma concentration following drug administration), etc.

**[0057]** A “drug-drug interaction” as used herein refers to a change in concentration in a patient of a first drug (*e.g.*, mitapivat ~~mitipivat~~) and consequential change in the effect of the first drug on the subject/patient resulting from administration of a second drug (*e.g.*, a CYP3A4/5 inducer, a CYP3A4/5 inhibitor). The change in concentration and consequential change in effect of the first drug resulting from the second drug typically occurs because of a change in activity or expression level of a detoxification enzyme that acts to clear the first drug from the patient (such as CYP3A4/5), wherein the change in activity or expression level of the detoxification enzyme is caused by the second drug. An increase in activity or expression level of the detoxification enzyme will tend to decrease the concentration of the first drug in the patient and its consequential effect on the patient, whereas a decrease in activity or expression level of the detoxification enzyme will tend to increase the concentration of the first drug in the patient and its consequential effect on the patient.

**[0058]** An “effect of a drug-drug interaction” as used herein refers to a detectable and measurable effect, such as a detectable symptom, on the subject/patient caused by the drug-drug interaction. Where the concentration of the first drug is increased, such as when mitapivat is co-administered with a CYP3A4/5 inhibitor, the “effect” typically refers to undesired phenomena such as an overdose of the first drug, liver failure, kidney failure, and the prevalence of side effects and adverse reactions. Where the concentration of the first drug is decreased, such as when mitapivat is co-administered with a CYP3A4/5 inducer, the “effect” typically refers to a decrease in the therapeutic efficacy of the first drug (*e.g.*, mitapivat). Exemplary effects of a drug-drug interaction as used herein further include headache, insomnia, nausea, viral upper respiratory tract infection, arthralgia, diarrhoea, fatigue, hot flush, influenza, vomiting, cough, dizziness, oropharyngeal pain, pyrexia, back pain, dysmenorrhoea, and gastroenteritis, and dyspepsia. Hypertriglyceridemia, hemolytic anemia, hemolysis, dizziness, etc.

**[0059]** In one embodiment, the present disclosure relates to a method of treating pyruvate kinase deficiency (PKD) in a subject, comprising administering to the subject an effective

amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inhibitor of cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5).

**[0060]** In another embodiment, the present disclosure relates to a method of treating sickle cell disease in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inhibitor of cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5).

**[0061]** In yet another embodiment, the present disclosure relates to a method of treating thalassemia in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inhibitor of cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5) or a p-gp inhibitor. In certain embodiments, the thalassemia is alpha thalassemia. In certain embodiments, the thalassemia is beta thalassemia.

**[0062]** In one embodiment, the method of any one of the suitable preceding embodiments further comprises:

(a) monitoring the subject for a symptom of a drug-drug interaction between mitapivat or a pharmaceutically acceptable salt thereof and the CYP3A4/5 inhibitor; and

(b) in the event that a symptom of the drug-drug interaction is present, adjusting the effective amount of mitapivat or a pharmaceutically acceptable salt thereof and/or the effective amount of the CYP3A4/5 inhibitor being administered to the subject to reduce the effect.

**[0063]** In some embodiments, the adjustment is to reduce the dose of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the adjustment is to reduce the dose frequency of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the adjustment is to reduce the dose and dose frequency of mitapivat or a pharmaceutically acceptable salt thereof. Details of the dose and/or dose frequency adjustment are shown in the dose and dose regime section.

**[0064]** In one embodiment, the method of the preceding embodiment further comprises:

(c) repeating [(a) and (b)] until there are no further detectable symptoms of the drug-drug interaction.

Likewise, steps (a) and (b) can be repeated as many times as necessary until there are no further detectable symptoms of the drug-drug interaction. Ways of monitoring the subject for a symptom of a drug-drug interaction between mitapivat or a pharmaceutically acceptable salt thereof and the CYP3A4/5 inhibitor as recited in step (a) are as described above.

**[0065]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject the effective amount of mitapivat or a pharmaceutically acceptable salt thereof and the effective amount of the CYP3A4/5 inducer (or inhibitor) sequentially.

**[0066]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject the effective amount of mitapivat or a pharmaceutically acceptable salt thereof and the effective amount of the CYP3A4/5 inducer (or inhibitor) concurrently.

**[0067]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject the effective amount of mitapivat or a pharmaceutically acceptable salt thereof and the effective amount of the CYP3A4/5 inducer (or inhibitor) sequentially and concurrently. As used herein, “co-administered” or “in combination with” encompasses administration of mitapivat or a pharmaceutically acceptable salt thereof and the CYP3A4/5 inducer (or inhibitor) sequentially and concurrently, or at different intervals. In another embodiment, for the method of any one of the embodiments, the CYP3A4/5 inducer is a mild CYP3A4/5 inducer. In another embodiment, for the method of any one of the embodiments, the CYP3A4/5 inhibitor is a mild CYP3A4/5 inhibitor. In another embodiment, for the methods of any one of the embodiments, the CYP3A4/5 inhibitor is a moderate CYP3A4/5 inhibitor.

**[0068]** In another aspect, the present disclosure provides methods of treating a disease, disorder or a condition with [[ ]]mitapivat in the absence of a CYP3A4/5 inducer or a CYP3A4/5 inhibitor or a p-gp inhibitor; use of mitapivat in the absence of a CYP3A4/5 inducer or a CYP3A4/5 inhibitor or a p-gp inhibitor for the manufacture of a medicament for treating disease, disorder or a condition; and mitapivat in the absence of a CYP3A4/5 inducer or a CYP3A4/5 inhibitor or a p-gp inhibitor for use in treating a disease, disorder or a condition.

**[0069]** As used herein, “in the absence of” when referring to a secondary drug co-administered with mitapivat (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor or a p-gp inhibitor), refers to levels of the secondary drug in the subject/patient that are sufficiently low that such that any change in the levels of the detoxification enzymes for mitapivat and the secondary drug results in no detectable symptoms of a drug-drug interaction between mitapivat and the secondary drug.

**[0070]** In one embodiment, the present disclosure relates to a method of treating pyruvate kinase deficiency (PKD) in a subject, comprising administering to the subject ☐ an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of an inducer of cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5).

**[0071]** In another embodiment, the present disclosure relates to a method of treating sickle cell disease in a subject, comprising administering to the subject ☐ an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of an inducer of cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5).

**[0072]** In yet another embodiment, the present disclosure relates to a method of treating thalassemia in a subject, comprising administering to the subject ☐ an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of an inducer of cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5). In some embodiments, the thalassemia is alpha thalassemia. In some embodiments, the thalassemia is beta thalassemia.

**[0073]** In one embodiment, in accordance with the method of any one of the suitable preceding embodiments, administration of a CYP3A4/5 inducer, if any, is terminated at a sufficient time interval prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof such that there is no detectable symptom of a drug-drug interaction ☐ between mitapivat or a pharmaceutically acceptable salt thereof and the CYP3A4/5 inducer. In certain embodiments, the CYP3A4/5 inducer is a strong CYP3A4/5 inducer. In certain embodiments, the CYP3A4/5 inducer is a moderate CYP3A4/5 inducer. In some embodiments, the CYP3A4/5 inducer is terminated at least 30 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inducer is terminated at least 28 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inducer is terminated at least 25 days prior to

the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inducer is terminated at least 21 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inducer is terminated at least 15 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inducer is terminated at least 14 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inducer is terminated at least 10 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inducer is terminated at least 7 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inducer is terminated at least 5 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof.

**[0074]** In one embodiment, the present disclosure relates to a method of treating pyruvate kinase deficiency (PKD) in a subject, comprising administering to the subject ☐ an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of an inhibitor of cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5).

**[0075]** In another embodiment, the present disclosure relates to a method of treating sickle cell disease in a subject, comprising administering to the subject ☐ an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of an inhibitor of cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5).

**[0076]** In yet another embodiment, the present disclosure relates to a method of treating thalassemia in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of an inhibitor of cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5). In certain embodiment, the thalassemia is alpha-thalassemia. In certain embodiment, the thalassemia is beta-thalassemia.

**[0077]** In one embodiment, in accordance with the method of any one of the suitable preceding embodiments, administration of a CYP3A4/5 inhibitor, if any, is terminated at a sufficient time interval prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof such that there is no detectable symptom of a drug-

drug interaction between mitapivat or a pharmaceutically acceptable salt thereof and the CYP3A4/5 inhibitor. In certain embodiments, the CYP3A4/5 inhibitor is a strong CYP3A4/5 inhibitor. In certain embodiments, the CYP3A4/5 inhibitor is a moderate CYP3A4/5 inhibitor. In some embodiments, the CYP3A4/5 inhibitor is terminated at least 21 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inhibitor is terminated at least 15 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inhibitor is terminated at least 14 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inhibitor is terminated at least 10 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inhibitor is terminated at least 7 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inhibitor is terminated at least 5 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inhibitor is terminated at least 3 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inhibitor is terminated at least 2 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inhibitor is terminated at least 1 day prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof.

**[0078]** In one embodiment, the present disclosure relates to a method of treating pyruvate kinase deficiency (PKD) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of a p-gp inhibitor.

**[0079]** In another embodiment, the present disclosure relates to a method of treating sickle cell disease in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of a p-gp inhibitor.

**[0080]** In yet another embodiment, the present disclosure relates to a method of treating thalassemia in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of a p-gp inhibitor. In

certain embodiment, the thalassemia is alpha-thalassemia. In certain embodiment, the thalassemia is beta-thalassemia.

**[0081]** In one embodiment, in accordance with the method of any one of the suitable preceding embodiments, administration of a p-gp inhibitor, if any, is terminated at a sufficient time interval prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof such that there is no detectable symptom of a drug-drug interaction between mitapivat or a pharmaceutically acceptable salt thereof and the p-gp inhibitor. In certain embodiments, the p-gp inhibitor is a strong p-gp inhibitor ([e.g.] e.g., amiodarone, azithromycin, captopril, carvedilol, clarithromycin, conivaptan, cyclosporine, diltiazem, dronedarone, erythromycin, felodipine, itraconazole, ketoconazole, lopinavir, ritonavir, quercetin, quinidine, ranolazine, ticagrelor, and erapamil). In some embodiments, the p-gp inhibitor is terminated at least 21 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the p-gp inhibitor is terminated at least 15 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the p-gp inhibitor is terminated at least 14 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the p-gp inhibitor is terminated at least 10 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the p-gp inhibitor is terminated at least 7 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the p-gp inhibitor is terminated at least 5 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the p-gp inhibitor is terminated at least 3 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the p-gp inhibitor is terminated at least 2 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the p-gp inhibitor is terminated at least 1 day prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof.

**[0082]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject [ ] about 1 mg to about 2500 mg, or about 5 mg to

2500 mg, or about 30 mg to 2500 mg, or about 5 mg to about 700 mg, or about 15 mg to about 700 mg, about 5 mg to about 360 mg, about 5 mg to about 300 mg, about 50 mg to about 300 mg, about 120 mg to about 360 mg, about 120 mg to about 300 mg, about 5 mg to about 120 mg, about 5 mg, about 20 mg, about 50 mg, about 100 mg, about 120 mg , about 200 mg, about 300 mg, or about 360 mg of mitapivat or a pharmaceutically acceptable salt thereof.

**[0083]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject [[ ]]about 1 mg to about 2500 mg of mitapivat or a pharmaceutically acceptable salt thereof.

**[0084]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject [[ ]]about about 5 mg to 2500 mg of mitapivat or a pharmaceutically acceptable salt thereof.

**[0085]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject [[ ]]about 5 mg to about 360 mg of mitapivat or a pharmaceutically acceptable salt thereof.

**[0086]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject [[ ]]about 5 mg to about 300 mg of mitapivat or a pharmaceutically acceptable salt thereof.

**[0087]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject [[ ]]about 50 mg to about 300 mg of mitapivat or a pharmaceutically acceptable salt thereof.

**[0088]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject about 5 mg of mitapivat or a pharmaceutically acceptable salt thereof.

**[0089]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject about 20 mg of mitapivat or a pharmaceutically acceptable salt thereof.

**[0090]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject about 50 mg of mitapivat or a pharmaceutically acceptable salt thereof.

**[0091]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject about 100 mg of mitapivat or a pharmaceutically acceptable salt thereof.

**[0092]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject the effective amount of mitapivat or a pharmaceutically acceptable salt thereof once daily.

**[0093]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject the effective amount of mitapivat or a pharmaceutically acceptable salt thereof twice daily.

**[0094]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject a sulfate salt of mitapivat, such as a sulfate salt of mitapivat having the structural formula depicted in the background of the present disclosure.

**[0095]** In one embodiment, in the method of any one of the suitable preceding embodiments, the CYP3A4/5 inducer is a strong inducer of CYP3A4/5.

**[0096]** In one embodiment, in the method of any one of the suitable preceding embodiments, the CYP3A4/5 inducer is a moderate inducer of CYP3A4/5.

**[0097]** In one embodiment, in the method of any one of the suitable preceding embodiments, the CYP3A4/5 inducer is a mild inducer of CYP3A4/5.

**[0098]** In one embodiment, in the method of any one of the suitable preceding embodiments, the inducer of CYP3A4/5 is a strong CYP3A4/5 inducer selected from a group consisting of apalutamide, enzalutamide, mitotane, phenytoin, efavirenz, nevirapine, carbamazepine, glucocorticoids, modafinil, oxcarbazepine, phenobarbital, henytoin, pioglitazone, rifabutin, rifampin, St. John's Wort, and troglitazone.

**[0099]** In one embodiment, in the method of any one of the suitable preceding embodiments, the inducer of CYP3A4/5 is a moderate CYP3A4/5 inducer selected from a group consisting of bosentan, etravirine, phenobarbital, and primidone.

**[00100]** In one embodiment, in the method of any one of the suitable preceding embodiments, the inducer of CYP3A4/5 is a mild CYP3A4/5 inducer selected from a group consisting of armodafinil, modafinil, and rufinamide.

**[00101]** In one embodiment, in the method of any one of the suitable preceding embodiments, the CYP3A4/5 inducer is selected from the group consisting of aminoglutethimide, bexarotene, bosentan, carbamazepine, dexamethasone, efavirenz, fosphenytoin, griseofulvin, modafinil, nafcillin, nevirapine, oxcarbazepine, phenobarbital, phenytoin, primidone, rifabutin, rifampin, rifapentine, St. John's wort, avasimibe, dabrafenib, mifepristone, etravirine, armodafinil, glycerol phenylbutyrate, asunaprevir, rifunamide, oritavancin, clobazam, felbamate, eslicarbazepine acetate, and genistein.

**[00102]** In one embodiment, in the method of any one of the suitable preceding embodiments, the CYP3A4/5 inducer is rifampin. In one aspect, rifampin is administered to the subject in the form of a capsule composition. In one aspect, about 0.1-1000 mg of rifampin is administered to the subject. In one aspect, about 100-1000 mg of rifampin is administered to the subject.

**[00103]** In one embodiment, the method of any one of the suitable preceding embodiments comprises administering about 600 mg of rifampin to the subject. In one aspect, the method of any one of the suitable preceding embodiments comprises administering about 600 mg of rifampin to the subject once daily.

**[00104]** In one embodiment, in the method of any one of the suitable preceding embodiments, the inhibitor of CYP3A4/5 is a strong inhibitor of CYP3A4/5.

**[00105]** In one embodiment, in the method of any one of the suitable preceding embodiments, the inhibitor of CYP3A4/5 is a moderate inhibitor of CYP3A4/5.

**[00106]** In one embodiment, in the method of any one of the suitable preceding embodiments, the inhibitor of CYP3A4/5 is a mild inhibitor of CYP3A4/5.

**[00107]** In one embodiment, in the method of any one of the suitable preceding embodiments, the inhibitor of CYP3A4/5 is selected from the group consisting of toamiodarone, amprenavir, aprepitant, atazanavir, chloramphenicol, clarithromycin, conivaptan, cyclosporine, darunavir, dasatinib, delavirdine, diltiazem, erythromycin, fluconazole, fluoxetine, fluvoxamine, fosamprenavir, imatinib, indinavir, isoniazid, itraconazole, ketoconazole, lapatinib, miconazole, nefazodone, nelfinavir, posaconazole, ritonavir, quinupristin, saquinavir, tamoxifen, telithromycin, troleandomycin, verapamil, voriconazole, grapefruit, Seville oranges, limes, pomelos, fruit juices, and vegetables from mustard green family.

**[00108]** In one embodiment, in the method of any one of the suitable preceding embodiments, the inhibitor of CYP3A4/5 is a strong CYP3A4/5 inhibitor selected from a group consisting of boceprevir, cobicistat, danoprevir, elvitegravir, ritonavir, grapefruit juice, indinavir, itraconazole, ketoconazole, lopinavir, paritaprevir, ombitasvir, dasabuvir, posaconazole, saquinavir, telaprevir, tipranavir, telithromycin, troleandomycin, nelfinavir, clarithromycin, nefazodone, saquinavir, suboxone, and voriconazole.

**[00109]** In one embodiment, in the method of any one of the suitable preceding embodiments, the inhibitor of CYP3A4/5 is a moderate CYP3A4/5 inhibitor selected from a group consisting of aprepitant, ciprofloxacin, conivaptan, crizotinib, cyclosporine, diltiazem, dronedarone, erythromycin, fluconazole, fluvoxamine, imatinib, tofisopam, and verapamil.

**[00110]** In one embodiment, in the method of any one of the suitable preceding embodiments, the inhibitor of CYP3A4/5 is a mild CYP3A4/5 inhibitor selected from a group consisting of chlorzoxazone, cilostazol, cimetidine, clotrimazole, fosaprepitant, istradefylline, ivacaftor, lomitapide, ranitidine, ranolazine, ticagrelor.

**[00111]** As used herein, the terms “weak inhibitor” and ‘mild inhibitor” are used interchangeably.

**[00112]** As used herein, the terms “weak inducer” and ‘mild inducer” are used interchangeably.

**[00113]** In one embodiment, in the method of any one of the suitable preceding embodiments, the inhibitor of CYP3A4/5 is itraconazole. In one aspect, about 0.1-1000 mg of itraconazole is administered to the subject. In one aspect, itraconazole is administered in the

form of an oral solution. In one aspect, about 50-500 mg of itraconazole is administered to the subject.

**[00114]** In one embodiment, the method of any one of the suitable preceding embodiments comprises administering about 200 mg of itraconazole to the subject. In one aspect, about 0.1-1000 mg of itraconazole is administered to the subject. In one aspect, about 50-500 mg of itraconazole is administered to the subject. In one aspect, about 200 mg of itraconazole is administered to the subject. In one aspect, about 200 mg of itraconazole is administered to the subject once daily. In one aspect, about 100 mg of itraconazole is administered to the subject. In one aspect, about 100 mg of itraconazole is administered to the subject once daily. In one aspect, itraconazole is administered in the form of an oral solution.

#### **Diseases, Disorders and Conditions**

**[00115]** Pyruvate kinase deficiency (PKD) is a glycolytic enzymopathy that results in life-long hemolytic anemia. In certain embodiments, the subject having PKD is a patient having at least 2 mutant alleles in PKLR gene. In certain embodiments, the subject having PKD is a patient having at least 2 mutant alleles in PKLR gene and at least one is a missense mutation. See Canu. et al., Blood Cells, Molecules and Diseases **2016**, 57, pp. 100-109. In certain embodiments, a subject having PKD has an Hb concentration less than or equal to 10.0 g/dL. In certain embodiments, the subject having PKD is an adult not under regular transfusion ([e.g.] *e.g.*, having had no more than 4 transfusion episodes in the 12-month period up to the treatment). In certain embodiments, the subject having PKD is an adult transfusion independent ([e.g.] *e.g.*, having no more than 3 units of RBCs transfused in the 12-month period prior to the treatment). In certain embodiments, the subject having PKD is an adult under regular transfusion ([e.g.] *e.g.*, having had at least 4 transfusion episodes ([e.g.] *e.g.*, at least 6 transfusion episodes) in the 12-month period prior to the treatment). In certain embodiments, the subject having PKD has a total number of at least 5 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 10 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 15 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 20 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 25 transfusion episodes

during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 30 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 40 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 50 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 60 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 70 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD is not homozygous for the R479H mutation or does not have 2 non-missense mutations in the PKLR gene. In certain embodiments, the subject having PKD, under regular transfusion, has hemoglobin (Hb)  $\leq 12.0$  g/dL (if male) or  $\leq 11.0$  g/dL (if female), prior to the treatment. In certain embodiments, the subject having PKD, under regular transfusion, has transfusion occurring on average less than or equal to once every three weeks. In certain embodiments, the subject having PKD has received at least 0.8 mg ([e.g.] e.g., at least 1.0 mg) folic acid daily ([e.g.] e.g., for at least 21 days) prior to the treatment. In certain embodiments, the subject with PKD under regular transfusion achieves a reduction in transfusion burden ([e.g.] e.g., at least 33% reduction in the number of RBC units transfused) during the 5 weeks, 10 weeks, 15 weeks, 20 weeks, or 24 weeks, 28 weeks, or 32 weeks of treatment. In certain embodiments, the subject having PKD, not under regular transfusion (having had no more than 4 transfusion episodes in the 12-month period prior to the treatment and/or no transfusion in the 3 months prior to the treatment), has hemoglobin (Hb)  $\leq 10.0$  g/dL regardless of gender prior to the treatment. In certain embodiments, the subject having PKD has undergone splenectomy. In certain embodiment, the subject having PKD is a child 18 years old or younger. In certain embodiments, the child is transfusion dependent.

**[00116]** Exemplified conditions related to PKD include, but are not limited to, anemias, cholecystolithiasis, gallstones, tachycardia, hemochromatosis, icteric sclera, splenomegaly, leg ulcers, jaundice, fatigue, and shortness of breath. As described herein, PKD is a deficiency of PKR. In certain embodiments, the deficiency of PKR is associated with a PKR mutation.

**[00117]** In certain embodiments, the subject with PKD achieves a hemoglobin response of at least 1.0 g/dL increase in Hb concentration after the treatment compared to the baseline of

prior to the treatment. In certain embodiments, the subject with PKD achieves a hemoglobin response of at least 1.5 g/dL increase in Hb concentration from baseline prior to the treatment. In certain embodiments, the subject with PKD achieves a hemoglobin response of at least 2.0 g/dL increase in Hb concentration from baseline prior to the treatment.

**[00118]** In an embodiment, the mutant PKR is selected from the group consisting of A31V, A36G, G37Q, R40W, R40Q, L73P, S80P, P82H, R86P, I90N, T93I, G95R, M107T, G111R, A115P, S120F, H121Q, S130P, S130Y, V134D, R135D, A137T, G143S, I153T, A154T, L155P, G159V, R163C, R163L, T164N, G165V, L167M, G169G, E172Q, W201R, I219T, A221Y, D221N, G222A, I224T, G232C, N253D, G263R, G263W, E266K, V269F, L272V, L272P, G275R, G275R, E277K, V280G, D281N, F287V, F287L, V288L, D293N, D293V, A295I, A295V, I310N, I314T, E315K, N316K, V320L, V320M, S330R, D331N, D331G, D331E, G332S, V335M, A336S, R337W, R337P, R337Q, D339N, D339Q, G341A, G341D, I342F, K348N, A352D, I357T, G358R, G358E, R359C, R359H, C360Y, N361D, G364D, K365M, V368F, T371I, L374P, S376I, T384M, R385W, R385K, E387G, D390N, A392T, N393D, N393S, N393K, A394S, A394D, A394V, V395L, D397V, G398A, M403I, G406R, E407K, E407G, T408P, T408A, T408I, K410E, G411S, G411A, Q421K, A423A, A423A, R426W, R426Q, E427A, E427N, A431T, R449C, I457V, G458D, A459V, V460M, A468V, A468G, A470D, T477A, R479C, R479H, S485F, R486W, R486L, R488Q, R490W, I494T, A495T, A495V, R498C, R498H, A503V, R504L, Q505E, V506I, R510Q, G511R, G511E, R518S, R531C, R532W, R532Q, E538D, G540R, D550V, V552M, G557A, R559G, R559P, N566K, M568V, R569Q, R569L, Q58X, E174X, W201X, E241X, R270X, E440X, R486X, Q501X, L508X, R510X, E538X, R559X. These mutations are described in Canu et al., *Blood Cells, Molecules and Diseases* **2016**, 57, pp. 100-109. In an embodiment, the mutant PKR is selected from G332S, G364D, T384M, K410E, R479H, R479K, R486W, R532W, R510Q, and R490W. In certain embodiments, the mutant PKR is selected from A468V, A495V, I90N, T408I, and Q421K, and R498H. In certain embodiments, the mutant PKR is R532W, K410E, or R510Q. In certain embodiments, the mutant PKR is R510Q, R486W, or R479H.

**[00119]** In other aspects, provided are methods of treating a disease selected from hemolytic anemia, hereditary spherocytosis, hereditary elliptocytosis, abetalipoproteinemia, Bassen-Kornzweig syndrome, and paroxysmal nocturnal hemoglobinuria in a subject in need thereof, comprising administering to the subject an effective amount of mitapivat or a

pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor). Also provided is mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor), for use in treating disease selected from hemolytic anemia, hereditary spherocytosis, hereditary elliptocytosis, abetalipoproteinemia, Bassen-Kornzweig syndrome, and paroxysmal nocturnal hemoglobinuria in a subject. Further provided is the use of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor), in the manufacture of a medicament for treating a disease selected from hemolytic anemia, hereditary spherocytosis, hereditary elliptocytosis, abetalipoproteinemia, Bassen-Kornzweig syndrome, and paroxysmal nocturnal hemoglobinuria in a subject in need thereof. In one aspect, the disease to be treated is hemolytic anemia.

**[00120]**   Thalassemia is an inherited blood disorder in which the normal ratio of  $\alpha$ - to  $\beta$ -globin production is disrupted due to a disease-causing variant in 1 or more of the globin genes. In certain embodiments, Alpha-globin aggregates (as found in  $\beta$ -thalassemia) readily precipitate, which disrupts the red blood cell (RBC) membrane and results in oxidative stress. In certain embodiments, Beta-globin tetramers (Hb H, found in  $\alpha$ -thalassemia) are generally more soluble, but are still unstable and can form precipitates. The imbalance of the globin chain synthesis can lead to a net reduction in Hb concentrations and has dramatic effects on the survival of RBC precursors, ultimately resulting in their premature destruction in the bone marrow and in extramedullary sites (Cappellini et al., 2014). In certain embodiments, the disorder results in large numbers of red blood cells being destroyed, which leads to anemia. In certain embodiments, the thalassemia is alpha thalassemia. In certain embodiments, the thalassemia is beta thalassemia. In other embodiments, the thalassemia is non-transfusion-dependent thalassemia[[ (]]. In other embodiments, the thalassemia is beta thalassemia intermedia. In other embodiments, the thalassemia is Hb E beta thalassemia. In other embodiments, the thalassemia is beta thalassemia with mutations of 1 or more alfa genes.

**[00121]**   In certain embodiments, the subject is an adult subject with thalassemia. In certain embodiments, the subject has thalassemia such as  $\beta$ -thalassemia intermedia, Hb E  $\beta$ -thalassemia,  $\alpha$ -thalassemia (Hb H disease), or  $\beta$ -thalassemia with mutations of 1 or more  $\alpha$  genes. In certain embodiments, the subject has non-transfusion-dependent thalassemia. In

certain embodiments, the subject has non-transfusion-dependent alpha thalassemia. In certain embodiments, the subject has beta-thalassemia or non-transfusion-dependent beta-thalassemia. In certain embodiments, the subject is an adult male subject with thalassemia such as beta-thalassemia or non-transfusion-dependent thalassemia. In certain embodiments, the subject is a female subject with thalassemia such as beta-thalassemia or non-transfusion-dependent thalassemia. In certain embodiments, the subject is an adult female subject with thalassemia such as beta-thalassemia or non-transfusion-dependent thalassemia. In certain embodiments, the subject has a hemoglobin concentration of less than or equal to 6.0 g/dL. In certain embodiments, the subject has a hemoglobin concentration of less than or equal to 7.0 g/dL. In certain embodiments, the subject has a hemoglobin concentration of less than or equal to 8.0 g/dL. In certain embodiments, the subject has a hemoglobin concentration of less than or equal to 9.0 g/dL. In certain aspects, the subject having non-transfusion-dependent thalassemia does not have a known history ([e.g.] e.g., has been diagnosed in the past) of Hb S or Hb C forms of thalassemia. In certain embodiments, the term “non-transfusion dependent” thalassemia refers to subjects with thalassemia having no more than 4 ([e.g.] e.g., five) units of RBCs transfused during a 24-week period up to the first day of administration of mitapivat and/or no RBC transfusions in the 8 weeks prior to the first day of administration of mitapivat. In certain embodiment, the subject is an adult over 18 years old. In certain embodiment, the subject is a child 18 years old or younger.

**[00122]** As used herein, sickle cell disease (SCD), Hemoglobin SS disease, and sickle cell anemia are used interchangeably. Sickle cell disease (SCD) is an inherited blood disorder caused by the presence of sickle hemoglobin (HbS). In certain embodiments, subjects with SCD have abnormal hemoglobin, called hemoglobin S or sickle hemoglobin, in their red blood cells. In certain embodiments, people having SCD have at least one abnormal gene ~~genes~~ causing the body to make hemoglobin S. In certain embodiments, people having SCD have two hemoglobin S genes, Hemoglobin SS.

**[00123]** In other aspects, provided herein are methods for treating anemia in a subject in need thereof comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug ([e.g.] e.g., a CYP3A4/5 inducer or a CYP3A4/5 inhibitor). Also provided is mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug ([e.g.] e.g., a

CYP3A4/5 inducer or a CYP3A4/5 inhibitor), for use in treating anemia in a subject in need thereof. Further provided is the use of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug ([*e.g.*]*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor), in the manufacture of a medicament for treating anemia. In one aspect, the anemia to be treated is dyserythropoietic anemia.

**[00124]** In certain embodiments, the anemia is a dyserythropoietic anemia such as congenital dyserythropoietic anemia type I, II, III, or IV. In certain embodiments, the anemia is hemolytic anemia. In certain embodiments, the hemolytic anemia is a congenital and/or hereditary form of hemolytic anemia such as PKD, sickle cell disease, thalassemias (*e.g.*, alpha or beta or non-transfusion-dependent thalassemia), hereditary spherocytosis, hereditary elliptocytosis), paroxysmal nocturnal hemoglobinuria, abeta-lipoproteinemia (Bassen-Kornzweig syndrome). In certain embodiments, the hemolytic anemia is acquired hemolytic anemia such as autoimmune hemolytic anemia, drug-induced hemolytic anemia. In certain embodiments, the hemolytic anemia is anemia as part of a multi-system disease, such as the anemia of Congenital Erythropoietic Purpura, Fanconi, Diamond-Blackfan.

**[00125]** As used herein, the term “anemia” refers to a deficiency of red blood cells (RBCs) and/or hemoglobin. As used herein, anemia includes all types of clinical anemia, for example (but not limited to): microcytic anemia, iron deficiency anemia, hemoglobinopathies, heme synthesis defect, globin synthesis defect, sideroblastic defect, normocytic anemia, anemia of chronic disease, aplastic anemia, hemolytic anemia, macrocytic anemia, megaloblastic anemia, pernicious anemia, dimorphic anemia, anemia of prematurity, Fanconi anemia, hereditary spherocytosis, sickle cell disease, warm autoimmune hemolytic anemia, cold agglutinin hemolytic anemia, osteopetrosis, thalassemia, and myelodysplastic syndrome.

**[00126]** In certain embodiments, anemia can be diagnosed on a complete blood count. In certain embodiments, anemia can be diagnosed based on the measurement of one or more markers of hemolysis (*e.g.*, RBC count, hemoglobin, reticulocytes, schistocytes, lactate Dehydrogenase (LDH), haptoglobin, bilirubin, and ferritin) and/or hemosiderinuria mean corpuscular volume (MCV) and/or red cell distribution width (RDW). In the context of the present invention, anemia is present if an individual has a hemoglobin (Hb) less than the desired level, for example, the Hb concentration of less than 14 g/dL, more preferably of less than 13 g/dL, more preferably of less than 12 g/dL, more preferably of less than 11 g/dL, or most preferably of less than 10 g/dL.

**[00127]** In certain embodiments, provided herein is a method of increasing the amount of hemoglobin in a subject by administering an effective amount of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug ([e.g.], e.g., a CYP3A4/5 inducer or a CYP3A4/5 inhibitor). In certain embodiments, also provided herein is a method of increasing the amount of hemoglobin in a subject having thalassemia comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug ([e.g.], e.g., a CYP3A4/5 inducer or a CYP3A4/5 inhibitor). Further provided is a method of increasing the amount of hemoglobin in subjects having non-transfusion-dependent thalassemia comprising administering an effective amount of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug ([e.g.], e.g., a CYP3A4/5 inducer or a CYP3A4/5 inhibitor) to the subject. In certain embodiments, the provided methods increase hemoglobin concentration in the subject. In certain embodiments, the provided methods increase Hb concentration to a desired level, for example, above 10 g/dL, more preferably above 11 g/dL, more preferably above 12 g/dL, more preferably above 13 g/dL, or most preferably above 14 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 0.5 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 1.0 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 1.5 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 2.0 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 2.5 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 3.0 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 3.5 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 4.0 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 4.5 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 5.0 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 5.5 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 6.0 g/dL. In certain embodiments, the increase in Hb concentration is determined from baseline at one or more assessment between week 1 and week 20 ([e.g.], e.g., between week 2 and week 15, between week 3 and week 15, and between week 4 and week 12) of treatment with an effective amount of mitapivat or a pharmaceutically acceptable salt thereof, in combination

with or in the absence of an effective amount of a secondary drug ([*e.g.*]*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor). In certain embodiments, the provided methods increase Hb concentration as described above in female subjects having thalassemia ([*e.g.*]*e.g.*, beta-thalassemia or non-transfusion-dependent thalassemia). In certain embodiments, the provided methods increase Hb concentration from baseline to about 12 g/dL in female subjects having thalassemia ([*e.g.*]*e.g.*, beta-thalassemia or non-transfusion-dependent thalassemia). In certain embodiments, the provided methods increase Hb concentration as described above in male subjects having thalassemia ([*e.g.*]*e.g.*, beta-thalassemia or non-transfusion-dependent thalassemia). In certain embodiments, the provided methods increase Hb concentration from baseline to about 13 g/dL in male subjects having thalassemia ([*e.g.*]*e.g.*, beta-thalassemia or non-transfusion-dependent thalassemia).

**[00128]** In some aspects, provided herein are methods for treating hemolytic anemia in a subject in need thereof comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug ([*e.g.*]*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor). Also provided is mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug ([*e.g.*]*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor), for use in treating hemolytic anemia in a subject in need thereof. Further provided is the use of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug ([*e.g.*]*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor), in the manufacture of a medicament for treating hemolytic anemia. In one aspect, the hemolytic anemia to be treated is hereditary and/or congenital hemolytic anemia, acquired hemolytic anemia, or anemia as part of a multi-system disease.

**[00129]** In some aspects, provided herein are methods for activating wild-type or mutant PKR in red blood cells in a subject in need thereof comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug ([*e.g.*]*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor). Also provided is mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug ([*e.g.*]*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor), for use in activating wild-type or mutant PKR in red blood cells in a subject in need thereof. Further

provided is the use of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*[[e.g.]]e.g.,* a CYP3A4/5 inducer or a CYP3A4/5 inhibitor), in the manufacture of a medicament for activating wild-type or mutant PKR in red blood cells.

**[00130]** Mitapivat is an activator of PKR mutants having lower activities compared to the wild type, thus are useful for methods of the present disclosure. Such mutations in PKR can affect enzyme activity (catalytic efficiency), regulatory properties (modulation by fructose biphosphate (FBP)/ATP), and/or thermostability of the enzyme. Examples of such mutations are described in Valentini et al., JBC 2002. Some examples of the mutants that are activated by the compounds described herein include G332S, G364D, T384M, R479H, R479K, R486W, R532W, R510Q, and R490W. Without being bound by theory, in certain embodiments, mitapivat affects the activities of PKR mutants by activating FBP non-responsive PKR mutants, restoring thermostability to mutants with decreased stability, or restoring catalytic efficiency to impaired mutants.

**[00131]** In certain embodiments, mitapivat increases the affinity of PKR to phosphoenolpyruvate (PEP). In certain embodiments, mitapivat restores the ability of RBCs to cover PEP and ADP to pyruvate and ATP.

**[00132]** In certain embodiments, provided herein are methods of reducing transfusion frequency of a subject with PKD comprising administering to the subject mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*[[e.g.]]e.g.,* a CYP3A4/5 inducer or a CYP3A4/5 inhibitor). In certain embodiments, the transfusion frequency is reduced by at least 5% in the number of RBC units transfused over at least 15 weeks. In certain embodiments, the transfusion frequency is reduced by at least 10% in the number of RBC units transfused over at least 15 weeks. In certain embodiments, the transfusion frequency is reduced by at least 15% in the number of RBC units transfused over at least 15 weeks. In certain embodiments, the transfusion frequency is reduced by at least 20% in the number of RBC units transfused over at least 15 weeks. In certain embodiments, the transfusion frequency is reduced by at least 25% in the number of RBC units transfused over at least 15 weeks. In certain embodiments, the transfusion frequency is reduced by at least 30% in the number of RBC units transfused over at least 15 weeks. In certain embodiments, the transfusion frequency is reduced by at least 35% in the number of RBC units transfused over at least 15 weeks. In

certain embodiments, the transfusion frequency is reduced by at least 40% in the number of RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 5% in the number of RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 10% in the number of RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 15% in the number of RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 20% in the number of RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 25% in the number of RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 30% in the number of RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 35% in the number of RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 40% in the number of RBC units transfused over at least 20 weeks.

**[00133]** In some aspects, provided herein are methods of evaluating the efficacy of mitapivat therapy (monotherapy or concomitant medications with a CYP3A4/5 inducer or a CYP3A4/5 inhibitor) in a subject, the method comprising: administering to the subject mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug ([*e.g.*] *e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor); and acquiring a value for the level of mitapivat, the level of 2,3-diphosphoglycerate (2,3-DPG), the level of adenosine triphosphate (ATP), or the activity of PKR in the subject, to thereby evaluate the efficacy of the mitapivat therapy. In some aspects, the value for the level is acquired by analyzing the plasma concentration of mitapivat and optionally, the metabolite known to be formed by CYP3A4/5. In some aspects, the level of 2,3-DPG is acquired by analyzing the blood concentration of 2,3-DPG. In some aspects, the level of ATP is acquired by analyzing the blood concentration of ATP. In some aspects, the activity of PKR is acquired by analyzing the blood concentration of a <sup>13</sup>C-label in the blood. In some aspects, the analysis is performed by sample analysis of bodily fluid. In some aspects, the bodily fluid is blood. In some aspects, the analysis is performed by mass spectroscopy. In some aspects, the analysis is performed by LC-MS.

**[00134]** In some aspects, provided herein are methods of treating a subject, the method comprising: administering to the subject an effective amount of mitapivat or a

pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug ([*e.g.*]*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor); and acquiring a value for the level of mitapivat and optionally its metabolite known to be formed by CYP3A4/5, the level of 2,3-diphosphoglycerate (2,3-DPG), the level of adenosine triphosphate (ATP), or the activity of PKR in the subject, to thereby treat the subject.

### EXEMPLIFICATIONS

[00135] As depicted in the Examples below, the drug-drug interactions between mitapivat sulfate and a CYP3A4/5 inducer ([*e.g.*]*e.g.*, rifampin) or a CYP3A4/5 inhibitor ([*e.g.*]*e.g.*, itraconazole) are studied. Mitapivat can be synthesized using the procedures described in International Patent Application Publication Nos. WO 2011/002817 and WO 2016/201227.

#### **Example 1: Mitapivat sulfate is primarily metabolized by CYP3A4**

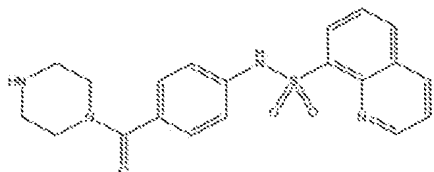
[00136] CYP-enzyme phenotyping using human liver microsomes and recombinant CYP enzymes revealed that mitapivat sulfate was primarily metabolized by CYP3A4/5 (>90%), with minor contributions from CYP2C9, CYP2C8, and CYP1A2. There was evidence of metabolism-dependent inhibition of CYP2C19 (largely reversible) and CYP3A4 (largely irreversible). Mitapivat sulfate was found to be a substrate and inhibitor for P-gp but not for breast cancer resistance protein (BCRP) and was found to be a potential inducer of human CYP2B6 and CYP3A4. Mitapivat sulfate appeared to be a mild inhibitor of CYP2C8, CYP2C9, xYP2C19, CYP2D6, and CYP3A4/5 enzymes (testosterone 6 $\beta$ -hydroxylation), bile salts export pump (BSEP), organic anion transporting polypeptide (OATP)1B1, organic anion transporter (OAT)3, and organic cation transporter (OCT)2, and of uridine-5'-diphosphoglucuronosyltransferase (UGT) 1A3, 1A4, and 1A9. Mitapivat sulfate does not appear to be an inhibitor of multidrug resistance-associated protein (MRP)2, MRP3, OATP1B3, and OAT1. The metabolite was found to be a mild inhibitor of CYP2C9 and CYP2C19 and was not an inhibitor of P-gp or BCRP under tested concentrations.

#### **Example 2: Drug-drug Interaction (DDI) Studies of Mitapivat Sulfate and a CYP3A4 Inducer or a CYP3A4 Inhibitor**

**[00117]** Twenty-eight (28) healthy, adult male and female (non-childbearing potential) subjects were enrolled in the study in total; 14 subjects in each study part (Parts 1 and 2). A minimum of 8 female subjects were enrolled in the study (*i.e.*, a minimum of 4 female subjects per study part). Each subject participated in either Part 1 or Part 2, but not both.

### **Part 1**

**[00118]** On Day 1 of Treatment Period 1, a single oral dose of 20 mg mitapivat sulfate was administered. Serial blood samples for plasma assay of mitapivat concentrations and its CYP3A4 metabolite, referred to herein as the “Metabolite” (structure below),



were collected from predose to 120 hours following administration of mitapivat sulfate. In Treatment Period 2, an oral dose of 200 mg itraconazole was administered once daily (QD) for 9 consecutive days (Day 1 through Day 9 of Treatment Period 2) with a single oral dose of 20 mg mitapivat sulfate coadministered on Day 5. Serial blood samples for plasma assay of mitapivat and the Metabolite concentrations were collected from predose to 120 hours following coadministration of mitapivat sulfate and itraconazole on Day 5.

**[00119]** In Treatment Period 1, mitapivat sulfate was administered orally with approximately 240 mL of water. In Treatment Period 2, on Days 1 to 4, itraconazole was administered alone immediately followed by approximately 220 mL of water, and on Day 5, itraconazole was administered first (no water) and was immediately followed by mitapivat sulfate administration with approximately 220 mL of water. Study drugs (mitapivat sulfate and itraconazole) were administered following an overnight fast of at least 10 hours on Day 1 of Treatment Period 1 (mitapivat sulfate only) and Day 5 of Treatment Period 2 (mitapivat sulfate and itraconazole), and subjects remained fasted for 4 hours after dosing. On all other dosing days, itraconazole was administered following a predose fast of at least 4 hours and subjects remained fasted for at least 2 hours after dosing.

### **Part 2**

**[00120]** On Day 1 of Treatment Period 1, a single oral dose of 50 mg mitapivat sulfate was administered. Serial blood samples for plasma assay of mitapivat and the Metabolite concentrations were collected from predose to 120 hours following administration of mitapivat sulfate. In Treatment Period 2, an oral dose of 600 mg rifampin was administered QD for 12 consecutive days (Day 1 through Day 12 of Treatment Period 2) with a single oral dose of 50 mg mitapivat sulfate coadministered on Day 8. Serial blood samples for plasma assay of mitapivat sulfate and the Metabolite concentrations were collected from predose to 120 hours following coadministration of mitapivat and rifampin on Day 8.

**[00121]** In Part 2, study drugs were administered with approximately 240 mL of water on all dosing days including the coadministration of mitapivat sulfate and rifampin on Day 8 of Treatment Period 2. Mitapivat sulfate and rifampin was administered following an overnight fast of at least 10 hours on Day 1 of Treatment Period 1 (mitapivat sulfate only) and Day 8 of Treatment Period 2 (both mitapivat sulfate and rifampin) and subjects remained fasted for 4 hours after dosing. On all other dosing days, rifampin was administered following a predose fast of at least 4 hours and subjects remained fasted for at least 2 hours after dosing. There was a washout period of 7 days between the mitapivat sulfate dose in Treatment Period 1 and the first itraconazole (Part 1) or rifampin (Part 2) dose in Treatment Period 2. All study drugs were consumed within 5 minutes for both Part 1 and Part 2.

## **Test and Reference Products, Dose, Duration and Mode of Administration**

### **Part 1**

**[00122]** Mitapivat sulfate (20 mg tablets) administered along with Itraconazole (also referred to as Sporanox®, 10 mg/mL oral solution, is considered as the test arm in this study. Mitapivat sulfate administered alone is considered as the reference arm in this study.

In Period 1, a single oral dose of 20 mg mitapivat sulfate was administered orally with approximately 240 mL of water. In Period 2, on Day 5 (coadministration of itraconazole and mitapivat sulfate), 200 mg itraconazole (20 mL of 10 mg/mL oral solution) was administered first (no water) and was immediately followed by 20 mg mitapivat sulfate (1 x 20 mg tablet) administration with approximately 220 mL of water. On all other dosing days, itraconazole was administered alone immediately followed by approximately 220 mL of water. Each subject received 2 doses of mitapivat sulfate and 9 doses of itraconazole in Part 1.

### **Part 2**

**[00123]** Mitapivat sulfate (50 mg tablets) administered along with Rifampin (also referred to as Rifadin®, 300 mg capsules) is considered as the test arm in this study. Mitapivat sulfate (50 mg tablets) administered alone is considered as the reference arm in this study.

**[00124]** In Period 1, a single oral dose of 50 mg mitapivat sulfate was administered orally. In Period 2, subjects were administered 600 mg rifampin (2 x 300 mg capsules) QD on Day 1 to Day 12 inclusive, with 50 mg mitapivat sulfate (1 x 50 mg tablet) coadministered on Day 8. Study drugs were administered with approximately 240 mL of water on all dosing days including the coadministration of mitapivat sulfate and rifampin on Day 8 of Period 2.

**[00125]** Each subject received 2 doses of mitapivat sulfate and 12 doses of rifampin in Part 2.

#### **Duration of Treatment:**

**[00126]** The total duration of participation including the screening period for each subject was approximately 49 to 62 days for Part 1 and approximately 61 or 63 days in Part 2 of the study.

**[00127]** In each study part, Treatment Period 1 was approximately 6 days and Treatment Period 2 was approximately 12 days; a washout phase was 7 days between both treatment periods in each study part.

#### **Criteria for Evaluation of Pharmacokinetics**

**[00128]** In Parts 1 and 2, for mitapivat and the Metabolite, blood samples were obtained for all subjects to determine the plasma concentrations of mitapivat, and the Metabolite, before mitapivat sulfate dosing (0 hour) on Day 1 of Period 1 (Parts 1 and 2) and Day 5 (Part 1) or Day 8 (Part 2) of Period 2 at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 16, 24, 48, 72, 96, and 120 hours postdose. Plasma concentrations of mitapivat and the Metabolite were determined using methods validated with respect to accuracy, precision, linearity, sensitivity, and specificity, with a lower limit of quantitation (LLOQ) of 0.5 ng/mL for both analytes. A noncompartmental PK approach was used to analyze individual plasma mitapivat and the Metabolite concentration time data (using Phoenix® WinNonlin® Version 7.0). The

following PK parameters were calculated:  $AUC_{0-t}$ ,  $AUC_{0-inf}$ ,  $AUC_{0-24}$ ,  $AUC_{\%extrap}$ ,  $C_{max}$ ,  $CL/F$  (mitapivat only),  $T_{last}$ ,  $T_{max}$ ,  $t_{1/2}$ , and  $V_z/F$  (mitapivat only).

### Statistical Methods for Pharmacokinetics

**[00129]** The plasma concentrations of mitapivat and the Metabolite were listed and summarized by treatment and collection time for all subjects in the PK Population. Plasma concentrations were presented with the same level of precision as received from the bioanalytical laboratory including sample size (n), arithmetic mean (Mean), standard deviation (SD), coefficient of variation (CV%), standard error of the mean (SEM), minimum, maximum, and median. Concentrations from excluded subjects were included in the plasma concentration tables, but were excluded from the summary statistics and noted as such in the tables. All BLQ values were presented as “BLQ” in the plasma concentration table listings and footnoted accordingly.

**[00130]** PK parameters were presented with a minimum of 3 significant figures. PK parameters were listed by subject, tabulated by treatment, and summarized using descriptive statistics (n, mean, SD, CV%, SEM, minimum, median, maximum, geometric mean (Geom. Mean) and geometric mean coefficient of variation (Geom. CV%). Time-based parameters (*i.e.*,  $T_{max}$  and  $t_{1/2}$ ) and treatment:reference AUC and  $C_{max}$  ratios were presented to 2 decimals. Excluded subjects were included in the PK parameter table listings, but were excluded from the summary statistics and noted as such in the tables.

**[00131]** To evaluate the effect of itraconazole and rifampin on mitapivat and the Metabolite (primary objective), plasma mitapivat and the Metabolite PK parameters ( $C_{max}$ ,  $AUC_{0-t}$ , and  $AUC_{0-inf}$ ) were natural log (ln) transformed before analysis. The ln-transformed value of the PK parameters was analyzed using a linear mixed effect model for each study part separately with treatment as a fixed effect and subject as a random effect. The linear mixed effect model included treatment as a fixed effect and subject as a random effect. The inferential results (least-squares means [LSMs], difference between LSMs, and 90% confidence intervals [CIs] of the difference) were back transformed to the original scale. Geometric LSMs, geometric mean ratios (GMRs), and 90% CIs were presented. The GMR of the LSMs were calculated from the back-transformed difference between the treatment LSMs. The GMRs were expressed as a percentage relative to mitapivat sulfate given alone for both Part 1 and Part 2. The 90% CIs for the ratios were derived by back-transformation of

the CIs obtained for the difference between the treatment LSMs. The CIs were expressed as a percentage relative to mitapivat sulfate given alone for both Part 1 and Part 2.

**[00132]** The median differences in Tmax for evaluation of the effect of itraconazole and rifampin on mitapivat and the Metabolite along with the range of the difference were calculated using the non parametric Wilcoxon Signed Rank test, and the p-value was presented. Note that Tmax was not ln-transformed for these analyses.

## **Pharmacokinetic Results**

### **Part 1**

**[00133]** Mean plasma mitapivat and the Metabolite concentration-time profiles following 20 mg mitapivat sulfate administered alone (Period 1) or coadministered with multiple doses of 200 mg itraconazole (Period 2) on linear and semi-log scales are presented in Figures 1 and 2.

### **Mitapivat**

**[00134]** Mitapivat plasma concentrations were detectable in all subjects at 0.5 hour (the first scheduled postdose sample) in both Periods 1 and 2. Mitapivat was still detectable up to the last sample time at 120 hours postdose in the majority of subjects in Period 1 and in all subjects in Period 2.

**[00135]** Measurable predose mitapivat concentrations were present in all but 2 subjects in Period 2 (two subjects were BLQ). As used herein, BLQ refers to below the limit of quantification. These predose concentrations were < 5% of the Cmax, so PK calculations were performed.

**[00136]** Mitapivat concentrations exhibited a characteristic multi-compartmental pharmacokinetic profile. When coadministered with itraconazole, mitapivat concentrations rose to a higher mean peak with a delay in time to peak by approximately 2 hours compared to when the drug was administered alone. The post-peak decline of the multi-compartmental behavior was slower after coadministration with itraconazole when compared to mitapivat administered alone. Overall, higher mitapivat concentrations were experienced for a longer time period when mitapivat was coadministered with itraconazole.

### **The Metabolite**

[00137] When mitapivat was administered alone, the Metabolite plasma concentrations were detectable in all but 1 subject at 0.5 hour. The Metabolite remained detectable all but 1 subject up to 48 hours postdose, with quantifiable the Metabolite out to 72 hours in 3 subjects and 96 hours in 1 subject. In Period 2 (coadministration with itraconazole), the Metabolite concentrations were delayed relative to administration of mitapivat alone. Concentrations were not measurable in all subjects until 1.5 hours postdose. Maximum concentrations were also reduced. However, the Metabolite residence was prolonged in the presence of itraconazole, with concentrations still detectable in all but 1 subject throughout the 120-hour sampling interval (in 1 subject the last measurable concentration was at 96 hours postdose).

[00138] The statistical comparisons of mitapivat PK parameters following 20 mg mitapivat sulfate administered alone (Period 1) or coadministered with multiple doses of 200 mg itraconazole (Period 2) PK parameters are summarized below in Table 1.

**Table 1**  
**Summary of Statistical Comparisons of Plasma Mitapivat Pharmacokinetic Parameters Following 20 mg Mitapivat Sulfate Co-Administered With Multiple Doses of 200 mg Itraconazole Versus Administered Alone (Part 1)**

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Mitapivat Sulfate + Itraconazole (Test) |    | Mitapivat sulfate alone (Reference) |    |        |                         |                   |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------|----|-------------------------------------|----|--------|-------------------------|-------------------|
| Parameter (unit)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Geometric LSM                           | n  | Geometric LSM                       | n  | GMR    | 90% Confidence Interval | Intra-subject CV% |
| AUC <sub>0-t</sub> (ng*hr/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | 8860                                    | 14 | 1885                                | 14 | 4.6998 | 4.2519 - 5.1949         | 15.05             |
| AUC <sub>0-inf</sub> (ng*hr/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 9475                                    | 14 | 1942                                | 14 | 4.8780 | 4.4154 - 5.3890         | 14.97             |
| C <sub>max</sub> (ng/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | 704.3                                   | 14 | 412.5                               | 14 | 1.7074 | 1.5815 - 1.8433         | 11.48             |
| Mitapivat sulfate alone: 20 mg mitapivat sulfate (1 x 20 mg tablet) at Hour 0 on Day 1 (Period 1)<br>Mitapivat sulfate + itraconazole: 200 mg itraconazole (20 mL of 10 mg/mL oral solution) QD on Days 1 to Day 9 inclusive (within ± 1 hour of dosing time on Day 1) with 20 mg mitapivat sulfate (1 x 20 mg tablet) coadministered at Hour 0 on Day 5 (Period 2)<br>Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the linear mixed effect model.<br>Geometric Mean Ratio (GMR) = test / reference<br>Intra-subject CV% = 100 x (square root (exp[MSE]-1), where MSE = Residual variance from linear mixed effect model. |                                         |    |                                     |    |        |                         |                   |

[00139] The coadministration of 200 mg itraconazole with 20 mg mitapivat sulfate increased mitapivat exposure relative to when mitapivat sulfate was administered alone. The geometric mean AUC<sub>0-t</sub> and AUC<sub>0-inf</sub> ratios of mitapivat in the presence and absence of itraconazole were 4.7 and 4.9, respectively. The geometric mean Cmax ratio of mitapivat in the presence and absence of itraconazole was 1.7. The intra-subject variability was low at approximately 15% for AUCs and 11% for Cmax.

[00140] The statistical comparisons of the Metabolite PK parameters following 20 mg mitapivat sulfate administered alone (Period 1) or coadministered with multiple doses of 200 mg itraconazole (Period 2) PK parameters are summarized below in Table 2.

**Table 2**  
**Summary of Statistical Comparisons of Plasma Metabolite Pharmacokinetic Parameters Following 20 mg Mitapivat Sulfate Co-Administered With Multiple Doses of 200 mg Itraconazole Versus Administered Alone (Part 1)**

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Mitapivat Sulfate + Itraconazole (Test) |    | Mitapivat sulfate alone (Reference) |    |        |                         |                   |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------|----|-------------------------------------|----|--------|-------------------------|-------------------|
| Parameter (unit)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Geometric LSM                           | n  | Geometric LSM                       | n  | GMR    | 90% Confidence Interval | Intra-subject CV% |
| AUC <sub>0-t</sub> (ng*hr/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | 300.0                                   | 14 | 197.1                               | 14 | 1.5218 | 1.4268 - 1.6231         | 9.65              |
| AUC <sub>0-inf</sub> (ng*hr/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 338.6                                   | 14 | 216.2                               | 14 | 1.5661 | 1.4784 - 1.6590         | 8.63              |
| C <sub>max</sub> (ng/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | 9.110                                   | 14 | 18.79                               | 14 | 0.4849 | 0.4441 - 0.5295         | 13.20             |
| Mitapivat sulfate alone: 20 mg mitapivat sulfate (1 x 20 mg tablet) at Hour 0 on Day 1 (Period 1)<br>Mitapivat sulfate + itraconazole: 200 mg itraconazole (20 mL of 10 mg/mL oral solution) QD on Days 1 to Day 9 inclusive (within ± 1 hour of dosing time on Day 1) with 20 mg mitapivat sulfate (1 x 20 mg tablet) coadministered at Hour 0 on Day 5 (Period 2)<br>Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the linear mixed effect model.<br>Geometric Mean Ratio (GMR) = test / reference<br>Intra-subject CV% = 100 x (square root (exp[MSE]-1), where MSE = Residual variance from linear mixed effect model. |                                         |    |                                     |    |        |                         |                   |

[00141] The coadministration of 200 mg itraconazole with 20 mg mitapivat sulfate increased total Metabolite exposure relative to when mitapivat sulfate was administered alone. The geometric mean AUC<sub>0-t</sub> and AUC<sub>0-inf</sub> ratios of the Metabolite in the presence and absence of itraconazole were 1.5 and 1.6, respectively. The geometric mean Cmax ratio of the Metabolite in the presence and absence of itraconazole was 0.5. The intra-subject variability was low at approximately 9% to 10% for AUCs and 13% for Cmax.

**Part 2**

[00142] Mean plasma mitapivat and metabolite the Metabolite concentration-time profiles following 50 mg mitapivat sulfate administered alone (Period 1) or coadministered with multiple doses of 600 mg rifampin (Period 2) on linear and semi-log scales are presented in Figures 3 and 4.

**Mitapivat**

**[00143]** Mitapivat plasma concentrations were detectable in all subjects at 0.5 hour (the 1st scheduled postdose sample) when given alone or with rifampin. Mitapivat was still detectable up to the last sample time at 120 hours postdose in all but 1 subject when given alone had a BLQ concentration at 120 hours). When coadministered with rifampin, detectable mitapivat concentrations were not present in any sample collections from any subject beyond 24 hours postdose (except 1 subject with detectable mitapivat at 48 hours).

**[00144]** Mitapivat concentrations increased to a lower and slightly earlier peak when coadministered with rifampin compared to when administered alone. The peak arithmetic mean mitapivat concentrations were observed at a nominal time of approximately 1 hour postdose for Period 1 and 0.5 hour postdose for Period 2. The mean concentrations of mitapivat then declined in a multi exponential manner for both periods; however, the post-peak initial decline in mitapivat concentrations was more rapid and to lower levels when coadministered with rifampin compared to dosing alone.

#### **The Metabolite**

**[00145]** The Metabolite plasma concentrations were detectable at 0.5 hour postdose in all subjects after mitapivat sulfate was given alone and in all but 1 subject (1 subject was reported as BLQ) when mitapivat sulfate was coadministered with rifampin. The Metabolite was still detectable in all but 4 subjects up to the last scheduled sampling time of 120 hours postdose when mitapivat sulfate was given alone. When mitapivat sulfate was coadministered with rifampin, The Metabolite remained detectable in all subjects up to 24 hours postdose, with quantifiable The Metabolite out to 48 hours in 6 subjects and 72 hours in 1 subject. No Metabolite concentrations were detectable in samples collected beyond 72 hours.

**[00146]** The peak arithmetic mean Metabolite concentrations were observed at a nominal time of approximately 2 hours postdose with mitapivat sulfate given alone and 1 hour when mitapivat sulfate was coadministered with rifampin. When mitapivat sulfate was given alone, higher mean concentrations The Metabolite were observed over the initial 1 hour postdose. Metabolite concentrations were higher when mitapivat sulfate was coadministered with rifampin beyond 1 hour. The mean concentrations of The Metabolite then declined in a multi exponential fashion for both treatments. Decline in Metabolite concentrations was more rapid when mitapivat sulfate was coadministered with rifampin.

[00147] The statistical comparisons of mitapivat PK parameters following 50 mg mitapivat sulfate administered alone (Period 1) or coadministered with multiple doses of 600 mg rifampin (Period 2) PK parameters are summarized below in Table 3.

**Table 3**  
**Summary of Statistical Comparisons of Plasma Mitapivat Pharmacokinetic Parameters Following 50 mg Mitapivat Sulfate Co-Administered With Multiple Doses of 600 mg Rifampin Versus Administered Alone (Part 2)**

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | Mitapivat sulfate + rifampin (Test) |    | Mitapivat sulfate alone (Reference) |    |        |                         |                   |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|----|-------------------------------------|----|--------|-------------------------|-------------------|
| Parameter (unit)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Geometric LSM                       | n  | Geometric LSM                       | n  | GMR    | 90% Confidence Interval | Intra-subject CV% |
| AUC <sub>0-t</sub> (ng*hr/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 486.4                               | 14 | 5565                                | 14 | 0.0874 | 0.0719 - 0.1062         | 29.73             |
| AUC <sub>0-inf</sub> (ng*hr/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 496.4                               | 14 | 5681                                | 14 | 0.0874 | 0.0719 - 0.1062         | 29.84             |
| C <sub>max</sub> (ng/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 292.4                               | 14 | 1263                                | 14 | 0.2316 | 0.1933 - 0.2773         | 27.45             |
| Mitapivat sulfate alone: 50 mg mitapivat sulfate (1 x 50 mg tablet) at Hour 0 on Day 1 (Period 1)<br>Mitapivat sulfate + rifampin: 600 mg rifampin (2 x 300 mg capsules) QD on Day 1 to Day 12 inclusive (within ± 1 hour of dosing time on Day 1) with 50 mg mitapivat sulfate (1 x 50 mg tablet) coadministered at Hour 0 on Day 8 (Period 2)<br>Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the linear mixed effect model.<br>Geometric Mean Ratio (GMR) = test / reference<br>Intra-subject CV% = 100 x (square root (exp[MSE]-1), where MSE = Residual variance from linear mixed effect model. |                                     |    |                                     |    |        |                         |                   |

[00148] The coadministration of 600 mg rifampin with 50 mg mitapivat sulfate decreased mitapivat exposure relative to when mitapivat sulfate was administered alone. The geometric mean AUC<sub>0-t</sub> and AUC<sub>0-inf</sub> ratios of mitapivat in the presence and absence of rifampin were 0.09. The geometric mean Cmax ratio of mitapivat in the presence and absence of rifampin was 0.23. The intra-subject variability was low at approximately 30% for AUCs and 27% for Cmax.

[00149] The statistical comparisons of Metabolite PK parameters following 50 mg mitapivat sulfate administered alone (Period 1) or co-administered with multiple doses of 600 mg rifampin (Period 2) PK parameters are summarized below in Table 4.

**Table 4**  
**Summary of Statistical Comparisons of Plasma Metabolite Pharmacokinetic Parameters Following 50 mg Mitapivat Sulfate Co-Administered With Multiple Doses of 600 mg Rifampin Versus Administered Alone (Part 2)**

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | Mitapivat sulfate + rifampin (Test) |    | Mitapivat sulfate alone (Reference) |    |        |                         |                   |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|----|-------------------------------------|----|--------|-------------------------|-------------------|
| Parameter (unit)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Geometric LSM                       | n  | Geometric LSM                       | n  | GMR    | 90% Confidence Interval | Intra-subject CV% |
| AUC <sub>0-t</sub> (ng*hr/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 230.5                               | 14 | 623.0                               | 14 | 0.3700 | 0.3316 - 0.4129         | 16.50             |
| AUC <sub>0-inf</sub> (ng*hr/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 244.9                               | 14 | 662.6                               | 14 | 0.3696 | 0.3321 - 0.4113         | 16.08             |
| C <sub>max</sub> (ng/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 64.47                               | 14 | 53.80                               | 14 | 1.1983 | 1.0642 - 1.3492         | 17.86             |
| Mitapivat sulfate alone: 50 mg mitapivat sulfate (1 x 50 mg tablet) at Hour 0 on Day 1 (Period 1)<br>Mitapivat sulfate + rifampin: 600 mg rifampin (2 x 300 mg capsules) QD on Day 1 to Day 12 inclusive (within ± 1 hour of dosing time on Day 1) with 50 mg mitapivat sulfate (1 x 50 mg tablet) coadministered at Hour 0 on Day 8 (Period 2)<br>Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the linear mixed effect model.<br>Geometric Mean Ratio (GMR) = test / reference<br>Intra-subject CV% = 100 x (square root (exp[MSE]-1), where MSE = Residual variance from linear mixed effect model. |                                     |    |                                     |    |        |                         |                   |

[00150] The coadministration of 600 mg rifampin with 50 mg mitapivat sulfate decreased the Metabolite exposure relative to when mitapivat sulfate was administered alone. The geometric mean AUC0-t and AUC0-inf ratios of the Metabolite in the presence versus absence of rifampin were 0.37. The geometric mean Cmax ratio of the Metabolite in the presence versus absence of rifampin was 1.2. The intra-subject variability was low at approximately 16% to 18% for AUCs and Cmax.

Conclusions

[00151] Total exposure of mitapivat increased in the presence of itraconazole compared to dosing of mitapivat sulfate alone.

[00152] Total exposure of mitapivat decreased in the presence of rifampin compared to dosing of mitapivat sulfate alone.

Example 3. Development of a PBPK Model for Mitapivat Sulfate to Evaluate the Potential for Drug-Drug Interactions

Part I

[00153] A physiologically based pharmacokinetic model (hereinafter “PBPK” model) for mitapivat sulfate based on the currently available in-vitro and clinical PK data is being developed. The developed PBPK model will be used to assess DDI liability with mitapivat sulfate acting as a victim of CYP3A4-mediated metabolism as well as a perpetrator of CYP and transporter mediated DDI.

**[00154]** Specifically, a PBPK model that includes a simple first order absorption model will be developed. Full PBPK and minimal PBPK distribution models will be evaluated, both of which consider liver and intestinal metabolism. The fraction of mitapivat sulfate absorbed will be estimated from mass balance data from the [14C] human ADME study (Clinical Study AG348-C-009). In-vitro data will be used to assign the relative contribution of CYP3A4 to the clearance of mitapivat sulfate.  $K_I$  and  $k_{inact}$  values for mechanism based inhibition of CYP3A4 and CYP3A4 induction ( $E_{max}$  and  $EC_{50}$ ) data by mitapivat sulfate will be incorporated within the PBPK model to assess the net DDI effects at steady state. Once an mitapivat sulfate PBPK model has been verified against the available clinical data, a series of DDI simulations assessing mitapivat sulfate as a victim or perpetrator of DDI will be performed in healthy volunteers.

#### **Experiments with mitapivat sulfate as a victim**

**[00155]** The impact of the co-administration of the strong CYP3A4 inhibitor, itraconazole, on the PK of mitapivat sulfate will be determined based on the following single and multiple-dose administration of 5, 20 and 50 mg BID.

**[00156]** The impact of the co-administration of the strong CYP3A4 inhibitor, ketoconazole, on the PK of mitapivat sulfate will be determined based following single and multiple-dose administration of 5, 20 and 50 mg BID.

**[00157]** The impact of the co-administration of the moderate CYP3A4 inhibitor fluconazole on the PK of mitapivat sulfate will be determined based on the following single- and multiple dose administration of 5, 20 and 50 mg BID.

**[00158]** The impact of the co-administration of the mild CYP3A4 inhibitor cimetidine on the PK of mitapivat sulfate will be determined based on the following single- and multiple dose administration of 5, 20 and 50 mg BID.

**[00159]** The impact of the co-administration of the strong CYP3A4 inducer, rifampin, on the PK of mitapivat sulfate will be determined based on the following single- and multiple dose administration of 5, 20 and 50 mg BID.

**[00160]** The impact of the co-administration of the moderate CYP3A4 inducer efavirenz on the PK of mitapivat sulfate will be determined following single- and multiple dose administration of 5, 20 and 50 mg BID.

#### **Experiments with mitapivat sulfate as perpetrator**

**[00161]** The extent to which 5 to 50 mg BID mitapivat sulfate affects the systemic exposure of CYP3A4 substrate midazolam when co-administered with mitapivat sulfate will be determined.

**[00162]** The effect of 5 to 50 mg mitapivat sulfate on the systemic exposures of CYP2B6 substrate bupropion when co-administered will be determined. CYP2B6 induction (Emax and EC50 ) data by mitapivat sulfate will be incorporated within the PBPK model. Sensitivity analysis will be conducted to gauge the impact of the uncertainty around Emax and EC50 on the predicted DDIs.

**[00163]** The effect of 5 to 50 mg AG mitapivat sulfate BID on the systemic exposures of CYP2C9 substrate warfarin when co-administered will be determined. CYP2C9 induction (Emax and EC50) data by mitapivat sulfate will be incorporated within the PBPK model. Sensitivity analysis will be conducted to gauge the impact of the uncertainty around Emax and EC50 on the predicted DDIs.

**[00164]** The effect of 5 to 50 mg mitapivat sulfate BID on the systemic exposures of CYP2C8/CYP3A4 substrate repaglinide when co-administered will be determined. CYP2C8 induction (Emax and EC50) data by mitapivat sulfate will be incorporated within the PBPK model. Sensitivity analysis will be conducted to gauge the impact of the uncertainty around Emax and EC50 on the predicted DDIs.

**[00165]** The effect of 5 to 50 mg mitapivat sulfate BID on the systemic exposures of CYP2C19 substrate omeprazole when co-administered will be determined. CYP2C19 induction (Emax and EC50) data by mitapivat sulfate will be incorporated within the PBPK model. Sensitivity analysis will be conducted to gauge the impact of the uncertainty around Emax and EC50 on the predicted DDIs.

**[00166]** The effect of mitapivat sulfate (5 to 50 mg BID) on the systemic exposures of OATP1B1/OATP1B3 (rosuvastatin), P-gp (digoxin), metformin (MATE1 and OCT2), OAT3 (methotrexate) substrate drugs when co-administered will be determined. The respective IC50 values for inhibition of OATP1B1, P-gp, MATE1, OCT2 and OAT3 by mitapivat sulfate will be incorporated within the PBPK model. Sensitivity analysis will be performed to assess the impact of these parameters on the DDI liability of mitapivat sulfate.

## **Part II**

**[00167]** The first order absorption component of the previously developed PBPK model will be extended using available pH solubility data to include the ADAM model. The recovery of mitapivat sulfate plasma concentration profiles after single and multiple dosing (up to 50 mg BID) to healthy volunteer subjects will be demonstrated. Once verified against the available clinical data, the modified PBPK model will be used to assess the impact of an increase in gastric pH on the pharmacokinetics of mitapivat sulfate via manipulation of system parameters (gastric pH).

**[00168]** While a number of embodiments have been described, the scope of this disclosure is to be defined by the appended claims, and not by the specific embodiments that have been represented by way of example. The contents of all references (including literature references, issued patents, published patent applications, and co-pending patent applications) cited throughout this application are hereby expressly incorporated herein in their entireties by reference. Unless otherwise defined, all technical and scientific terms used herein are accorded the meaning commonly known to one with ordinary skill in the art.

(PATENT)

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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In re Utility Application of:  
Varsha Venkatachalam IYER *et al.*

Application No.: 16/900,610

Confirmation No.: 1054

Filed: June 12, 2020

Art Unit: 1621

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For: MITAPIVAT THERAPY AND MODULATORS  
OF CYTOCHROME P450

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Examiner: George W. Kosturko

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

**AMENDMENT AFTER ALLOWANCE UNDER 37 C.F.R. § 1.312**

Dear Sir:

Applicant submits this paper pursuant to 37 C.F.R. § 1.312 after a Notice of Allowance was mailed on May 3, 2023. This paper is being filed with the payment of the issue fee for this application.

**Amendments to the Specification** begin on page 2 of this paper.

**Remarks** begin on page 3 of this paper.

Application No.: 16/900,610  
Amendment after Allowance

Docket No.: 128748-01502

**AMENDMENTS TO THE SPECIFICATION**

Please replace the originally filed specification with the attached substitute specification, submitted concurrently herewith.

Application No.: 16/900,610  
Amendment after Allowance

Docket No.: 128748-01502

### **REMARKS**

In the specification, [0021] and [0022] are amended to include the inadvertent omission of the word “salt” following “pharmaceutically acceptable”. Other obvious and inadvertent typographical and spacing errors are also updated. The substitute specification is submitted with markings and is also accompanied by a clean version (without markings) as required by 37 C.F.R. § 1.125(c). No new matter has been added.

The amendments to the specification merely correct formal matters and “require no substantial amount of additional work” by the Examiner. See MPEP §§ 714.16 and 714.16(d). Applicant therefore requests that the amendment be approved and entered.

Dated: August 2, 2023

Respectfully submitted,

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| Electronic Acknowledgement Receipt   |                                                     |
|--------------------------------------|-----------------------------------------------------|
| EFS ID:                              | 48395011                                            |
| Application Number:                  | 16900610                                            |
| International Application Number:    |                                                     |
| Confirmation Number:                 | 1054                                                |
| Title of Invention:                  | MITAPIVAT THERAPY AND MODULATORS OF CYTOCHROME P450 |
| First Named Inventor/Applicant Name: | Varsha Venkatachalam Iyer                           |
| Customer Number:                     | 190109                                              |
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| Submitted with Payment                                                                                         | yes              |
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| Payment was successfully received in RAM                                                                       | \$ 1500          |
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| Deposit Account                                                                                                |                  |
| Authorized User                                                                                                |                  |
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| Document Number | Document Description                                                                                                      | File Name                                          | File Size(Bytes)/ Message Digest           | Multi Part /.zip | Pages (if appl.) |
| 1               | Request for Continued Examination (RCE)                                                                                   | 128748_01502_Request_for_Continued_Examination.pdf | 1667017                                    | no               | 3                |
|                 |                                                                                                                           |                                                    | 3e0929f5b4fbae6d5b201dc8798812c457b02deaab |                  |                  |
| Warnings:       |                                                                                                                           |                                                    |                                            |                  |                  |
| Information:    |                                                                                                                           |                                                    |                                            |                  |                  |
| 2               | Amendment Submitted/Entered with Filing of Continued Prosecution Application (CPA)/Request for Continued Examination(RCE) | 128748_01502_Aug_Amendment.pdf                     | 35399                                      | no               | 6                |
|                 |                                                                                                                           |                                                    | 22bc5f8b989ac8835d5f7233001bfbdb451fdbaa4  |                  |                  |
| Warnings:       |                                                                                                                           |                                                    |                                            |                  |                  |
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| 3               | Amendment Submitted/Entered with Filing of Continued Prosecution Application (CPA)/Request for Continued Examination(RCE) | 128748_01502_Application_Aug_Marked.pdf            | 254393                                     | no               | 63               |
|                 |                                                                                                                           |                                                    | 4f0faab1cf779edc0b7cb2a322b1aaf7300904d2   |                  |                  |
| Warnings:       |                                                                                                                           |                                                    |                                            |                  |                  |
| Information:    |                                                                                                                           |                                                    |                                            |                  |                  |
| 4               | Amendment Submitted/Entered with Filing of Continued Prosecution Application (CPA)/Request for Continued Examination(RCE) | 128748_01502_Application_Aug_Clean.pdf             | 252421                                     | no               | 63               |
|                 |                                                                                                                           |                                                    | 49ca0762f8d7efd9ac597a42607153875fcdab4    |                  |                  |
| Warnings:       |                                                                                                                           |                                                    |                                            |                  |                  |
| Information:    |                                                                                                                           |                                                    |                                            |                  |                  |
| 5               | ePetition Request Form                                                                                                    | petition-request.pdf                               | 39763                                      | no               | 2                |
|                 |                                                                                                                           |                                                    | 9de7e88db085f6eebae4a2334440d04c4f72f08c   |                  |                  |
| Warnings:       |                                                                                                                           |                                                    |                                            |                  |                  |
| Information:    |                                                                                                                           |                                                    |                                            |                  |                  |
| 6               | Fee Worksheet (SB06)                                                                                                      | fee-info.pdf                                       | 40343                                      | no               | 2                |
|                 |                                                                                                                           |                                                    | 136c218e7f03935e1a2d1b0d0f33dd192a83a939   |                  |                  |
| Warnings:       |                                                                                                                           |                                                    |                                            |                  |                  |
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| Total Files Size (in bytes):                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | 2289336 |
| <p><b>This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.</b></p> <p><b><u>New Applications Under 35 U.S.C. 111</u></b><br/>If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.</p> <p><b><u>National Stage of an International Application under 35 U.S.C. 371</u></b><br/>If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.</p> <p><b><u>New International Application Filed with the USPTO as a Receiving Office</u></b><br/>If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.</p> |         |

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                |             |            |                               |                    |          |      |
|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------|-------------|------------|-------------------------------|--------------------|----------|------|
| REQUEST FOR CONTINUED EXAMINATION(RCE)TRANSMITTAL<br>(Submitted Only via EFS-Web)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                |             |            |                               |                    |          |      |
| Application Number                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 16900610       | Filing Date | 2020-06-12 | Docket Number (if applicable) | 128748-01502       | Art Unit | 1653 |
| First Named Inventor                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | Varsha V. Iyer |             |            | Examiner Name                 | George W. Kosturko |          |      |
| <p><b>This is a Request for Continued Examination (RCE) under 37 CFR 1.114 of the above-identified application.</b><br/>Request for Continued Examination (RCE) practice under 37 CFR 1.114 does not apply to any utility or plant application filed prior to June 8, 1995, or to any design application. The Instruction Sheet for this form is located at WWW.USPTO.GOV</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                |             |            |                               |                    |          |      |
| SUBMISSION REQUIRED UNDER 37 CFR 1.114                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                |             |            |                               |                    |          |      |
| <p>Note: If the RCE is proper, any previously filed unentered amendments and amendments enclosed with the RCE will be entered in the order in which they were filed unless applicant instructs otherwise. If applicant does not wish to have any previously filed unentered amendment(s) entered, applicant must request non-entry of such amendment(s).</p>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                |             |            |                               |                    |          |      |
| <div><div><input type="checkbox"/></div><div>Previously submitted. If a final Office action is outstanding, any amendments filed after the final Office action may be considered as a submission even if this box is not checked.</div></div> <div><div><input type="checkbox"/></div><div>Consider the arguments in the Appeal Brief or Reply Brief previously filed on _____</div></div> <div><div><input type="checkbox"/></div><div>Other _____</div></div> <div><div><input checked="" type="checkbox"/></div><div>Enclosed</div></div> <div><div><input type="checkbox"/></div><div>Amendment/Reply</div></div> <div><div><input type="checkbox"/></div><div>Information Disclosure Statement (IDS)</div></div> <div><div><input type="checkbox"/></div><div>Affidavit(s)/ Declaration(s)</div></div> <div><div><input checked="" type="checkbox"/></div><div>Other</div><div>e-Petition - withdrawal from issue after payment of the issue fee</div></div> |                |             |            |                               |                    |          |      |
| MISCELLANEOUS                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                |             |            |                               |                    |          |      |
| <div><div><input type="checkbox"/></div><div>Suspension of action on the above-identified application is requested under 37 CFR 1.103(c) for a period of months _____<br/>(Period of suspension shall not exceed 3 months; Fee under 37 CFR 1.17(i) required)</div></div> <div><div><input type="checkbox"/></div><div>Other _____</div></div>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                |             |            |                               |                    |          |      |
| FEES                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                |             |            |                               |                    |          |      |
| <div><div><input checked="" type="checkbox"/></div><div><p>The RCE fee under 37 CFR 1.17(e) is required by 37 CFR 1.114 when the RCE is filed.</p><p>The Director is hereby authorized to charge any underpayment of fees, or credit any overpayments, to Deposit Account No 50-4876</p></div></div>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                |             |            |                               |                    |          |      |
| SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                |             |            |                               |                    |          |      |
| <div><div><input checked="" type="checkbox"/></div><div>Patent Practitioner Signature</div></div> <div><div><input type="checkbox"/></div><div>Applicant Signature</div></div>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                |             |            |                               |                    |          |      |

| Signature of Registered U.S. Patent Practitioner |                  |                     |            |
|--------------------------------------------------|------------------|---------------------|------------|
| Signature                                        | /Andrew R. Ehle/ | Date (YYYY-MM-DD)   | 2023-08-04 |
| Name                                             | Andrew R. Ehle   | Registration Number | 78384      |

This collection of information is required by 37 CFR 1.114. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.11 and 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450.

*If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.*

## Privacy Act Statement

The Privacy Act of 1974 (P.L. 93-579) requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b)(2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether the Freedom of Information Act requires disclosure of these records.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspections or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 C.F.R. § 1.6(a)(4).

Dated: August 4, 2023 Signature: /Andrew R. Ehle/  
(Andrew R. Ehle)

Docket No.: 128748-01502  
(PATENT)

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Utility Application of:  
Varsha Venkatachalam Iyer et al.

Application No.: 16/900,610

Confirmation No.: 1054

Filed: June 12, 2020

Art Unit: 1621

For: MITAPIVAT THERAPY AND MODULATORS  
OF CYTOCHROME P450

Examiner: George W. Kosturko

MS RCE  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

**REQUEST FOR CONTINUED EXAMINATION UNDER 37 C.F.R. § 1.114**

Further to payment of the Issue Fee and 37 C.F.R. § 1.312 Amendment filed on August 2, 2023, Applicant requests reconsideration of this application in view of the following amendments. Filed concurrently herewith is an electronic Petition to Withdraw Application from Issue under 37 § CFR 1.313(c)(2).

**Amendments to the Specification** begin on page 2 of this paper.

**Amendments to the Claims** begin on page 3 of this paper.

**Remarks** begin on page 6 of this paper.

**AMENDMENTS TO THE SPECIFICATION**

Please amend the specification in accordance with the substitute specification, submitted concurrently herewith.

**AMENDMENTS TO THE CLAIMS**

1-58. (Cancelled)

59. (Currently Amended) A method of treating hemolytic anemia in a subject, comprising administering to the subject about 10 mg/day, about 40 mg/day, about 100 mg/day, or about 200 mg/day of mitapivat or a pharmaceutically acceptable salt thereof and a moderate inducer of cytochrome P450 3A4/5 (CYP3A4/5).

60. (Currently Amended) The method of claim 59, wherein the subject is administered about 10 mg/day of mitapivat or a pharmaceutically acceptable salt thereof.

61. (Currently Amended) The method of claim 60, wherein the subject is administered about 5 mg of mitapivat or a pharmaceutically acceptable salt thereof twice per day.

62. (Currently Amended) The method of claim 59, wherein the subject is administered about 40 mg/day of mitapivat or a pharmaceutically acceptable salt thereof.

63. (Currently Amended) The method of claim 62, wherein the subject is administered about 20 mg of mitapivat or a pharmaceutically acceptable salt thereof twice per day.

64. (Currently Amended) The method of claim 59, wherein the subject is administered about 100 mg/day of mitapivat or a pharmaceutically acceptable salt thereof.

65. (Currently Amended) The method of claim 64, wherein the subject is administered about 50 mg of mitapivat or a pharmaceutically acceptable salt thereof twice per day.

66. (Currently Amended) The method of claim 59, wherein the subject is administered about 200 mg/day of mitapivat or a pharmaceutically acceptable salt thereof.

67. **(Currently Amended)** The method of claim 66, wherein the subject is administered about 100 mg of mitapivat or a pharmaceutically acceptable salt thereof twice per day.

68. **(Currently Amended)** The method of claim 59, wherein the mitapivat or a pharmaceutically acceptable salt thereof is orally administered as part of a tablet composition.

69. **(Currently Amended)** The method of claim 61, wherein the mitapivat or a pharmaceutically acceptable salt thereof is orally administered as part of a tablet composition.

70. **(Currently Amended)** The method of claim 63, wherein the mitapivat or a pharmaceutically acceptable salt thereof is orally administered as part of a tablet composition.

71. **(Currently Amended)** The method of claim 65, wherein the mitapivat or a pharmaceutically acceptable salt thereof is orally administered as part of a tablet composition.

72. **(Currently Amended)** The method of claim 67, wherein the mitapivat or a pharmaceutically acceptable salt thereof is orally administered as part of a tablet composition.

73. **(Previously Presented)** The method of claim 68, wherein the tablet composition further comprises microcrystalline cellulose, mannitol, croscarmellose sodium, and sodium stearyl fumarate.

74. **(Previously Presented)** The method of claim 69, wherein the tablet composition further comprises microcrystalline cellulose, mannitol, croscarmellose sodium, and sodium stearyl fumarate.

75. **(Previously Presented)** The method of claim 70, wherein the tablet composition further comprises microcrystalline cellulose, mannitol, croscarmellose sodium, and sodium stearyl fumarate.

76. **(Previously Presented)** The method of claim 71, wherein the tablet composition further comprises microcrystalline cellulose, mannitol, croscarmellose sodium, and sodium stearyl fumarate.

77. **(Previously Presented)** The method of claim 72, wherein the tablet composition further comprises microcrystalline cellulose, mannitol, croscarmellose sodium, and sodium stearyl fumarate.

**REMARKS****I. Amendments to the Specification**

In the specification, paragraphs [0021] and [0022] are amended to include the inadvertent omission of the word “salt” following “pharmaceutically acceptable” and to correct the spelling of “mitapivat”. Paragraph [0081] has been amended to remove duplicative instances of the word “inhibitor”. Paragraphs [00117] through [00168] have been updated to paragraphs [00137] through [00188]. Other obvious and inadvertent typographical and spacing errors are updated throughout the specification. The substitute specification is submitted with markings and is also accompanied by a clean version (without markings) as required by 37 C.F.R. § 1.125(c). To avoid confusion, these amendments include those filed with Applicant’s Amendment under § 312 on August 2, 2023, since this prior § 312 Amendment has not yet been entered. *No new matter has been added.*

**II. Amendments to the Claims**

Claims 59-72 have been amended to specify that the method comprises administering mitapivat or a pharmaceutically acceptable salt thereof. Support for this amendment can be found, at least, for example, in paragraph [0031] and throughout the specification as filed. With entry of these amendments, claims 59-77 are pending. *No new matter has been added.*

Dated: August 4, 2023

Respectfully submitted,

Electronic signature: /Andrew R. Ehle/  
Andrew R. Ehle  
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Attorney/Agent For Applicant

**MITAPIVAT THERAPY AND MODULATORS OF CYTOCHROME P450**

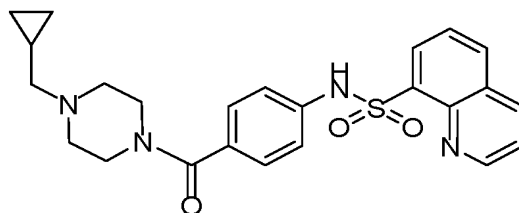
**RELATED APPLICATIONS**

The application claims the benefit of U.S. Provisional Application Serial No. 62/861,600, filed June 14, 2019, the entire teachings of which are incorporated herein by reference.

**BACKGROUND**

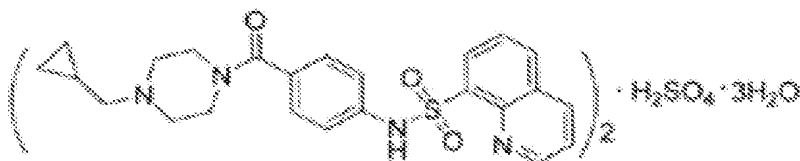
[0001] Pyruvate kinase deficiency (PKD) is a disease of the red blood cells caused by a deficiency of the pyruvate kinase R (PKR) enzyme due to recessive mutations of PKLR gene. PKR activators can be beneficial to treat PKD, thalassemia (*e.g.*, beta-thalassemia), abetalipoproteinemia or Bassen-Kornzweig syndrome, sickle cell disease, paroxysmal nocturnal hemoglobinuria, anemia (*e.g.*, congenital anemias) (*e.g.*, enzymopathies), hemolytic anemia (*e.g.*, hereditary and/or congenital hemolytic anemia, acquired hemolytic anemia, chronic hemolytic anemia caused by phosphoglycerate kinase deficiency, anemia of chronic diseases, non-spherocytic hemolytic anemia or hereditary spherocytosis). Treatment of PKD is supportive, including blood transfusions, splenectomy, chelation therapy to address iron overload, and/or interventions for other disease-related morbidity. Currently, however, there is no approved medicine that treats the underlying cause of PKD, and thus the etiology of life-long hemolytic anemia.

[0002] Mitapivat, also known as AG-348 or by its chemical name *N*-(4-(4-(cyclopropylmethyl)piperazine-1-carbonyl)phenyl)quinoline-8-sulfonamide, and is represented by the following structural formula:



is an allosteric activator of pyruvate kinase R (PKR). See, *e.g.*, International Patent Application Publication Nos. WO 2011/002817 and WO 2016/201227.

[0003] Mitapivat sulfate ~~that~~ is represented by the following structural formula:



and is currently in Phase 2 and Phase 3 clinical trials as a disease-altering therapy in patients with PKD, hemolytic anemia and thalassemia. See, e.g., U.S. Clinical Trials Identifier Nos. NCT03692052, NCT03559699, NCT03548220, NCT03853798, and NCT02476916.

#### SUMMARY

[0004] Non-clinical studies described in Example 1 show that the PKR activator, mitapivat, is primarily metabolized by the cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5) enzymes (*i.e.*, >90%), with minor contributions from other detoxification enzymes, namely CYP2C8, CYP2C9, and CYP1A2. It has also been found that in a clinical trial, total exposure of mitapivat increased in the presence of itraconazole, a strong CYP3A4A and p-gp inhibitor, compared with dosing of mitapivat sulfate alone. It has further been found that total exposure of mitapivat decreased in the presence of rifampin, a strong CYP3A4A and p-gp inducer, compared with dosing of mitapivat sulfate alone.

[0005] Accordingly, the present disclosure provides methods of treating a disease, disorder or a condition with mitapivat or a pharmaceutically acceptable salt thereof and a CYP3A4/5 inducer or a CYP3A4/5 inhibitor. In another aspect, the present disclosure provides methods of treating a disease, disorder or a condition with mitapivat or a pharmaceutically acceptable salt thereof and a p-glycoprotein (p-gp) inhibitor. As used herein, “CYP3A4/5” means cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5). In some embodiments, the pharmaceutically acceptable salt is mitapivat sulfate.

[0006] Specifically, in one embodiment, the present disclosure relates to a method of treating pyruvate kinase deficiency (PKD) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inhibitor of cytochrome P450 3A4/5 (CYP3A4/5)[[ ]]. In another embodiment, the present disclosure relates to a method of treating sickle cell disease in a subject, comprising administering to the subject an effective amount of mitapivat or a

pharmaceutically acceptable salt thereof and an effective amount of an inhibitor of cytochrome P450 3A4/5 (CYP3A4/5). In another embodiment, the present disclosure relates to a method of treating thalassemia ([e.g.] e.g., alpha-thalassemia and beta-thalassemia) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inhibitor of cytochrome P450 3A4/5 (CYP3A4/5). In another embodiment, the present disclosure relates to a method of treating hemolytic anemia in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inhibitor of cytochrome P450 3A4/5 (CYP3A4/5). In some embodiments, the inhibitor of CYP3A4/5 is a moderate CYP3A4/5 inhibitor. In some embodiments, the inhibitor of CYP3A4/5 is a mild CYP3A4/5 inhibitor.

**[0007]** In another embodiment, the present disclosure relates to a method of treating pyruvate kinase deficiency (PKD) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inducer of cytochrome P450 3A4/5 (CYP3A4/5)[[ ]]. In another embodiment, the present disclosure relates to a method of treating sickle cell disease in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inducer of cytochrome P450 3A4/5 (CYP3A4/5). In another embodiment, the present disclosure relates to a method of treating thalassemia ([e.g.] e.g., alpha-thalassemia and/or beta-thalassemia) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inducer of cytochrome P450 3A4/5 (CYP3A4/5). In another embodiment, the present disclosure relates to a method of treating hemolytic anemia in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inducer of cytochrome P450 3A4/5 (CYP3A4/5). In some embodiments, the inducer of CYP3A4/5 is a moderate CYP3A4/5 inducer. In some embodiments, the inhibitor of CYP3A4/5 is a mild CYP3A4/5 inducer.

**[0008]** Specifically, in one embodiment, the present disclosure relates to a method of treating pyruvate kinase deficiency (PKD) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of a p-glycoprotein inhibitor. In another embodiment, the present disclosure

relates to a method of treating sickle cell disease in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of a p-glycoprotein inhibitor. In another embodiment, the present disclosure relates to a method of treating thalassemia ([e.g.] e.g., alpha-thalassemia and beta-thalassemia) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of a p-glycoprotein inhibitor. In another embodiment, the present disclosure relates to a method of treating hemolytic anemia in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of a p-glycoprotein inhibitor. In some embodiments, the p-glycoprotein inhibitor is a moderate p-glycoprotein inhibitor. In some embodiments, the p-glycoprotein inhibitor is a mild p-glycoprotein inhibitor.

**[0009]** The present disclosure further provides methods of treating a disease, disorder or a condition with [  ]mitapivat or a pharmaceutically acceptable salt thereof in the absence of a CYP3A4/5 inducer or a CYP3A4/5 inhibitor or p-glycoprotein inhibitor.

**[0010]** Specifically, in one embodiment, the present disclosure relates to a [  ]method of treating pyruvate kinase deficiency (PKD) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of an inducer or an inhibitor of cytochrome P450 3A4/5 (CYP3A4/5), respectively. In another embodiment, the present disclosure relates to a method of treating sickle cell disease in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of an inducer or an inhibitor of cytochrome P450 3A4/5 (CYP3A4/5), respectively. In yet another embodiment, the present disclosure relates to a [  ]method of treating thalassemia ([e.g.] e.g., alpha-thalassemia and/or beta-thalassemia) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of an inducer or an inhibitor of cytochrome P450 3A4/5 (CYP3A4/5), respectively. In yet another embodiment, the present disclosure relates to a method of treating ~~hemolytic~~ hemolytic anemia in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of an inducer or an inhibitor of cytochrome P450 3A4/5 (CYP3A4/5), respectively. In some embodiments, the inducer of CYP3A4/5 is a strong inducer of CYP3A4/5. In some embodiments, the inducer of

CYP3A4/5 is a moderate inducer of CYP3A4/5. In some embodiments, the inhibitor of CYP3A4/5 is a strong inhibitor of CYP3A4/5. In some embodiments, the inhibitor of CYP3A4/5 is a moderate inhibitor of CYP3A4/5.

**[0011]** In another embodiment, the present disclosure relates to a method of treating pyruvate kinase deficiency (PKD) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of a p-glycoprotein inhibitor. In another embodiment, the present disclosure relates to a method of treating sickle cell disease in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of a p-glycoprotein inhibitor. In yet another embodiment, the present disclosure relates to a method of treating thalassemia ([e.g.]e.g., alpha-thalassemia and/or beta-thalassemia) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of a p-glycoprotein inhibitor. In yet another embodiment, the present disclosure relates to a method of treating ~~hemolytic~~ hemolytic anemia in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of a p-glycoprotein inhibitor. In some embodiments, the p-glycoprotein inhibitor is a strong p-glycoprotein inhibitor. In some embodiments, ~~In some embodiments~~, the p-glycoprotein inhibitor is a mild p-glycoprotein inhibitor.

#### BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a graph showing Arithmetic Mean (SD) Plasma Mitapivat and Metabolite Concentration-Time Profiles following administration of 20 mg mitapivat sulfate alone (Treatment Period 1) and co-administered with multiple doses of 200 mg itraconazole (Treatment Period 2) on linear scale (Part 1). “Metabolite” refers to the CYP3A4 metabolite of mitapivat (see Example 2).

Figure 2 is a graph showing Arithmetic Mean Plasma Mitapivat and Metabolite Concentration-Time Profiles following administration of 20 mg mitapivat sulfate alone (Treatment Period 1) and co-administered with multiple doses of 200 mg itraconazole (Treatment Period 2) on semi-log scale (Part 1) (see Example 2).

Figure 3 is a graph showing Arithmetic Mean (SD) Plasma Mitapivat and Metabolite Concentration-Time Profiles following administration of 50 mg mitapivat sulfate alone

(Treatment Period 1) and co-administered with multiple doses of 600 mg rifampin on linear scale (Treatment Period 2, Part 2, see Example 2).

Figure 4 is a graph showing Arithmetic Mean Plasma Mitapivat and Metabolite Concentration-Time Profiles following administration of 50 mg mitapivat sulfate alone (Treatment Period 1) and co-administered with multiple doses of 600 mg rifampin on semi-log scale (Treatment Period 2, Part 2, see Example 2).

### DETAILED DESCRIPTION

#### Definitions

##### [0012]

A “CYP3A4/5 modulator” refers to any substance, including, but not limited to a small molecule (with a molecular weight of  $\leq 1000$  Da, or  $\leq 750$  Da, or  $\leq 500$  Da), a nucleoside, a nucleotide, a nucleobase, a sugar, a nucleic acid, an amino acid, a polysaccharide, a peptide, a polypeptide, a protein, an antibody, an aptamer, a natural or synthetic plant-based substance, or a complex or a composition comprising any combination of the aforementioned substances, that can modify or alter the activity and/or expression levels of the enzyme cytochrome P450 3A4 (CYP3A4; EC 1.14.13.97) or 3A5 (CYP3A5; EC1.14.14.1). Briefly, the cytochrome P450 (CYP) is a superfamily of detoxification enzymes that are responsible for the oxidative and reductive metabolic transformation of drug medications and the ultimate “clearance”, which is the elimination of the drug from the body as determined by the measured plasma levels of the drug and optionally, the drug metabolites, such as the metabolite known to be formed by CYP3A4/5. CYP3A4 and CYP3A5 are two of such enzymes and are largely prevalent in the liver.

[0013] A CYP3A4/5 modulator may be a CYP3A4/5 inducer or a CYP3A4/5 inhibitor. A “CYP3A4/5 inducer” or “inducer of CYP3A4/5” is a CYP3A4/5 modulator as defined above that can increase, enhance, stimulate, induce, accelerate or augment the CYP3A4/5 activity from its existing state or native state, such as by at least about 0.1%, 0.2%, 0.5%, 1%, 2%, 3%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% and 100% or more relative to a reference level, *e.g.*, the level of the CYP3A4/5 activity and/or expression prior to the exposure or contact with the CYP3A4/5 inducer, including all ranges and values therebetween not expressly mentioned above. The CYP3A4/5 inducer activity may cause mitapivat or a pharmaceutically acceptable

salt thereof that is co-administered with the CYP3A4/5 inducer to be metabolized and eliminated more quickly from the body, as determined by the measured plasma levels of the compound and its metabolites.

**[0014]** A “CYP3A4/5 inhibitor” or “inhibitor of CYP3A4/5” is a CYP3A4/5 modulator as defined above that can decrease, reduce, inhibit, decelerate, attenuate, or suppress the CYP3A4/5 activity and/or expression from its existing state or native state, such as by at least about 0.1%, 0.2%, 0.5%, 1%, 2%, 3%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% and 100% or more relative to a reference level, *e.g.*, the level of the CYP3A4/5 activity and/or expression prior to the exposure or contact with the CYP3A4/5 inhibitor, including all ranges and values therebetween not expressly mentioned above. The CYP3A4/5 inhibitor may cause mitapivat or a pharmaceutically acceptable salt thereof that is co-administered with the CYP3A4/5 inhibitor to be metabolized and eliminated more slowly from the body, as determined by the measured plasma levels of the compound and its metabolites.

**[0015]** If an investigational drug is a CYP inducer, it can be classified as a strong, moderate, or mild inducer based on its effect on an index CYP substrate. The convention is to categorize CYP induction in the following ways:

**[0016]** A strong inducer decreases the AUC of a sensitive index CYP by  $\geq 80$  percent.

**[0017]** A moderate inducer decreases the AUC of a sensitive index CYP substrate by  $\geq 50$  to  $< 80$  percent.

**[0018]** A mild inducer decreases the AUC of a sensitive index CYP substrate by  $\geq 20$  to  $< 50$  percent.[“”]

**[0019]** A CYP inhibitor can be classified as a strong, moderate, or mild inhibitor based on its effect on an index CYP substrate. A strong inhibitor increases the AUC of a sensitive index CYP substrate  $\geq 5$ -fold. A moderate inhibitor increases the AUC of a sensitive index CYP substrate by  $\geq 2$ - to  $< 5$ -fold. A mild inhibitor increases the AUC of a sensitive index CYP substrate by  $\geq 1.25$ - to  $< 2$ - fold.[“”]

**[0020]** A p-glycoprotein inhibitor refers to any substance, including, but not limited to a small molecule (with a molecular weight of  $\leq 1000$  Da, or  $\leq 750$  Da, or  $\leq 500$  Da), a

nucleoside, a nucleotide, a nucleobase, a sugar, a nucleic acid, an amino acid, a polysaccharide, a peptide, a polypeptide, a protein, an antibody, an aptamer, a natural or synthetic plant-based substance, or a complex or a composition comprising any combination of the aforementioned substances, that can decrease, reduce, inhibit, decelerate, attenuate, or suppress the p-glycoprotein activity and/or expression from its existing state or native state, such as by at least about 0.1%, 0.2%, 0.5%, 1%, 2%, 3%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% and 100% or more relative to a reference level, *e.g.*, the level of the p-glycoprotein. In some embodiments, the p-glycoprotein inhibitor is a strong p-glycoprotein inhibitor, selected from the group consisting of amiodarone, azithromycin, captopril, carvedilol, clarithromycin, conivaptan, cyclosporine, diltiazem, dronedarone, erythromycin, felodipine, itraconazole, ketoconazole, lopinavir, ritonavir, quercetin, quinidine, ranolazine, ticagrelor, and erapamil.

**[0021]** The terms “effective amount” and “therapeutically effective amount” are used interchangeably throughout the present disclosure, whether referring to ~~mitavipat~~ mitapivat or a pharmaceutically acceptable salt thereof, a CYP3A4/5 inducer, or a CYP3A4/5 inhibitor.

**[0022]** An “effective amount” of ~~mitavipat~~ mitapivat or a pharmaceutically acceptable salt thereof is an amount sufficient to provide a therapeutic benefit in the treatment of pyruvate kinase deficiency (PKD), thalassemia (*e.g.*, ☐ alfa-thalassemia, beta-thalassemia or non-transfusion-dependent thalassemia), abetalipoproteinemia or Bassen-Kornzweig syndrome, sickle cell disease, paroxysmal nocturnal hemoglobinuria, anemia (*e.g.*, congenital anemias (*e.g.*, enzymopathies), hemolytic anemia (*e.g.*, hereditary and/or congenital hemolytic anemia, acquired hemolytic anemia, chronic hemolytic anemia, chronic hemolytic anemia caused by phosphoglycerate kinase deficiency, anemia of chronic diseases, non-spherocytic hemolytic anemia or hereditary spherocytosis), as well as all other conditions disclosed in the section “Diseases, Disorders and Conditions” in the present disclosure, *i.e.*, conditions that are ☐ responsive to treatment with a PKR activator such as ~~mitavipat~~ mitapivat or a pharmaceutically acceptable salt thereof. Such diseases, disorders and conditions are hereinafter referred to as “a PKR activator-responsive condition” when referring to any one of these conditions or “PKR activator-responsive conditions” when referring to these conditions collectively. Additionally or alternatively, an “effective amount” of ~~mitavipat~~ mitapivat or a pharmaceutically acceptable salt thereof is an amount sufficient to delay or minimize one or more effects or symptoms associated with these conditions. In one

aspect, an “effective amount” of ~~mitavipat~~ mitapivat or a pharmaceutically acceptable salt thereof means an amount of ~~mitavipat~~ mitapivat or a pharmaceutically acceptable salt thereof, alone or in combination with other therapies, which provides a therapeutic benefit in the treatment of the condition. The term “effective amount” can encompass an amount that improves overall therapy, reduces or avoids effects, symptoms, signs, or causes of the condition, and/or enhances the therapeutic efficacy of another therapeutic agent. In certain embodiments, an “effective amount” of ~~mitavipat~~ mitapivat or a pharmaceutically acceptable salt thereof is an amount sufficient for eliciting measurable activation of wild-type or mutant PKR. In certain embodiments, an “effective amount” of ~~mitavipat~~ mitapivat or a pharmaceutically acceptable salt thereof is an amount sufficient for regulating 2,3-diphosphoglycerate levels in blood in need thereof or for treating PKR activator-responsive conditions or treating diseases or conditions that are associated with increased 2,3-diphosphoglycerate levels (*e.g.*, liver diseases). In certain embodiments, an effective amount of ~~mitavipat~~ mitapivat or a pharmaceutically acceptable salt thereof is an amount sufficient for eliciting measurable activation of wild-type or mutant PKR and for regulating 2,3-diphosphoglycerate levels in blood in need thereof or for treating PKR activator-responsive conditions or treating diseases or conditions that are associated with increased 2,3-diphosphoglycerate levels ([*e.g.*,]*e.g.*, liver diseases). In one aspect, the effective amount is the amount required to generate a subject’s hemoglobin response of  $\geq 1.0$  g/dL (such as  $\geq 1.5$  g/dL or  $\geq 2.0$  g/dL) increase in Hb concentration from baseline. In one aspect, the subject’s baseline Hb concentration is the average of all available Hb concentrations before treatment with a compound described herein. In certain aspects, the effective amount is the amount required to reduce the patient’s transfusion burden. In one aspect, the effective amount of mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) is between about 0.01-100 mg/kg body weight/day of mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate), *e.g.*, about 0.1-100 mg/kg body weight/day.

**[0023]** Concomitant medications will be commonly used with mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate). An “effective amount” of the drug (*e.g.*, an effective amount of a CYP3A4/5 inducer or an effective amount of a CYP3A4/5 inhibitor) being co-administered with mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) is an amount sufficient to provide a desired therapeutic benefit. Drugs that are being co-administered with mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate), including some of the current antiarrhythmics,

antibiotics, antidepressants, antifungals, calcium channel blockers, H<sub>2</sub> receptor antagonists, non-nucleoside reverse transcriptase inhibitors (NNRTI), protease inhibitors, etc., will sometimes be CYP3A4/5 inducers or CYP3A4/5 inhibitors. In such instances, the amount of mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) or the amount of the drug being co-administered should be adjusted to reduce effects of a drug-drug interaction between mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) and the co-administered drug.

**[0024]** In one aspect, an effective amount of a CYP3A4/5 inducer is between about 0.1-1000 mg/kg/day of a CYP3A4/5 inducer, *e.g.*, about 1.0-1000 mg/kg/day, depending on the drug that is co-administered with.

**[0025]** In one aspect, an effective amount of a CYP3A4/5 inhibitor is between about 0.1-1000 mg/kg/day of a CYP3A4/5 inhibitor, *e.g.*, about 1.0-1000 mg/kg/day, depending on the drug that is co-administered with.

**[0026]** In one aspect, an effective amount of a p-gp inhibitor is between about 0.1-1000 mg/kg/day of a p-gp inhibitor, *e.g.*, about 1.0-1000 mg/kg/day, depending on the drug that is co-administered with.

**[0027]** As used herein, reduction in transfusion burden means at least 20% reduction in the number of RBC units transfused within at least 5 weeks of treatment. In certain embodiments, the reduction in transfusion burden is  $\geq 33\%$  reduction in the number of RBC units transfused within at least 5 weeks of treatment. In certain embodiments, reduction of transfusion burden is  $\geq 33\%$  reduction in the number of RBC units transfused within at least 10 weeks (*[[e.g.,]]e.g.*, at least 20 weeks or at least 24 weeks) of treatment.

**[0028]** The term “activating” as used herein means an agent that (measurably) increases the activity of wild type pyruvate kinase R (wt PKR) or causes wild type pyruvate kinase R (wt PKR) activity to increase to a level that is greater than wt PKR’s basal levels of activity or an agent that (measurably) increases the activity of a mutant pyruvate kinase R (mPKR) or causes mutant pyruvate kinase R (mPKR) activity to increase to a level that is greater than that mutant PKR’s basal levels of activity, for examples, to a level that is 20%, 40%, 50%, 60%, 70%, 80%, 90% or 100% of the activity of wild type PKR.

**[0029]** The terms “treatment,” “treat,” and “treating” refer to reversing, alleviating, reducing the likelihood of developing, or inhibiting the progress of a disease or disorder, or one or more effects or symptoms thereof, as described herein. In some embodiments, treatment may be administered after one or more symptoms have developed, *i.e.*, therapeutic treatment. In other embodiments, treatment may be administered in the absence of symptoms. For example, treatment may be administered to a susceptible individual prior to the onset of symptoms (*e.g.*, in light of a history of symptoms and/or in light of genetic or other susceptibility factors), *i.e.*, prophylactic treatment. Treatment may also be continued after symptoms have resolved, for example to reduce the likelihood of or delay their recurrence.

**[0030]** As used herein the terms “subject” and “patient” may be used interchangeably, and means a mammal in need of treatment with mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate).

**[0031]** The term “pharmaceutically acceptable salt” when referring to a pharmaceutically acceptable salt of mitapivat, refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response, and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art, for example, Berge et al. describe pharmaceutically acceptable salts in detail in *J. Pharmaceutical Sciences*, 1977, 66, 1-19. Pharmaceutically acceptable salts of mitapivat include those derived from suitable inorganic and organic acids. Examples of pharmaceutically acceptable acid addition salts are salts of an amino group formed with inorganic acids, such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid, and perchloric acid or with organic acids, such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid, or malonic acid or by using other methods known in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, besylate bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, gentisate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate,

persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, tosylate p-toluenesulfonate, undecanoate, valerate salts, and the like. Preferably, a pharmaceutically acceptable salt of mitapivat is a sulfate salt.

**[0032]** As used herein, the terms “about” and “approximately” are used herein to mean within the typical ranges of tolerance in the art. In one embodiment, “about” means within 2 standard deviations from the mean value. In one embodiment, “about” means  $\pm 10\%$ . In one embodiment, “about” means  $\pm 5\%$ . When “about” is present before a series of numbers of number ranges, it is understood that the term can apply ~~applies~~ to any and each of the numbers and ranges recited in the series.

### **Compositions and Administration**

**[0033]** The effective amount of mitapivat, in accordance with a method of the invention, may be administered in the form of a pharmaceutical composition comprising mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate), together with a pharmaceutically acceptable carrier.

**[0034]** Pharmaceutical compositions of mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) can be prepared by any method known in the art of pharmacology. In general, such preparatory methods include the steps of bringing of mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) into association with a carrier and/or one or more other accessory ingredients, and then, if necessary and/or desirable, shaping and/or packaging the product into a desired single- or multi-dose unit.

**[0035]** Pharmaceutically acceptable carriers used in the manufacture of provided pharmaceutical compositions include inert diluents, dispersing and/or granulating agents, surface active agents and/or emulsifiers, disintegrating agents, binding agents, preservatives, buffering agents, lubricating agents, and/or oils. Exemplary carriers, diluents, granulating and/or dispersing agents, surface active agents and/or emulsifiers, binding agents, preservatives, antioxidants, chelating agent, antifungal preservatives, alcohol preservatives, acidic preservatives, other types of preservatives, buffering agents, lubricating agents, and natural oils that can be included in a pharmaceutical composition of [[ ]]mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) as disclosed in International Patent Application No. WO2019/104134.

**[0036]** Pharmaceutical compositions of mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, transmucosally, or in an ophthalmic preparation. The term “parenteral” as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection or infusion techniques. In one aspect, the pharmaceutical compositions provided herewith are orally administered in an orally acceptable dosage form including, but not limited to, capsules, tablets, emulsions and aqueous suspensions, dispersions and solutions. In the case of tablets for oral use, carriers which are commonly used include one or more substances selected from microcrystalline cellulose, mannitol, croscarmellose sodium, and sodium stearyl fumarate. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions and/or emulsions are administered orally, the active ingredient may be suspended or dissolved in an oily phase is combined with emulsifying and/or suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added.

**[0037]** A secondary drug can be co-administered with mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate). The effective amount of a secondary drug that is co-administered with mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate), such as a CYP3A4/5 inducer or a CYP3A4/5 inhibitor or a p-gp inhibitor, if used in a method of the invention, may also be administered in the form of a pharmaceutical composition comprising the CYP3A4/5 inducer or the CYP3A4/5 inhibitor or the p-gp inhibitor, together with a pharmaceutically acceptable carrier. Pharmaceutical compositions of the secondary drug may be prepared and administered as described above for pharmaceutical compositions of mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate).

**[0038]** In one aspect, mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) is formulated as a tablet composition together with a pharmaceutically acceptable carrier, in accordance with the disclosures of International Patent Application No. WO2019/104134.

**[0039]** In one embodiment, in therapies comprising mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) and a secondary drug (*e.g.*, a CYP3A4/5

inducer or a CYP3A4/5 inhibitor), mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) and the secondary drug are administered “concurrently” to the subject, which means that the subject is administered both drugs on the same day on all days of the treatment period. In one embodiment, mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) and the secondary drug are administered “sequentially” to the subject, which means that the subject is administered either of the two drugs on all days of the treatment period, but not both drugs. In another embodiment, mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) and the secondary drug are administered “sequentially and concurrently” to the subject, which means that the subject is administered either of the two drugs on certain days of the treatment period, but not both drugs; and the subject is administered both drugs on the same day on other days of the treatment period.

### **Doses and Dosing Regimens**

**[0040]** The amount of mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) that may be combined with carrier materials to produce a composition in a single dosage form will vary depending upon the subject to be treated and the particular mode of administration. For example, [ ] a specific dosage and treatment regimen for any particular subject will depend upon a variety of factors, including age, body weight, general health, sex, diet, time of administration, rate of excretion, drug combination, the judgment of the treating physician, and the severity of the particular disease being treated. The amount of mitapivat or a pharmaceutically acceptable salt thereof will also depend upon the particular form (*e.g.*, free base, salt form, crystalline form, etc.) in the composition. In one aspect, a provided composition may be formulated such that a dosage equivalent to about 0.001 to about 100 mg/kg body weight/day of mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, about 0.5 to about 100 mg/kg of mitapivat or a pharmaceutically acceptable salt thereof) can be administered to a subject receiving these compositions. Alternatively, dosages equivalent to 1 mg/kg and 1000 mg/kg of mitapivat or a pharmaceutically acceptable salt thereof every 4 to 120 hours is also acceptable.

**[0041]** In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose of equivalent to about 2 mg to about 3000 mg of mitapivat. In certain embodiments, the dose is an oral dose. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 2

[illegible]

[illegible]

of [[ ]]mitapivat. In certain embodiment, the pharmaceutically acceptable salt of mitapivat is mitapivat sulfate.

**[0042]** In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 2 mg to about 3000 mg of [[ ]]mitapivat per day. In certain embodiments, [[ ]]mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 5 mg to about 500 mg of [[ ]]mitapivat of per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 5 mg to about 200 mg of [[ ]]mitapivat per day. In certain embodiments, [[ ]]mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 5 mg of [[ ]]mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 5 mg to about 10 mg of [[ ]]mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose of about 15 mg equivalent to mitapivat per day. In certain embodiments, [[ ]]mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 20 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 25 mg of [[ ]]mitapivat per day. In certain embodiments, [[ ]]mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 30 mg of [[ ]]mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 35 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 40 mg of [[ ]]mitapivat per day. In certain embodiments, [[ ]]mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 45 mg of [[ ]]mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 50 mg of [[ ]]mitapivat thereof per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 55 mg of [[ ]]mitapivat thereof per day. In certain embodiments, [[ ]]mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 60 mg of [[ ]]mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated

for administration at a dose equivalent to about 70 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 80 mg of [[ ]]mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 90 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 100 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 110 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 120 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 130 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 140 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 150 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 160 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 170 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 180 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 190 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 200 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 210 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 220 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 230 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for

administration at a dose equivalent to about 240 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 250 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 260 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 270 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 280 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 290 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 300 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 300-360 mg of mitapivat per day. Dosing can be once, twice, or three times daily. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 5 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 10 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 15 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 20 mg of mitapivat twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 25 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 30 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 35 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 40 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 45 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent

to about 50 mg of mitapivat twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 55 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 60 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 65 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 70 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 75 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 80 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 85 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 90 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 95 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 100 mg of mitapivat twice per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 5 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 10 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 15 mg of mitapivat thereof once per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 20 mg of mitapivat once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 25 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 30 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 35 mg of mitapivat thereof once per day. In

another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 40 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 45 mg of mitapivat thereof once per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 50 mg of mitapivat once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 55 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 60 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 65 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 70 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 75 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 80 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 85 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 95 mg of mitapivat thereof once per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 100 mg of mitapivat once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 110 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 120 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 130 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 140 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 150 mg of mitapivat thereof once per day. In

another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 160 mg to about 200 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 200 mg to about 360 mg of mitapivat thereof once per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 5 mg of mitapivat once every other day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 20 mg of mitapivat once every other day. In one aspect, mitapivat or a pharmaceutically acceptable salt ~~thereof~~ thereof is formulated for administration at a dose equivalent to about 50 mg of mitapivat once every other day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 100 mg of mitapivat once every other day. In certain embodiment, the pharmaceutically acceptable salt of mitapivat is mitapivat sulfate.

**[0043]** In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof by about 70% to about 80%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof by about 60% to about 70%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof by about 50% to about 60%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof by about 40% to about 50%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof by about 30% to about 40%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof by about 20% to about 30%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof by about 10% to about 20%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof by about 50%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 400 mg of mitapivat to the dose equivalent to about 200 mg. In

certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 300 mg of mitapivat to the dose equivalent to about 150 mg. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 200 mg of mitapivat to the dose equivalent to about 100 mg. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 100 mg of mitapivat to the dose equivalent to about 50 mg. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 50 mg of mitapivat to the dose equivalent to about 25 mg. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 20 mg of mitapivat to the dose equivalent to about 10 mg. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 10 mg of mitapivat to the dose equivalent to about 5 mg. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 5 mg of mitapivat to the dose equivalent to about 2.5 mg.

**[0044]** In certain embodiments, for any of the methods presented herein, further comprising reducing the dose frequency of mitapivat or a pharmaceutically acceptable salt thereof by about 70% to about 80%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose frequency of mitapivat or a pharmaceutically acceptable salt thereof by about 60% to about 70%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose frequency of mitapivat or a pharmaceutically acceptable salt thereof by about 50% to about 60%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose frequency of mitapivat or a pharmaceutically acceptable salt thereof by about 40% to about 50%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose frequency of mitapivat or a pharmaceutically acceptable salt thereof by about 30% to about 40%. In certain embodiments, for any of the methods

presented herein, further comprising reducing the dose frequency of mitapivat or a pharmaceutically acceptable salt thereof by about 20% to about 30%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose frequency of mitapivat or a pharmaceutically acceptable salt thereof by about 10% to about 20%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose frequency of mitapivat or a pharmaceutically acceptable salt thereof by about 50%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose frequency of mitapivat or a pharmaceutically acceptable salt thereof from twice daily to once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose frequency of mitapivat or a pharmaceutically acceptable salt thereof from once daily to once every other day.

**[0045]** In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 400 mg of mitapivat twice daily to the dose equivalent to about 200 mg twice daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 300 mg of mitapivat twice daily to the dose equivalent to about 150 mg twice daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 200 mg of mitapivat twice daily to the dose equivalent to about 100 mg twice daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 100 mg of mitapivat twice daily to the dose equivalent to about 50 mg twice daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 50 mg of mitapivat twice daily to the dose equivalent to about 25 mg twice daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 20 mg of mitapivat twice daily to the dose equivalent to about 10 mg twice daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 10 mg of mitapivat twice daily to the dose equivalent to about 5 mg twice daily. In certain embodiments, for any of the methods presented herein, further

comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 5 mg twice daily of mitapivat to the dose equivalent to about 2.5 mg twice daily.

**[0046]** In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 400 mg of mitapivat once daily to the dose equivalent to about 200 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 300 mg of mitapivat once daily to the dose equivalent to about 150 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 200 mg of mitapivat once daily to the dose equivalent to about 100 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 100 mg of mitapivat once daily to the dose equivalent to about 50 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 50 mg of mitapivat once daily to the dose equivalent to about 25 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 20 mg of mitapivat once daily to the dose equivalent to about 10 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 10 mg of mitapivat once daily to the dose equivalent to about 5 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 5 mg once daily of mitapivat to the dose equivalent to about 2.5 mg once daily.

**[0047]** In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 400 mg of mitapivat twice daily to the dose equivalent to about 200 mg once daily. In certain embodiments, for any of the methods presented herein, further

comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 300 mg of mitapivat twice daily to the dose equivalent to about 150 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 200 mg of mitapivat twice daily to the dose equivalent to about 100 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 100 mg of mitapivat twice daily to the dose equivalent to about 50 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 50 mg of mitapivat twice daily to the dose equivalent to about 25 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 20 mg of mitapivat twice daily to the dose equivalent to about 10 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 10 mg of mitapivat twice daily to the dose equivalent to about 5 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 5 mg twice daily of mitapivat to the dose equivalent to about 2.5 mg once daily.

**[0048]** In some embodiments, mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) is present in the tablet composition in an amount equivalent to about 1 to about 200 mg of mitapivat. In some embodiments, mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) is present in the tablet composition in an amount equivalent to about 1 to about 150 mg of mitapivat. In some embodiments, mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) is present in the tablet composition in an amount equivalent to about 1 to about 100 mg of mitapivat. In some embodiments, mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) is present in the tablet composition in an amount equivalent to about 5 mg of mitapivat. In some embodiments, mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) is present in the tablet composition in an amount equivalent to about 20 mg of mitapivat. In some embodiments, mitapivat or a pharmaceutically acceptable salt thereof

(e.g., mitapivat sulfate) is present in the tablet composition in an amount equivalent to about 50 mg of mitapivat. In some embodiments, mitapivat or a pharmaceutically acceptable salt thereof (e.g., mitapivat sulfate) is present in the tablet composition in an amount equivalent to about 75 mg of mitapivat. In some embodiments, mitapivat or a pharmaceutically acceptable salt thereof (e.g., mitapivat sulfate) is present in a tablet composition in an amount equivalent to about 100 mg of mitapivat. In certain embodiment, the pharmaceutically acceptable salt of mitapivat is mitapivat sulfate.

**[0049]** As used herein, the dose amount of mitapivat or a pharmaceutically acceptable salt thereof (e.g., mitapivat sulfate) is based on the equivalence to the free-base form of mitapivat. For example, “mitapivat or a pharmaceutically acceptable salt thereof present in the composition in an amount equivalent to about 2.0 mg of mitapivat” means about 2.0 mg of free-base mitapivat or about 2.33 mg of mitapivat sulfate having the structural formula as depicted in the background of the present disclosure.

**Methods of Treatment (Mitapivat Mitapivat Monotherapy and Concomitant Medications)**

**[0050]** In one aspect, the present disclosure provides methods of treating a disease, disorder or condition with [[ ]]mitapivat or a pharmaceutically acceptable salt thereof and a CYP3A4/5 inducer or a CYP3A4/5 inhibitor or a p-gp inhibitor; use of mitapivat and a CYP3A4/5 inducer or a CYP3A4/5 inhibitor or a p-gp inhibitor for the manufacture of a medicament for treating a disease, disorder or condition; and mitapivat or a pharmaceutically acceptable salt thereof and a CYP3A4/5 inducer or a CYP3A4/5 inhibitor or a p-gp inhibitor for use in treating a disease, disorder or condition. The disease, disorder or condition treated is pyruvate kinase deficiency (PKD), sickle cell disease, or thalassemia as further discussed in the following embodiments, and any one disease, disorder or condition described in the section “Diseases, Disorders and Conditions” provided in the present disclosure.

**[0051]** In one embodiment, the present disclosure relates to a method of treating pyruvate kinase deficiency (PKD) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inducer of cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5).

**[0052]** In another embodiment, the present disclosure relates to a method of treating sickle cell disease in a subject, comprising administering to the subject an effective amount of

mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inducer of cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5).

**[0053]** In yet another embodiment, the present disclosure relates to a method of treating thalassemia in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inducer of cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5). In certain embodiments, the thalassemia is alpha thalassemia. In certain embodiments, the thalassemia is beta thalassemia.

**[0054]** In one embodiment, the method of any one of the preceding embodiments further comprises:

- (a) monitoring the subject for an effect of a drug-drug interaction between mitapivat or a pharmaceutically acceptable salt thereof and the CYP3A4/5 inducer; and
- (b) in the event that an effect of the drug-drug interaction is present, adjusting the effective amount of mitapivat or a pharmaceutically acceptable salt thereof and/or the effective amount of the CYP3A4/5 inducer being administered to the subject to reduce the effect.

**[0055]** In some embodiments, the adjustment is to reduce the dose of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the adjustment is to reduce the dose frequency of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the adjustment is to reduce the dose and dose frequency of mitapivat or a pharmaceutically acceptable salt thereof. Details of the dose and/or dose frequency adjustment are shown in the dose and dose regime section.

**[0056]** In one embodiment, the method of the preceding embodiment further comprises:

- (c) repeating (a) and (b) until there are no further detectable symptoms of the drug-drug interaction.

Steps (a) and (b) can be repeated as many times as necessary until there are no further detectable symptoms of the drug-drug interaction. In one embodiment, step (a) comprises measuring and analyzing the pharmacokinetic (PK) such as AUC (*i.e.*, area under the plasma concentration-time curve, such as AUC<sub>0-24</sub>, AUC<sub>0-t</sub>, AUC<sub>0-inf</sub>, AUC<sub>%estrap</sub>) and C<sub>max</sub> values (*i.e.*, the maximum (or peak) serum concentration that a drug achieves in a specified

compartment or test area of the body after the drug has been administered and before the administration of a second dose). In one embodiment, step (a) comprises measuring and analyzing other PK parameters such as but not limited to  $CL/F$  (*i.e.*, apparent total body clearance of the drug from plasma),  $V_z/F$  (*i.e.*, apparent volume of distribution during terminal phase after non-intravenous administration)[[ ]],  $t_{1/2}$  (elimination half-life),  $t_{last}$ ,  $t_{max}$  (time to reach maximum (peak) plasma concentration following drug administration), etc.

**[0057]** A “drug-drug interaction” as used herein refers to a change in concentration in a patient of a first drug (*e.g.*, ~~mitapivat~~ ~~mitipivat~~) and consequential change in the effect of the first drug on the subject/patient resulting from administration of a second drug (*e.g.*, a CYP3A4/5 inducer, a CYP3A4/5 inhibitor). The change in concentration and consequential change in effect of the first drug resulting from the second drug typically occurs because of a change in activity or expression level of a detoxification enzyme that acts to clear the first drug from the patient (such as CYP3A4/5), wherein the change in activity or expression level of the detoxification enzyme is caused by the second drug. An increase in activity or expression level of the detoxification enzyme will tend to decrease the concentration of the first drug in the patient and its consequential effect on the patient, whereas a decrease in activity or expression level of the detoxification enzyme will tend to increase the concentration of the first drug in the patient and its consequential effect on the patient.

**[0058]** An “effect of a drug-drug interaction” as used herein refers to a detectable and measurable effect, such as a detectable symptom, on the subject/patient caused by the drug-drug interaction. Where the concentration of the first drug is increased, such as when mitapivat is co-administered with a CYP3A4/5 inhibitor, the “effect” typically refers to undesired phenomena such as an overdose of the first drug, liver failure, kidney failure, and the prevalence of side effects and adverse reactions. Where the concentration of the first drug is decreased, such as when mitapivat is co-administered with a CYP3A4/5 inducer, the “effect” typically refers to a decrease in the therapeutic efficacy of the first drug (*e.g.*, mitapivat). Exemplary effects of a drug-drug interaction as used herein further include headache, insomnia, nausea, viral upper respiratory tract infection, arthralgia, diarrhoea, fatigue, hot flush, influenza, vomiting, cough, dizziness, oropharyngeal pain, pyrexia, back pain, dysmenorrhoea, and gastroenteritis, and dyspepsia.[[.]] ~~Hypertiglyceridemia~~ hypertriglyceridemia, hemolytic anemia, hemolysis, dizziness, etc.

**[0059]** In one embodiment, the present disclosure relates to a method of treating pyruvate kinase deficiency (PKD) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inhibitor of cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5).

**[0060]** In another embodiment, the present disclosure relates to a method of treating sickle cell disease in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inhibitor of cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5).

**[0061]** In yet another embodiment, the present disclosure relates to a method of treating thalassemia in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inhibitor of cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5) or a p-gp inhibitor. In certain embodiments, the thalassemia is alpha thalassemia. In certain embodiments, the thalassemia is beta thalassemia.

**[0062]** In one embodiment, the method of any one of the suitable preceding embodiments further comprises:

(a) monitoring the subject for a symptom of a drug-drug interaction between mitapivat or a pharmaceutically acceptable salt thereof and the CYP3A4/5 inhibitor; and

(b) in the event that a symptom of the drug-drug interaction is present, adjusting the effective amount of mitapivat or a pharmaceutically acceptable salt thereof and/or the [[ ]]effective amount of the CYP3A4/5 inhibitor being administered to the subject to reduce the effect.

**[0063]** In some embodiments, the adjustment is to reduce the dose of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the adjustment is to reduce the dose frequency of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the adjustment is to reduce the dose and dose frequency of mitapivat or a pharmaceutically acceptable salt thereof. Details of the dose and/or dose frequency adjustment are shown in the dose and dose regime section.

**[0064]** In one embodiment, the method of the preceding embodiment further comprises:

(c) repeating [[ ]](a) and (b) until there are no further detectable symptoms of the drug-drug interaction.

Likewise, steps (a) and (b) can be repeated as many times as necessary until there are no further detectable symptoms of the drug-drug interaction. Ways of monitoring the subject for a symptom of a drug-drug interaction between mitapivat or a pharmaceutically acceptable salt thereof and the CYP3A4/5 inhibitor as recited in step (a) are as described above.

**[0065]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject the effective amount of mitapivat or a pharmaceutically acceptable salt thereof and the effective amount of the CYP3A4/5 inducer (or inhibitor) sequentially.

**[0066]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject the effective amount of mitapivat or a pharmaceutically acceptable salt thereof and the effective amount of the CYP3A4/5 inducer (or inhibitor) concurrently.

**[0067]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject the effective amount of mitapivat or a pharmaceutically acceptable salt thereof and the effective amount of the CYP3A4/5 inducer (or inhibitor) sequentially and concurrently. As used herein, “co-administered” or “in combination with” encompasses administration of mitapivat or a pharmaceutically acceptable salt thereof and the CYP3A4/5 inducer (or inhibitor) sequentially and concurrently, or at different intervals. In another embodiment, for the method of any one of the embodiments, the CYP3A4/5 inducer is a mild CYP3A4/5 inducer. In another embodiment, for the method of any one of the embodiments, the CYP3A4/5 inhibitor is a mild CYP3A4/5 inhibitor. In another embodiment, for the methods of any one of the embodiments, the CYP3A4/5 inhibitor is a moderate CYP3A4/5 inhibitor.

**[0068]** In another aspect, the present disclosure provides methods of treating a disease, disorder or a condition with [[ ]]mitapivat in the absence of a CYP3A4/5 inducer or a CYP3A4/5 inhibitor or a p-gp inhibitor; use of mitapivat in the absence of a CYP3A4/5 inducer or a CYP3A4/5 inhibitor or a p-gp inhibitor for the manufacture of a medicament for treating disease, disorder or a condition; and mitapivat in the absence of a CYP3A4/5 inducer

or a CYP3A4/5 inhibitor or a p-gp inhibitor for use in treating a disease, disorder or a condition.

**[0069]** As used herein, “in the absence of” when referring to a secondary drug co-administered with mitapivat (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor or a p-gp inhibitor), refers to levels of the secondary drug in the subject/patient that are sufficiently low that such that any change in the levels of the detoxification enzymes for mitapivat and the secondary drug results in no detectable symptoms of a drug-drug interaction between mitapivat and the secondary drug.

**[0070]** In one embodiment, the present disclosure relates to a method of treating pyruvate kinase deficiency (PKD) in a subject, comprising administering to the subject ☐ an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of an inducer of cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5).

**[0071]** In another embodiment, the present disclosure relates to a method of treating sickle cell disease in a subject, comprising administering to the subject ☐ an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of an inducer of cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5).

**[0072]** In yet another embodiment, the present disclosure relates to a method of treating thalassemia in a subject, comprising administering to the subject ☐ an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of an inducer of cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5). In some embodiments, the thalassemia is alpha thalassemia. In some embodiments, the thalassemia is beta thalassemia.

**[0073]** In one embodiment, in accordance with the method of any one of the suitable preceding embodiments, administration of a CYP3A4/5 inducer, if any, is terminated at a sufficient time interval prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof such that there is no detectable symptom of a drug-drug interaction ☐ between mitapivat or a pharmaceutically acceptable salt thereof and the CYP3A4/5 inducer. In certain embodiments, the CYP3A4/5 inducer is a strong CYP3A4/5 inducer. In certain embodiments, the CYP3A4/5 inducer is a moderate CYP3A4/5 inducer. In some embodiments, the CYP3A4/5 inducer is terminated at least 30 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inducer is terminated at least 28 days prior to

the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inducer is terminated at least 25 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inducer is terminated at least 21 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inducer is terminated at least 15 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inducer is terminated at least 14 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inducer is terminated at least 10 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inducer is terminated at least 7 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inducer is terminated at least 5 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof.

**[0074]** In one embodiment, the present disclosure relates to a method of treating pyruvate kinase deficiency (PKD) in a subject, comprising administering to the subject ☐ an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of an inhibitor of cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5).

**[0075]** In another embodiment, the present disclosure relates to a method of treating sickle cell disease in a subject, comprising administering to the subject ☐ an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of an inhibitor of cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5).

**[0076]** In yet another embodiment, the present disclosure relates to a method of treating thalassemia in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of an inhibitor of cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5). In certain embodiment, the thalassemia is alpha-thalassemia. In certain embodiment, the thalassemia is beta-thalassemia.

**[0077]** In one embodiment, in accordance with the method of any one of the suitable preceding embodiments, administration of a CYP3A4/5 inhibitor, if any, is terminated at a

sufficient time interval prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof such that there is no detectable symptom of a drug-drug interaction between mitapivat or a pharmaceutically acceptable salt thereof and the CYP3A4/5 inhibitor. In certain embodiments, the CYP3A4/5 inhibitor is a strong CYP3A4/5 inhibitor. In certain embodiments, the CYP3A4/5 inhibitor is a moderate CYP3A4/5 inhibitor. In some embodiments, the CYP3A4/5 inhibitor is terminated at least 21 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inhibitor is terminated at least 15 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inhibitor is terminated at least 14 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inhibitor is terminated at least 10 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inhibitor is terminated at least 7 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inhibitor is terminated at least 5 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inhibitor is terminated at least 3 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inhibitor is terminated at least 2 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inhibitor is terminated at least 1 day prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof.

**[0078]** In one embodiment, the present disclosure relates to a method of treating pyruvate kinase deficiency (PKD) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of a p-gp inhibitor.

**[0079]** In another embodiment, the present disclosure relates to a method of treating sickle cell disease in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of a p-gp inhibitor.

**[0080]** In yet another embodiment, the present disclosure relates to a method of treating thalassemia in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of a p-gp inhibitor. In certain embodiment, the thalassemia is alpha-thalassemia. In certain embodiment, the thalassemia is beta-thalassemia.

**[0081]** In one embodiment, in accordance with the method of any one of the suitable preceding embodiments, administration of a p-gp inhibitor, if any, is terminated at a sufficient time interval prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof such that there is no detectable symptom of a drug-drug interaction between mitapivat or a pharmaceutically acceptable salt thereof and the p-gp inhibitor. In certain embodiments, the p-gp inhibitor is a strong p-gp inhibitor ([e.g.] e.g., amiodarone, azithromycin, captopril, carvedilol, clarithromycin, conivaptan, cyclosporine, diltiazem, dronedarone, erythromycin, felodipine, itraconazole, ketoconazole, lopinavir, ritonavir, quercetin, quinidine, ranolazine, ticagrelor, and erapamil). In some embodiments, the p-gp inhibitor ~~inhibitor~~ is terminated at least 21 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the p-gp inhibitor ~~inhibitor~~ is terminated at least 15 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the p-gp inhibitor ~~inhibitor~~ is terminated at least 14 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the p-gp inhibitor ~~inhibitor~~ is terminated at least 10 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the p-gp inhibitor ~~inhibitor~~ is terminated at least 7 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the p-gp inhibitor ~~inhibitor~~ is terminated at least 5 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the p-gp inhibitor ~~inhibitor~~ is terminated at least 3 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the p-gp inhibitor ~~inhibitor~~ is terminated at least 2 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the p-gp inhibitor ~~inhibitor~~ is terminated at least 1 day prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof.

**[0082]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject ☐ about 1 mg to about 2500 mg, or about 5 mg to 2500 mg, or about 30 mg to 2500 mg, or about 5 mg to about 700 mg, or about 15 mg to about 700 mg, about 5 mg to about 360 mg, about 5 mg to about 300 mg, about 50 mg to about 300 mg, about 120 mg to about 360 mg, about 120 mg to about 300 mg, about 5 mg to about 120 mg, about 5 mg, about 20 mg, about 50 mg, about 100 mg, about 120 mg, about 200 mg, about 300 mg, or about 360 mg of mitapivat or a pharmaceutically acceptable salt thereof.

**[0083]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject ☐ about 1 mg to about 2500 mg of mitapivat or a pharmaceutically acceptable salt thereof.

**[0084]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject ☐ about ~~about~~ 5 mg to 2500 mg of mitapivat or a pharmaceutically acceptable salt thereof.

**[0085]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject ☐ about 5 mg to about 360 mg of mitapivat or a pharmaceutically acceptable salt thereof.

**[0086]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject ☐ about 5 mg to about 300 mg of mitapivat or a pharmaceutically acceptable salt thereof.

**[0087]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject ☐ about 50 mg to about 300 mg of mitapivat or a pharmaceutically acceptable salt thereof.

**[0088]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject about 5 mg of mitapivat or a pharmaceutically acceptable salt thereof.

**[0089]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject about 20 mg of mitapivat or a pharmaceutically acceptable salt thereof.

**[0090]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject about 50 mg of mitapivat or a pharmaceutically acceptable salt thereof.

**[0091]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject about 100 mg of mitapivat or a pharmaceutically acceptable salt thereof.

**[0092]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject the effective amount of mitapivat or a pharmaceutically acceptable salt thereof once daily.

**[0093]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject the effective amount of mitapivat or a pharmaceutically acceptable salt thereof twice daily.

**[0094]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject a sulfate salt of mitapivat, such as a sulfate salt of mitapivat having the structural formula depicted in the background of the present disclosure.

**[0095]** In one embodiment, in the method of any one of the suitable preceding embodiments, the CYP3A4/5 inducer is a strong inducer of CYP3A4/5.

**[0096]** In one embodiment, in the method of any one of the suitable preceding embodiments, the CYP3A4/5 inducer is a moderate inducer of CYP3A4/5.

**[0097]** In one embodiment, in the method of any one of the suitable preceding embodiments, the CYP3A4/5 inducer is a mild inducer of CYP3A4/5.

**[0098]** In one embodiment, in the method of any one of the suitable preceding [[  
]]embodiments, the inducer of CYP3A4/5 is a strong CYP3A4/5 inducer selected from a group consisting of apalutamide, enzalutamide, mitotane, phenytoin, efavirenz, nevirapine, carbamazepine, glucocorticoids, modafinil, oxcarbazepine, phenobarbital, henytoin, pioglitazone, rifabutin, rifampin, St. John's Wort, and troglitazone.

**[0099]** In one embodiment, in the method of any one of the suitable preceding [[  
]]embodiments, the inducer of CYP3A4/5 is a moderate CYP3A4/5 inducer selected from a group consisting of bosentan, etravirine, phenobarbital, and primidone.

**[00100]** In one embodiment, in the method of any one of the suitable preceding [[  
]]embodiments, the inducer of CYP3A4/5 is a mild CYP3A4/5 inducer selected from a group consisting of armodafinil, modafinil, and rufinamide.

**[00101]** In one embodiment, in the method of any one of the suitable preceding embodiments, the CYP3A4/5 inducer is selected from the group consisting of aminoglutethimide, bexarotene, bosentan, carbamazepine, dexamethasone, efavirenz, fosphenytoin, griseofulvin, modafinil, nafcillin, nevirapine, oxcarbazepine, phenobarbital, phenytoin, primidone, rifabutin, rifampin, rifapentine, St. John's wort, avasimibe, dabrafenib, mifepristone, etravirine, armodafinil, glycerol phenylbutyrate, asunaprevir, rifunamide, oritavancin, clobazam, felbamate, eslicarbazepine acetate, and genistein.

**[00102]** In one embodiment, in the method of any one of the suitable preceding embodiments, the CYP3A4/5 inducer is rifampin. In one aspect, rifampin is administered to the subject in the form of a capsule composition. In one aspect, about 0.1-1000 mg of rifampin is administered to the subject. In one aspect, about 100-1000 mg of rifampin is administered to the subject.

**[00103]** In one embodiment, the method of any one of the suitable preceding embodiments comprises administering about 600 mg of rifampin to the subject. In one aspect, the method of any one of the suitable preceding embodiments comprises administering about 600 mg of rifampin to the subject once daily.

**[00104]** In one embodiment, in the method of any one of the suitable preceding embodiments, the inhibitor of CYP3A4/5 is a strong inhibitor of CYP3A4/5.

**[00105]** In one embodiment, in the method of any one of the suitable preceding [[  
]]embodiments, the inhibitor of CYP3A4/5 is a moderate inhibitor of CYP3A4/5.

**[00106]** In one embodiment, in the method of any one of the suitable preceding [[  
]]embodiments, the inhibitor of CYP3A4/5 is a mild inhibitor of CYP3A4/5.

**[00107]** In one embodiment, in the method of any one of the suitable preceding embodiments, the inhibitor of CYP3A4/5 is selected from the group consisting of toamiodarone, amprenavir, aprepitant, atazanavir, chloramphenicol, clarithromycin, conivaptan, cyclosporine, darunavir, dasatinib, delavirdine, diltiazem, erythromycin, fluconazole, fluoxetine, fluvoxamine, fosamprenavir, imatinib, indinavir, isoniazid, itraconazole, ketoconazole, lapatinib, miconazole, nefazodone, nelfinavir, posaconazole, ritonavir, quinupristin, saquinavir, tamoxifen, telithromycin, troleandomycin, verapamil, voriconazole, grapefruit, Seville oranges, limes, pomelos, fruit juices, and vegetables from mustard green family.

**[00108]** In one embodiment, in the method of any one of the suitable preceding [[ ]]embodiments, the inhibitor of CYP3A4/5 is a strong CYP3A4/5 inhibitor selected from a group consisting of boceprevir, cobicistat, danoprevir, elvitegravir, ritonavir, grapefruit juice, indinavir, itraconazole, ketoconazole, lopinavir, paritaprevir, ombitasvir, dasabuvir, posaconazole, saquinavir, telaprevir, tipranavir, telithromycin, troleandomycin, nelfinavir, clarithromycin, nefazodone, saquinavir, suboxone, and voriconazole.

**[00109]** In one embodiment, in the method of any one of the suitable preceding [[ ]]embodiments, the inhibitor of CYP3A4/5 is a moderate CYP3A4/5 inhibitor selected from a group consisting of aprepitant, ciprofloxacin, conivaptan, crizotinib, cyclosporine, diltiazem, dronedarone, erythromycin, fluconazole, fluvoxamine, imatinib, tofisopam, and verapamil.

**[00110]** In one embodiment, in the method of any one of the suitable preceding [[ ]]embodiments, the inhibitor of CYP3A4/5 is a mild CYP3A4/5 inhibitor selected from a group consisting of chlorzoxazone, cilostazol, cimetidine, clotrimazole, fosaprepitant, istradefylline, ivacaftor, lomitapide, ranitidine, ranolazine, ticagrelor.

**[00111]** As used herein, the terms “weak inhibitor” and ‘mild inhibitor” are used interchangeably.

**[00112]** As used herein, the terms “weak inducer” and ‘mild inducer” are used interchangeably.

**[00113]** In one embodiment, in the method of any one of the suitable preceding [[ ]]embodiments, the inhibitor of CYP3A4/5 is itraconazole. In one aspect, about 0.1-1000 mg of [[ ]]itraconazole is administered to the subject. In one aspect, itraconazole is administered

in the form of an oral solution. In one aspect, about 50-500 mg of [[ ]]itraconazole is administered to the subject.

**[00114]** In one embodiment, the method of any one of the suitable preceding [[ ]]embodiments comprises administering about 200 mg of itraconazole to the subject. In one aspect, about 0.1-1000 mg of [[ ]]itraconazole is administered to the subject. In one aspect, about 50-500 mg of [[ ]]itraconazole is administered to the subject. In one aspect, about 200 mg of [[ ]]itraconazole is administered to the subject. In one aspect, about 200 mg of [[ ]]itraconazole is administered to the subject once daily. In one aspect, about 100 mg of [[ ]]itraconazole is administered to the subject. In one aspect, about 100 mg of [[ ]]itraconazole is administered to the subject once daily. In one aspect, itraconazole is administered in the form of an oral solution.

### **Diseases, Disorders and Conditions**

**[00115]** Pyruvate kinase deficiency (PKD) is a glycolytic enzymopathy that results in life-long hemolytic anemia. In certain embodiments, the subject having PKD is a patient having at least 2 mutant alleles in PKLR gene. In certain embodiments, the subject having PKD is a patient having at least 2 mutant alleles in PKLR gene and at least one is a missense mutation. See Canu[[.]] et[[.]] al., Blood Cells, Molecules and Diseases **2016**, 57, pp. 100-109. In certain embodiments, a subject having PKD has an Hb concentration less than or equal to 10.0 g/dL. In certain embodiments, the subject having PKD is an adult not under regular transfusion ([[e.g.]]e.g., having had no more than 4 transfusion episodes in the 12-month period up to the treatment). In certain embodiments, the subject having PKD is an adult transfusion independent ([[e.g.]]e.g., having no more than 3 units of RBCs transfused in the 12-month period prior to the treatment). In certain embodiments, the subject having PKD is an adult under regular transfusion ([[e.g.]]e.g., having had at least 4 transfusion episodes ([[e.g.]]e.g., at least 6 transfusion episodes) in the 12-month period prior to the treatment). In certain embodiments, the subject having PKD has a total number of at least 5 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 10 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 15 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 20 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 25 transfusion episodes

during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 30 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 40 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 50 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 60 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 70 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD is not homozygous for the R479H mutation or does not have 2 non-missense mutations in the PKLR gene. In certain embodiments, the subject having PKD, under regular transfusion, has hemoglobin (Hb)  $\leq 12.0$  g/dL (if male) or  $\leq 11.0$  g/dL (if female), prior to the treatment. In certain embodiments, the subject having PKD, under regular transfusion, has transfusion occurring on average less than or equal to once every three weeks. In certain embodiments, the subject having PKD has received at least 0.8 mg ([e.g.] e.g., at least 1.0 mg) folic acid daily ([e.g.] e.g., for at least 21 days) prior to the treatment. In certain embodiments, the subject with PKD under regular transfusion achieves a reduction in transfusion burden ([e.g.] e.g., at least 33% reduction in the number of RBC units transfused) during the 5 weeks, 10 weeks, 15 weeks, 20 weeks, or 24 weeks, 28 weeks, or 32 weeks of treatment. In certain embodiments, the subject having PKD, not under regular transfusion (having had no more than 4 transfusion episodes in the 12-month period prior to the treatment and/or no transfusion in the 3 months prior to the treatment), has hemoglobin (Hb)  $\leq 10.0$  g/dL regardless of gender prior to the treatment. In certain embodiments, the subject having PKD has undergone splenectomy. In certain embodiment, the subject having PKD is a child 18 years old or younger. In certain embodiments, the child is transfusion dependent.

**[00116]** Exemplified conditions related to PKD include, but are not limited to, anemias, cholecystolithiasis, gallstones, tachycardia, hemochromatosis, icteric sclera, splenomegaly, leg ulcers, jaundice, fatigue, and shortness of breath. As described herein, PKD is a deficiency of PKR. In certain embodiments, the deficiency of PKR is associated with a PKR mutation.

**[00117]** In certain embodiments, the subject with PKD achieves a hemoglobin response of at least 1.0 g/dL increase in Hb concentration after the treatment compared to the baseline of

prior to the treatment. In certain embodiments, the subject with PKD achieves a hemoglobin response of at least 1.5 g/dL increase in Hb concentration from baseline prior to the treatment. In certain embodiments, the subject with PKD achieves a hemoglobin response of at least 2.0 g/dL increase in Hb concentration from baseline prior to the treatment.

**[00118]** In an embodiment, the mutant PKR is selected from the group consisting of A31V, A36G, G37Q, R40W, R40Q, L73P, S80P, P82H, R86P, I90N, T93I, G95R, M107T, G111R, A115P, S120F, H121Q, S130P, S130Y, V134D, R135D, A137T, G143S, I153T, A154T, L155P, G159V, R163C, R163L, T164N, G165V, L167M, G169G, E172Q, W201R, I219T, A221Y, D221N, G222A, I224T, G232C, N253D, G263R, G263W, E266K, V269F, L272V, L272P, G275R, G275R, E277K, V280G, D281N, F287V, F287L, V288L, D293N, D293V, A295I, A295V, I310N, I314T, E315K, N316K, V320L, V320M, S330R, D331N, D331G, D331E, G332S, V335M, A336S, R337W, R337P, R337Q, D339N, D339Q, G341A, G341D, I342F, K348N, A352D, I357T, G358R, G358E, R359C, R359H, C360Y, N361D, G364D, K365M, V368F, T371I, L374P, S376I, T384M, R385W, R385K, E387G, D390N, A392T, N393D, N393S, N393K, A394S, A394D, A394V, V395L, D397V, G398A, M403I, G406R, E407K, E407G, T408P, T408A, T408I, K410E, G411S, G411A, Q421K, A423A, A423A, R426W, R426Q, E427A, E427N, A431T, R449C, I457V, G458D, A459V, V460M, A468V, A468G, A470D, T477A, R479C, R479H, S485F, R486W, R486L, R488Q, R490W, I494T, A495T, A495V, R498C, R498H, A503V, R504L, Q505E, V506I, R510Q, G511R, G511E, R518S, R531C, R532W, R532Q, E538D, G540R, D550V, V552M, G557A, R559G, R559P, N566K, M568V, R569Q, R569L, Q58X, E174X, W201X, E241X, R270X, E440X, R486X, Q501X, L508X, R510X, E538X, R559X. These mutations are described in Canu et al., *Blood Cells, Molecules and Diseases* **2016**, 57, pp. 100-109. In an embodiment, the mutant PKR is selected from G332S, G364D, T384M, K410E, R479H, R479K, R486W, R532W, R510Q, and R490W. In certain embodiments, the mutant PKR is selected from A468V, A495V, I90N, T408I, and Q421K, and R498H. In certain embodiments, the mutant PKR is R532W, K410E, or R510Q. In certain embodiments, the mutant PKR is R510Q, [[R486W, or R479H.

**[00119]** In other aspects, provided are methods of treating a disease selected from hemolytic anemia, hereditary spherocytosis, hereditary elliptocytosis, abetalipoproteinemia, Bassen-Kornzweig syndrome, and paroxysmal nocturnal hemoglobinuria in a subject in need thereof, comprising administering to the subject an effective amount of mitapivat or a

pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor). Also provided is mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor), for use in treating disease selected from hemolytic anemia, hereditary spherocytosis, hereditary elliptocytosis, abetalipoproteinemia, Bassen-Kornzweig syndrome, and paroxysmal nocturnal hemoglobinuria in a subject. Further provided is the use of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor), in the manufacture of a medicament for treating a disease selected from hemolytic anemia, hereditary spherocytosis, hereditary elliptocytosis, abetalipoproteinemia, Bassen-Kornzweig syndrome, and paroxysmal nocturnal hemoglobinuria in a subject in need thereof. In one aspect, the disease to be treated is hemolytic anemia.

**[00120]**   Thalassemia is an inherited blood disorder in which the normal ratio of  $\alpha$ - to  $\beta$ -globin production is disrupted due to a disease-causing variant in 1 or more of the globin genes. In certain embodiments, Alpha-globin aggregates (as found in  $\beta$ -thalassemia) readily precipitate, which disrupts the red blood cell (RBC) membrane and results in oxidative stress. In certain embodiments, Beta-globin tetramers (Hb H, found in  $\alpha$ -thalassemia) are generally more soluble, but are still unstable and can form precipitates. The imbalance of the globin chain synthesis can lead to a net reduction in Hb concentrations and has dramatic effects on the survival of RBC precursors, ultimately resulting in their premature destruction in the bone marrow and in extramedullary sites (Cappellini et al., 2014). In certain embodiments, the disorder results in large numbers of red blood cells being destroyed, which leads to anemia. In certain embodiments, the thalassemia is alpha thalassemia. In certain embodiments, the thalassemia is beta thalassemia. In other embodiments, the thalassemia is non-transfusion-dependent thalassemia[[ (]]. In other embodiments, the thalassemia is beta thalassemia intermedia. In other embodiments, the thalassemia is Hb E beta thalassemia. In other embodiments, the thalassemia is beta thalassemia with mutations of 1 or more alpha genes.

**[00121]**   In certain embodiments, the subject is an adult subject with thalassemia. In certain embodiments, the subject has thalassemia such as  $\beta$ -thalassemia intermedia, Hb E  $\beta$ -thalassemia,  $\alpha$ -thalassemia (Hb H disease), or  $\beta$ -thalassemia with mutations of 1 or more  $\alpha$  genes. In certain embodiments, the subject has non-transfusion-dependent thalassemia. In

certain embodiments, the subject has non-transfusion-dependent alpha thalassemia. In certain embodiments, the subject has beta-thalassemia or non-transfusion-dependent beta-thalassemia. In certain embodiments, the subject is an adult male subject with thalassemia such as beta-thalassemia or non-transfusion-dependent thalassemia. In certain embodiments, the subject is a female subject with thalassemia such as beta-thalassemia or non-transfusion-dependent thalassemia. In certain embodiments, the subject is an adult female subject with thalassemia such as beta-thalassemia or non-transfusion-dependent thalassemia. In certain embodiments, the subject has a hemoglobin concentration of less than or equal to 6.0 g/dL. In certain embodiments, the subject has a hemoglobin concentration of less than or equal to 7.0 g/dL. In certain embodiments, the subject has a hemoglobin concentration of less than or equal to 8.0 g/dL. In certain embodiments, the subject has a hemoglobin concentration of less than or equal to 9.0 g/dL. In certain aspects, the subject having non-transfusion-dependent thalassemia does not have a known history ([e.g.] e.g., has been diagnosed in the past) of Hb S or Hb C forms of thalassemia. In certain embodiments, the term “non-transfusion dependent” thalassemia refers to subjects with thalassemia having no more than 4 ([e.g.] e.g., five) units of RBCs transfused during a 24-week period up to the first day of administration of mitapivat and/or no RBC transfusions in the 8 weeks prior to the first day of administration of mitapivat. In certain embodiment, the subject is an adult over 18 years old. In certain embodiment, the subject is a child 18 years old or younger.

**[00122]** As used herein, sickle cell disease (SCD), Hemoglobin SS disease, and sickle cell anemia are used interchangeably. Sickle cell disease (SCD) is an inherited blood disorder caused by the presence of sickle hemoglobin (HbS). In certain embodiments, subjects with SCD have abnormal hemoglobin, called hemoglobin S or sickle hemoglobin, in their red blood cells. In certain embodiments, people having SCD have at least one abnormal gene ~~genes~~ causing the body to make hemoglobin S. In certain embodiments, people having SCD have two hemoglobin S genes, Hemoglobin SS.

**[00123]** In other aspects, provided herein are methods for treating anemia in a subject in need thereof comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug ([e.g.] e.g., a CYP3A4/5 inducer or a CYP3A4/5 inhibitor). Also provided is mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug ([e.g.] e.g., a

CYP3A4/5 inducer or a CYP3A4/5 inhibitor), for use in treating anemia in a subject in need thereof. Further provided is the use of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug ([e.g.]e.g., a CYP3A4/5 inducer or a CYP3A4/5 inhibitor), in the manufacture of a medicament for treating anemia. In one aspect, the anemia to be treated is dyserythropoietic anemia.

**[00124]** In certain embodiments, the anemia is a dyserythropoietic anemia such as congenital dyserythropoietic anemia type I, II, III, or IV. In certain embodiments, the anemia is hemolytic anemia. In certain embodiments, the hemolytic anemia is a congenital and/or hereditary form of hemolytic anemia such as PKD, sickle cell disease, thalassemias (*e.g.*, alpha or beta or non-transfusion-dependent thalassemia), hereditary spherocytosis, hereditary elliptocytosis), paroxysmal nocturnal hemoglobinuria, abeta-lipoproteinemia (Bassen-Kornzweig syndrome). In certain embodiments, the hemolytic anemia is acquired hemolytic anemia such as autoimmune [  ]hemolytic anemia, drug-induced hemolytic anemia. In certain embodiments, the hemolytic anemia is anemia as part of a multi-system disease, such as the anemia of Congenital Erythropoietic Purpura, Fanconi, Diamond-Blackfan.

**[00125]** As used herein, the term “anemia” refers to a deficiency of red blood cells (RBCs) and/or hemoglobin. As used herein, anemia includes all types of clinical anemia, for example (but not limited to): microcytic anemia, iron deficiency anemia, hemoglobinopathies, heme synthesis defect, globin synthesis defect, sideroblastic defect, normocytic anemia, anemia of chronic disease, aplastic anemia, hemolytic anemia, macrocytic anemia, megaloblastic anemia, pernicious anemia, dimorphic anemia, anemia of prematurity, Fanconi anemia, hereditary spherocytosis, sickle cell disease, warm autoimmune hemolytic anemia, cold agglutinin hemolytic anemia, osteopetrosis, thalassemia, and myelodysplastic syndrome.

**[00126]** In certain embodiments, anemia can be diagnosed on a complete blood count. In certain embodiments, anemia can be diagnosed based on the measurement of one or more markers of hemolysis (*e.g.*, RBC count, hemoglobin, reticulocytes, schistocytes, lactate Dehydrogenase (LDH), haptoglobin, bilirubin, and ferritin) and/or hemosiderinuria mean corpuscular volume (MCV) and/or red cell distribution width (RDW). In the context of the present invention, anemia is present if an individual has a hemoglobin (Hb) less than the desired level, for example, the Hb concentration of less than 14 g/dL, more preferably of less than 13 g/dL, more preferably of less than 12 g/dL, more preferably of less than 11 g/dL, or most preferably of less than 10 g/dL.

**[00127]** In certain embodiments, provided herein is a method of increasing the amount of hemoglobin in a subject by administering an effective amount of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug ([e.g.], e.g., a CYP3A4/5 inducer or a CYP3A4/5 inhibitor). In certain embodiments, also provided herein is a method of increasing the amount of hemoglobin in a subject having thalassemia comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug ([e.g.], e.g., a CYP3A4/5 inducer or a CYP3A4/5 inhibitor). Further provided is a method of increasing the amount of hemoglobin in subjects having non-transfusion-dependent thalassemia comprising administering an effective amount of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug ([e.g.], e.g., a CYP3A4/5 inducer or a CYP3A4/5 inhibitor) to the subject. In certain embodiments, the provided methods increase hemoglobin concentration in the subject. In certain embodiments, the provided methods increase Hb concentration to a desired level, for example, above 10 g/dL, more preferably above 11 g/dL, more preferably above 12 g/dL, more preferably above 13 g/dL, or most preferably above 14 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 0.5 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 1.0 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 1.5 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 2.0 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 2.5 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 3.0 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 3.5 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 4.0 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 4.5 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 5.0 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 5.5 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 6.0 g/dL. In certain embodiments, the increase in Hb concentration is determined from baseline at one or more assessment between week 1 and week 20 ([e.g.], e.g., between week 2 and week 15, between week 3 and week 15, and between week 4 and week 12) of treatment with an effective amount of mitapivat or a pharmaceutically acceptable salt thereof, in combination

with or in the absence of an effective amount of a secondary drug ([*e.g.*]*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor). In certain embodiments, the provided methods increase Hb concentration as described above in female subjects having thalassemia ([*e.g.*]*e.g.*, beta-thalassemia or non-transfusion-dependent thalassemia). In certain embodiments, the provided methods increase Hb concentration from baseline to about 12 g/dL in female subjects having thalassemia ([*e.g.*]*e.g.*, beta-thalassemia or non-transfusion-dependent thalassemia). In certain embodiments, the provided methods increase Hb concentration as described above in male subjects having thalassemia ([*e.g.*]*e.g.*, beta-thalassemia or non-transfusion-dependent thalassemia). In certain embodiments, the provided methods increase Hb concentration from baseline to about 13 g/dL in male subjects having thalassemia ([*e.g.*]*e.g.*, beta-thalassemia or non-transfusion-dependent thalassemia).

**[00128]** In some aspects, provided herein are methods for treating hemolytic anemia in a subject in need thereof comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug ([*e.g.*]*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor). Also provided is mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug ([*e.g.*]*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor), for use in treating hemolytic anemia in a subject in need thereof. Further provided is the use of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug ([*e.g.*]*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor), in the manufacture of a medicament for treating hemolytic anemia. In one aspect, the hemolytic anemia to be treated is hereditary and/or congenital hemolytic anemia, acquired hemolytic anemia, or anemia as part of a multi-system disease.

**[00129]** In some aspects, provided herein are methods for activating wild-type or mutant PKR in red blood cells in a subject in need thereof comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug ([*e.g.*]*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor). Also provided is mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug ([*e.g.*]*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor), for use in activating wild-type or mutant PKR in red blood cells in a subject in need thereof. Further

provided is the use of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug ([[e.g.]]e.g., a CYP3A4/5 inducer or a CYP3A4/5 inhibitor), in the manufacture of a medicament for activating wild-type or mutant PKR in red blood cells.

**[00130]** Mitapivat is an activator of PKR mutants having lower activities compared to the wild type, thus are useful for methods of the present disclosure. Such mutations in PKR can affect enzyme activity (catalytic efficiency), regulatory properties (modulation by fructose biphosphate (FBP)/ATP), and/or thermostability of the enzyme. Examples of such mutations are described in Valentini et al., JBC 2002. Some examples of the mutants that are activated by the compounds described herein include G332S, G364D, T384M, R479H, R479K, R486W, R532W, R510Q, and R490W. Without being bound by theory, in certain embodiments, mitapivat affects the activities of PKR mutants by activating FBP non-responsive PKR mutants, restoring thermostability to mutants with decreased stability, or restoring catalytic efficiency to impaired mutants.

**[00131]** In certain embodiments, mitapivat increases the affinity of PKR to phosphoenolpyruvate (PEP). In certain embodiments, mitapivat restores the ability of RBCs to cover PEP and ADP to pyruvate and ATP.

**[00132]** In certain embodiments, provided herein are methods of reducing transfusion frequency of a subject with PKD comprising administering to the subject mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug ([[e.g.]]e.g., a CYP3A4/5 inducer or a CYP3A4/5 inhibitor). In certain embodiments, the transfusion frequency is reduced by at least 5% in the number of RBC units transfused over at least 15 weeks. In certain embodiments, the transfusion frequency is reduced by at least 10% in the number of RBC units transfused over at least 15 weeks. In certain embodiments, the transfusion frequency is reduced by at least 15% in the number of RBC units transfused over at least 15 weeks. In certain embodiments, the transfusion frequency is reduced by at least 20% in the number of RBC units transfused over at least 15 weeks. In certain embodiments, the transfusion frequency is reduced by at least 25% in the number of RBC units transfused over at least 15 weeks. In certain embodiments, the transfusion frequency is reduced by at least 30% in the number of RBC units transfused over at least 15 weeks. In certain embodiments, the transfusion frequency is reduced by at least 35% in the number of RBC units transfused over at least 15 weeks. In

certain embodiments, the transfusion frequency is reduced by at least 40% in the number of RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 5% in the number of RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 10% in the number of RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 15% in the number of RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 20% in the number of RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 25% in the number of RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 30% in the number of RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 35% in the number of RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 40% in the number of RBC units transfused over at least 20 weeks.

**[00133]** In some aspects, provided herein are methods of evaluating the efficacy of ~~mitavipat~~ mitapivat therapy (monotherapy or concomitant medications with a CYP3A4/5 inducer or a CYP3A4/5 inhibitor) in a subject, the method comprising: administering to the subject mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*[[e.g.]]e.g.,* a CYP3A4/5 inducer or a CYP3A4/5 inhibitor); and acquiring a value for the level of mitapivat, the level of 2,3-diphosphoglycerate (2,3-DPG), the level of adenosine triphosphate (ATP), or the activity of PKR in the subject, to thereby evaluate the efficacy of the mitapivat therapy. In some aspects, the value for the level is acquired by analyzing the plasma concentration of mitapivat and optionally, the metabolite known to be formed by CYP3A4/5. In some aspects, the level of 2,3-DPG is acquired by analyzing the blood concentration of 2,3-DPG. In some aspects, the level of ATP is acquired by analyzing the blood concentration of ATP. In some aspects, the activity of PKR is acquired by analyzing the blood concentration of a <sup>13</sup>C-label in the blood. In some aspects, the analysis is performed by sample analysis of bodily fluid. In some aspects, the bodily fluid is blood. In some aspects, the analysis is performed by mass spectroscopy. In some aspects, the analysis is performed by LC-MS.

**[00134]** In some aspects, provided herein are methods of treating a subject, the method comprising: administering to the subject an effective amount of mitapivat or a

pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug ([*e.g.*]*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor); and acquiring a value for the level of mitapivat and optionally its metabolite known to be formed by CYP3A4/5, the level of 2,3-diphosphoglycerate (2,3-DPG), the level of adenosine triphosphate (ATP), or the activity of PKR in the subject, to thereby treat the subject.

### EXEMPLIFICATIONS

[00135] As depicted in the Examples below, the drug-drug interactions between mitapivat sulfate and a CYP3A4/5 inducer ([*e.g.*]*e.g.*, rifampin) or a CYP3A4/5 inhibitor ([*e.g.*]*e.g.*, itraconazole) are studied. Mitapivat can be synthesized using the procedures described in International Patent Application Publication Nos. WO 2011/002817 and WO 2016/201227.

#### **Example 1: Mitapivat sulfate is primarily metabolized by CYP3A4**

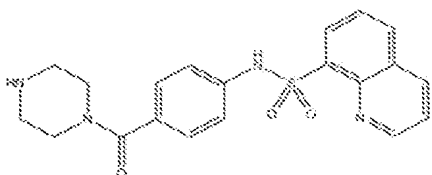
[00136] CYP-enzyme phenotyping using human liver microsomes and recombinant CYP enzymes revealed that mitapivat sulfate was primarily metabolized by CYP3A4/5 (>90%), with minor contributions from CYP2C9, CYP2C8, and CYP1A2. There was evidence of metabolism-dependent inhibition of CYP2C19 (largely reversible) and CYP3A4 (largely irreversible). Mitapivat sulfate was found to be a substrate and inhibitor for P-gp but not for breast cancer resistance protein (BCRP) and was found to be a potential inducer of human CYP2B6 and CYP3A4. Mitapivat sulfate appeared to be a mild inhibitor of CYP2C8, CYP2C9, xYP2C19, CYP2D6, and CYP3A4/5 enzymes (testosterone 6 $\beta$ -hydroxylation), bile salts export pump (BSEP), organic anion transporting polypeptide (OATP)1B1, organic anion transporter (OAT)3, and organic cation transporter (OCT)2, and of uridine-5'-diphospho-glucuronosyltransferase (UGT) 1A3, 1A4, and 1A9. Mitapivat sulfate does not appear to be an inhibitor of multidrug resistance-associated protein (MRP)2, MRP3, OATP1B3, and OAT1. The metabolite was found to be a mild inhibitor of CYP2C9 and CYP2C19 and was not an inhibitor of P-gp or BCRP under tested concentrations.

#### **Example 2: Drug-drug Interaction (DDI) Studies of Mitapivat Sulfate and a CYP3A4 Inducer or a CYP3A4 Inhibitor**

**[00117]**— **[00137]** Twenty-eight (28) healthy, adult male and female (non-childbearing potential) subjects were enrolled in the study in total; 14 subjects in each study part (Parts 1 and 2). A minimum of 8 female subjects were enrolled in the study (*i.e.*, a minimum of 4 female subjects per study part). Each subject participated in either Part 1 or Part 2, but not both.

### **Part 1**

**[00118]**— **[00138]** On Day 1 of Treatment Period 1, a single oral dose of 20 mg mitapivat sulfate was administered. Serial blood samples for plasma assay of mitapivat concentrations and its CYP3A4 metabolite, referred to herein as the “Metabolite” (structure below),



were collected from predose to 120 hours following administration of mitapivat sulfate. In Treatment Period 2, an oral dose of 200 mg itraconazole was administered once daily (QD) for 9 consecutive days (Day 1 through Day 9 of Treatment Period 2) with a single oral dose of 20 mg mitapivat sulfate coadministered on Day 5. Serial blood samples for plasma assay of mitapivat and the Metabolite concentrations were collected from predose to 120 hours following coadministration of mitapivat sulfate and itraconazole on Day 5.

**[00119]**— **[00139]** In Treatment Period 1, mitapivat sulfate was administered orally with approximately 240 mL of water. In Treatment Period 2, on Days 1 to 4, itraconazole was administered alone immediately followed by approximately 220 mL of water, and on Day 5, itraconazole was administered first (no water) and was immediately followed by mitapivat sulfate administration with approximately 220 mL of water. Study drugs (mitapivat sulfate and itraconazole) were administered following an overnight fast of at least 10 hours on Day 1 of Treatment Period 1 (mitapivat sulfate only) and Day 5 of Treatment Period 2 (mitapivat sulfate and itraconazole), and subjects remained fasted for 4 hours after dosing. On all other dosing days, itraconazole was administered following a predose fast of at least 4 hours and subjects remained fasted for at least 2 hours after dosing.

**Part 2**

~~[00120]~~— [00140] On Day 1 of Treatment Period 1, a single oral dose of 50 mg mitapivat sulfate was administered. Serial blood samples for plasma assay of mitapivat and the Metabolite concentrations were collected from predose to 120 hours following administration of mitapivat sulfate. In Treatment Period 2, an oral dose of 600 mg rifampin was administered QD for 12 consecutive days (Day 1 through Day 12 of Treatment Period 2) with a single oral dose of 50 mg mitapivat sulfate coadministered on Day 8. Serial blood samples for plasma assay of mitapivat sulfate and the Metabolite concentrations were collected from predose to 120 hours following coadministration of mitapivat and rifampin on Day 8.

~~[00121]~~— [00141] In Part 2, study drugs were administered with approximately 240 mL of water on all dosing days including the coadministration of mitapivat sulfate and rifampin on Day 8 of Treatment Period 2. Mitapivat sulfate and rifampin was administered following an overnight fast of at least 10 hours on Day 1 of Treatment Period 1 (mitapivat sulfate only) and Day 8 of Treatment Period 2 (both mitapivat sulfate and rifampin) and subjects remained fasted for 4 hours after dosing. On all other dosing days, rifampin was administered following a predose fast of at least 4 hours and subjects remained fasted for at least 2 hours after dosing. There was a washout period of 7 days between the mitapivat sulfate dose in Treatment Period 1 and the first itraconazole (Part 1) or rifampin (Part 2) dose in Treatment Period 2. All study drugs were consumed within 5 minutes for both Part 1 and Part 2.

**Test and Reference Products, Dose, Duration and Mode of Administration****Part 1**

~~[00122]~~— [00142] Mitapivat sulfate (20 mg tablets) administered along with Itraconazole (also referred to as Sporanox®, 10 mg/mL oral solution, is considered as the test arm in this study. Mitapivat sulfate administered alone is considered as the reference arm in this study.

In Period 1, a single oral dose of 20 mg mitapivat sulfate was administered orally with approximately 240 mL of water. In Period 2, on Day 5 (coadministration of itraconazole and mitapivat sulfate), 200 mg itraconazole (20 mL of 10 mg/mL oral solution) was administered first (no water) and was immediately followed by 20 mg mitapivat sulfate (1 x 20 mg tablet)

administration with approximately 220 mL of water. On all other dosing days, itraconazole was administered alone immediately followed by approximately 220 mL of water.

Each subject received 2 doses of mitapivat sulfate and 9 doses of itraconazole in Part 1.

## **Part 2**

**[00123]**— **[00143]** Mitapivat sulfate (50 mg tablets) administered along with Rifampin (also referred to as Rifadin®, 300 mg capsules) is considered as the test arm in this study. Mitapivat sulfate (50 mg tablets) administered alone is considered as the reference arm in this study.

**[00124]**— **[00144]** In Period 1, a single oral dose of 50 mg mitapivat sulfate was administered orally. In Period 2, subjects were administered 600 mg rifampin (2 x 300 mg capsules) QD on Day 1 to Day 12 inclusive, with 50 mg mitapivat sulfate (1 x 50 mg tablet) coadministered on Day 8. Study drugs were administered with approximately 240 mL of water on all dosing days including the coadministration of mitapivat sulfate and rifampin on Day 8 of Period 2.

**[00125]**— **[00145]** Each subject received 2 doses of mitapivat sulfate and 12 doses of rifampin in Part 2.

## **Duration of Treatment:**

**[00126]**— **[00146]** The total duration of participation including the screening period for each subject was approximately 49 to 62 days for Part 1 and approximately 61 or 63 days in Part 2 of the study.

**[00127]**— **[00147]** In each study part, Treatment Period 1 was approximately 6 days and Treatment Period 2 was approximately 12 days; a washout phase was 7 days between both treatment periods in each study part.

## **Criteria for Evaluation of Pharmacokinetics**

**[00128]**— **[00148]** In Parts 1 and 2, for mitapivat and the Metabolite, blood samples were obtained for all subjects to determine the plasma concentrations of mitapivat, and the Metabolite, before mitapivat sulfate dosing (0 hour) on Day 1 of Period 1 (Parts 1 and 2) and Day 5 (Part 1) or Day 8 (Part 2) of Period 2 at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 16, 24, 48, 72,

96, and 120 hours postdose. Plasma concentrations of mitapivat and the Metabolite were determined using methods validated with respect to accuracy, precision, linearity, sensitivity, and specificity, with a lower limit of quantitation (LLOQ) of 0.5 ng/mL for both analytes. A noncompartmental PK approach was used to analyze individual plasma mitapivat and the Metabolite concentration time data (using Phoenix® WinNonlin® Version 7.0). The following PK parameters were calculated:  $AUC_{0-t}$ ,  $AUC_{0-inf}$ ,  $AUC_{0-24}$ ,  $AUC_{\%extrap}$ ,  $C_{max}$ ,  $CL/F$  (mitapivat only),  $T_{last}$ ,  $T_{max}$ ,  $t_{1/2}$ , and  $V_z/F$  (mitapivat only).

### Statistical Methods for Pharmacokinetics

~~[00129]~~— [00149] The plasma concentrations of mitapivat and the Metabolite were listed and summarized by treatment and collection time for all subjects in the PK Population. Plasma concentrations were presented with the same level of precision as received from the bioanalytical laboratory including sample size (n), arithmetic mean (Mean), standard deviation (SD), coefficient of variation (CV%), standard error of the mean (SEM), minimum, maximum, and median. Concentrations from excluded subjects were included in the plasma concentration tables, but were excluded from the summary statistics and noted as such in the tables. All BLQ values were presented as “BLQ” in the plasma concentration table listings and footnoted accordingly.

~~[00130]~~— [00150] PK parameters were presented with a minimum of 3 significant figures. PK parameters were listed by subject, tabulated by treatment, and summarized using descriptive statistics (n, mean, SD, CV%, SEM, minimum, median, maximum, geometric mean (Geom. Mean) and geometric mean coefficient of variation (Geom. CV%). Time-based parameters (*i.e.*,  $T_{max}$  and  $t_{1/2}$ ) and treatment:reference AUC and  $C_{max}$  ratios were presented to 2 decimals. Excluded subjects were included in the PK parameter table listings, but were excluded from the summary statistics and noted as such in the tables.

~~[00131]~~— [00151] To evaluate the effect of itraconazole and rifampin on mitapivat and the Metabolite [](primary objective), plasma mitapivat and the Metabolite PK parameters ( $C_{max}$ ,  $AUC_{0-t}$ , and  $AUC_{0-inf}$ ) were natural log (ln) transformed before analysis. The ln-transformed value of the PK parameters was analyzed using a linear mixed effect model for each study part separately with treatment as a fixed effect and subject as a random effect. The linear mixed effect model included treatment as a fixed effect and subject as a random effect. The inferential results (least-squares means [LSMs], difference between LSMs, and 90% confidence intervals [CIs] of the difference) were back transformed to the original scale.

Geometric LSMs, geometric mean ratios (GMRs), and 90% CIs were presented. The GMR of the LSMs were calculated from the back-transformed difference between the treatment LSMs. The GMRs were expressed as a percentage relative to mitapivat sulfate given alone for both Part 1 and Part 2. The 90% CIs for the ratios were derived by back-transformation of the CIs obtained for the difference between the treatment LSMs. The CIs were expressed as a percentage relative to mitapivat sulfate given alone for both Part 1 and Part 2.

**[00132]**— **[00152]** The median differences in Tmax for evaluation of the effect of itraconazole and rifampin on mitapivat and the Metabolite along with the range of the difference were calculated using the non parametric Wilcoxon Signed Rank test, and the p-value was presented. Note that Tmax was not ln-transformed for these analyses.

## Pharmacokinetic Results

### Part 1

**[00133]**— **[00153]** Mean plasma mitapivat and the Metabolite concentration-time profiles following 20 mg mitapivat sulfate administered alone (Period 1) or coadministered with multiple doses of 200 mg itraconazole (Period 2) on linear and semi-log scales are presented in Figures 1 and 2.

### Mitapivat

**[00134]**— **[00154]** Mitapivat plasma concentrations were detectable in all subjects at 0.5 hour (the first scheduled postdose sample) in both Periods 1 and 2. Mitapivat was still detectable up to the last sample time at 120 hours postdose in the majority of subjects in Period 1 and in all subjects in Period 2.

**[00135]**— **[00155]** Measurable predose mitapivat concentrations were present in all but 2 subjects in Period 2 (two subjects were BLQ). As used herein, BLQ refers to below the limit of quantification. These predose concentrations were < 5% of the Cmax, so PK calculations were performed.

**[00136]**— **[00156]** Mitapivat concentrations exhibited a characteristic multi-compartmental pharmacokinetic profile. When coadministered with itraconazole, mitapivat concentrations rose to a higher mean peak with a delay in time to peak by approximately 2 hours compared to when the drug was administered alone. The post-peak decline of the multi-compartmental behavior was slower after coadministration with itraconazole when

compared to mitapivat administered alone. Overall, higher mitapivat concentrations were experienced for a longer time period when mitapivat was coadministered with itraconazole.

The Metabolite

~~[00137]~~— [00157] When mitapivat was administered alone, the Metabolite plasma concentrations were detectable in all but 1 subject at 0.5 hour. The Metabolite remained detectable all but 1 subject up to 48 hours postdose, with quantifiable the Metabolite out to 72 hours in 3 subjects and 96 hours in 1 subject. In Period 2 (coadministration with itraconazole), the Metabolite concentrations were delayed relative to administration of mitapivat alone. Concentrations were not measurable in all subjects until 1.5 hours postdose. Maximum concentrations were also reduced. However, the Metabolite residence was prolonged in the presence of itraconazole, with concentrations still detectable in all but 1 subject throughout the 120-hour sampling interval (in 1 subject the last measurable concentration was at 96 hours postdose).

~~[00138]~~— [00158] The statistical comparisons of mitapivat PK parameters following 20 mg mitapivat sulfate administered alone (Period 1) or coadministered with multiple doses of 200 mg itraconazole (Period 2) PK parameters are summarized below in Table 1.

Table 1  
Summary of Statistical Comparisons of Plasma Mitapivat Pharmacokinetic Parameters Following 20 mg Mitapivat Sulfate Co-Administered With Multiple Doses of 200 mg Itraconazole Versus Administered Alone (Part 1)

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Mitapivat Sulfate + Itraconazole (Test) |    | Mitapivat sulfate alone (Reference) |    |        |                         |                   |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------|----|-------------------------------------|----|--------|-------------------------|-------------------|
| Parameter (unit)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Geometric LSM                           | n  | Geometric LSM                       | n  | GMR    | 90% Confidence Interval | Intra-subject CV% |
| AUC <sub>0-t</sub> (ng*hr/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | 8860                                    | 14 | 1885                                | 14 | 4.6998 | 4.2519 - 5.1949         | 15.05             |
| AUC <sub>0-inf</sub> (ng*hr/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 9475                                    | 14 | 1942                                | 14 | 4.8780 | 4.4154 - 5.3890         | 14.97             |
| C <sub>max</sub> (ng/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | 704.3                                   | 14 | 412.5                               | 14 | 1.7074 | 1.5815 - 1.8433         | 11.48             |
| Mitapivat sulfate alone: 20 mg mitapivat sulfate (1 x 20 mg tablet) at Hour 0 on Day 1 (Period 1)<br>Mitapivat sulfate + itraconazole: 200 mg itraconazole (20 mL of 10 mg/mL oral solution) QD on Days 1 to Day 9 inclusive (within ± 1 hour of dosing time on Day 1) with 20 mg mitapivat sulfate (1 x 20 mg tablet) coadministered at Hour 0 on Day 5 (Period 2)<br>Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the linear mixed effect model.<br>Geometric Mean Ratio (GMR) = test / reference<br>Intra-subject CV% = 100 x (square root (exp[MSE]-1), where MSE = Residual variance from linear mixed effect model. |                                         |    |                                     |    |        |                         |                   |

~~[00139]~~— [00159] The coadministration of 200 mg itraconazole with 20 mg mitapivat sulfate increased mitapivat exposure relative to when mitapivat sulfate was administered

alone. The geometric mean AUC<sub>0-t</sub> and AUC<sub>0-inf</sub> ratios of mitapivat in the presence and absence of itraconazole were 4.7 and 4.9, respectively. The geometric mean C<sub>max</sub> ratio of mitapivat in the presence and absence of itraconazole was 1.7. The intra-subject variability was low at approximately 15% for AUCs and 11% for C<sub>max</sub>.

~~[00140]~~ — [00160] The statistical comparisons of the Metabolite PK parameters following 20 mg mitapivat sulfate administered alone (Period 1) or coadministered with multiple doses of 200 mg itraconazole (Period 2) PK parameters are summarized below in Table 2.

**Table 2**  
**Summary of Statistical Comparisons of Plasma Metabolite Pharmacokinetic Parameters Following 20 mg Mitapivat Sulfate Co-Administered With Multiple Doses of 200 mg Itraconazole Versus Administered Alone (Part 1)**

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Mitapivat Sulfate + Itraconazole (Test) |    | Mitapivat sulfate alone (Reference) |    |        |                         |                   |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------|----|-------------------------------------|----|--------|-------------------------|-------------------|
| Parameter (unit)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Geometric LSM                           | n  | Geometric LSM                       | n  | GMR    | 90% Confidence Interval | Intra-subject CV% |
| AUC <sub>0-t</sub> (ng*hr/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | 300.0                                   | 14 | 197.1                               | 14 | 1.5218 | 1.4268 - 1.6231         | 9.65              |
| AUC <sub>0-inf</sub> (ng*hr/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 338.6                                   | 14 | 216.2                               | 14 | 1.5661 | 1.4784 - 1.6590         | 8.63              |
| C <sub>max</sub> (ng/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | 9.110                                   | 14 | 18.79                               | 14 | 0.4849 | 0.4441 - 0.5295         | 13.20             |
| Mitapivat sulfate alone: 20 mg mitapivat sulfate (1 x 20 mg tablet) at Hour 0 on Day 1 (Period 1)<br>Mitapivat sulfate + itraconazole: 200 mg itraconazole (20 mL of 10 mg/mL oral solution) QD on Days 1 to Day 9 inclusive (within ± 1 hour of dosing time on Day 1) with 20 mg mitapivat sulfate (1 x 20 mg tablet) coadministered at Hour 0 on Day 5 (Period 2)<br>Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the linear mixed effect model.<br>Geometric Mean Ratio (GMR) = test / reference<br>Intra-subject CV% = 100 x (square root (exp[MSE]-1), where MSE = Residual variance from linear mixed effect model. |                                         |    |                                     |    |        |                         |                   |

~~[00141]~~ — [00161] The coadministration of 200 mg itraconazole with 20 mg mitapivat sulfate increased total Metabolite exposure relative to when mitapivat sulfate was administered alone. The geometric mean AUC<sub>0-t</sub> and AUC<sub>0-inf</sub> ratios of the Metabolite in the presence and absence of itraconazole were 1.5 and 1.6, respectively. The geometric mean C<sub>max</sub> ratio of the Metabolite in the presence and absence of itraconazole was 0.5. The intra-subject variability was low at approximately 9% to 10% for AUCs and 13% for C<sub>max</sub>.

**Part 2**

**[00142]**— **[00162]** Mean plasma mitapivat and metabolite the Metabolite concentration-time profiles following 50 mg mitapivat sulfate administered alone (Period 1) or coadministered with multiple doses of 600 mg rifampin (Period 2) on linear and semi-log scales are presented in Figures 3 and 4.

### **Mitapivat**

**[00143]**— **[00163]** Mitapivat plasma concentrations were detectable in all subjects at 0.5 hour (the 1st scheduled postdose sample) when given alone or with rifampin. Mitapivat was still detectable up to the last sample time at 120 hours postdose in all but 1 subject when given alone had a BLQ concentration at 120 hours). When coadministered with rifampin, detectable mitapivat concentrations were not present in any sample collections from any subject beyond 24 hours postdose (except 1 subject with detectable mitapivat at 48 hours).

**[00144]**— **[00164]** Mitapivat concentrations increased to a lower and slightly earlier peak when coadministered with rifampin compared to when administered alone. The peak arithmetic mean mitapivat concentrations were observed at a nominal time of approximately 1 hour postdose for Period 1 and 0.5 hour postdose for Period 2. The mean concentrations of mitapivat then declined in a multi exponential manner for both periods; however, the post-peak initial decline in mitapivat concentrations was more rapid and to lower levels when coadministered with rifampin compared to dosing alone.

### **The Metabolite**

**[00145]**— **[00165]** The Metabolite plasma concentrations were detectable at 0.5 hour postdose in all subjects after mitapivat sulfate was given alone and in all but 1 subject (1 subject was reported as BLQ) when mitapivat sulfate was coadministered with rifampin. The Metabolite was still detectable in all but 4 subjects up to the last scheduled sampling time of 120 hours postdose when mitapivat sulfate was given alone. When mitapivat sulfate was coadministered with rifampin, ~~The~~ the Metabolite remained detectable in all subjects up to 24 hours postdose, with quantifiable ~~The~~ Metabolite out to 48 hours in 6 subjects and 72 hours in 1 subject. No Metabolite concentrations were detectable in samples collected beyond 72 hours.

**[00146]**— **[00166]** The peak arithmetic mean Metabolite concentrations were observed at a nominal time of approximately 2 hours postdose with mitapivat sulfate given alone and 1 hour when mitapivat sulfate was coadministered with rifampin. When mitapivat sulfate was

given alone, higher mean concentrations ~~The~~ of the Metabolite were observed over the initial 1 hour postdose. Metabolite concentrations were higher when mitapivat sulfate was coadministered with rifampin beyond 1 hour. The mean concentrations of the ~~The~~ Metabolite then declined in a multi exponential fashion for both treatments. Decline in Metabolite concentrations was more rapid when mitapivat sulfate was coadministered with rifampin.

~~[00147]~~ — [00167] The statistical comparisons of mitapivat PK parameters following 50 mg mitapivat sulfate administered alone (Period 1) or coadministered with multiple doses of 600 mg rifampin (Period 2) PK parameters are summarized below in Table 3.

**Table 3**  
**Summary of Statistical Comparisons of Plasma Mitapivat Pharmacokinetic Parameters Following 50 mg Mitapivat Sulfate Co-Administered With Multiple Doses of 600 mg Rifampin Versus Administered Alone (Part 2)**

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | Mitapivat sulfate + rifampin (Test) |    | Mitapivat sulfate alone (Reference) |    |        |                         |                   |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|----|-------------------------------------|----|--------|-------------------------|-------------------|
| Parameter (unit)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Geometric LSM                       | n  | Geometric LSM                       | n  | GMR    | 90% Confidence Interval | Intra-subject CV% |
| AUC <sub>0-t</sub> (ng*hr/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 486.4                               | 14 | 5565                                | 14 | 0.0874 | 0.0719 - 0.1062         | 29.73             |
| AUC <sub>0-inf</sub> (ng*hr/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 496.4                               | 14 | 5681                                | 14 | 0.0874 | 0.0719 - 0.1062         | 29.84             |
| C <sub>max</sub> (ng/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 292.4                               | 14 | 1263                                | 14 | 0.2316 | 0.1933 - 0.2773         | 27.45             |
| Mitapivat sulfate alone: 50 mg mitapivat sulfate (1 x 50 mg tablet) at Hour 0 on Day 1 (Period 1)<br>Mitapivat sulfate + rifampin: 600 mg rifampin (2 x 300 mg capsules) QD on Day 1 to Day 12 inclusive (within ± 1 hour of dosing time on Day 1) with 50 mg mitapivat sulfate (1 x 50 mg tablet) coadministered at Hour 0 on Day 8 (Period 2)<br>Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the linear mixed effect model.<br>Geometric Mean Ratio (GMR) = test / reference<br>Intra-subject CV% = 100 x (square root (exp[MSE]-1), where MSE = Residual variance from linear mixed effect model. |                                     |    |                                     |    |        |                         |                   |

~~[00148]~~ — [00168] The coadministration of 600 mg rifampin with 50 mg mitapivat sulfate decreased mitapivat exposure relative to when mitapivat sulfate was administered alone. The geometric mean AUC<sub>0-t</sub> and AUC<sub>0-inf</sub> ratios of mitapivat in the presence and absence of rifampin were 0.09. The geometric mean Cmax ratio of mitapivat in the presence and absence of rifampin was 0.23. The intra-subject variability was low at approximately 30% for AUCs and 27% for Cmax.

~~[00149]~~ — [00169] The statistical comparisons of Metabolite PK parameters following 50 mg mitapivat sulfate administered alone (Period 1) or co-administered with multiple doses of 600 mg rifampin (Period 2) PK parameters are summarized below in Table 4.

**Table 4**  
**Summary of Statistical Comparisons of Plasma Metabolite Pharmacokinetic Parameters Following 50 mg Mitapivat Sulfate Co-Administered With Multiple Doses of 600 mg Rifampin Versus Administered Alone (Part 2)**

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | Mitapivat sulfate + rifampin (Test) |    | Mitapivat sulfate alone (Reference) |    |        |                         |                   |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|----|-------------------------------------|----|--------|-------------------------|-------------------|
| Parameter (unit)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Geometric LSM                       | n  | Geometric LSM                       | n  | GMR    | 90% Confidence Interval | Intra-subject CV% |
| AUC <sub>0-t</sub> (ng*hr/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 230.5                               | 14 | 623.0                               | 14 | 0.3700 | 0.3316 - 0.4129         | 16.50             |
| AUC <sub>0-inf</sub> (ng*hr/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 244.9                               | 14 | 662.6                               | 14 | 0.3696 | 0.3321 - 0.4113         | 16.08             |
| C <sub>max</sub> (ng/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 64.47                               | 14 | 53.80                               | 14 | 1.1983 | 1.0642 - 1.3492         | 17.86             |
| Mitapivat sulfate alone: 50 mg mitapivat sulfate (1 x 50 mg tablet) at Hour 0 on Day 1 (Period 1)<br>Mitapivat sulfate + rifampin: 600 mg rifampin (2 x 300 mg capsules) QD on Day 1 to Day 12 inclusive (within ± 1 hour of dosing time on Day 1) with 50 mg mitapivat sulfate (1 x 50 mg tablet) coadministered at Hour 0 on Day 8 (Period 2)<br>Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the linear mixed effect model.<br>Geometric Mean Ratio (GMR) = test / reference<br>Intra-subject CV% = 100 x (square root (exp[MSE]-1), where MSE = Residual variance from linear mixed effect model. |                                     |    |                                     |    |        |                         |                   |

**[00150]**— **[00170]** The coadministration of 600 mg rifampin with 50 mg mitapivat sulfate decreased the Metabolite exposure relative to when mitapivat sulfate was administered alone. The geometric mean AUC<sub>0-t</sub> and AUC<sub>0-inf</sub> ratios of the Metabolite in the presence versus absence of rifampin were 0.37. The geometric mean C<sub>max</sub> ratio of the Metabolite in the presence versus absence of rifampin was 1.2. The intra-subject variability was low at approximately 16% to 18% for AUCs and C<sub>max</sub>.

**Conclusions**

**[00151]**— **[00171]** Total exposure of mitapivat increased in the presence of itraconazole compared to dosing of mitapivat sulfate alone.

**[00152]**— **[00172]** Total exposure of mitapivat decreased in the presence of rifampin compared to dosing of mitapivat sulfate alone.

**Example 3. Development of a PBPK Model for Mitapivat Sulfate to Evaluate the Potential for Drug-Drug Interactions**

**Part I**

**[00153]**— **[00173]** A physiologically based pharmacokinetic model (hereinafter “PBPK” model) for mitapivat sulfate based on the currently available in-vitro and clinical PK data is being developed. The developed PBPK model will be used to assess DDI liability with mitapivat sulfate acting as a victim of CYP3A4-mediated metabolism as well as a perpetrator of CYP and transporter mediated DDI.

**[00154]**— **[00174]** Specifically, a PBPK model that includes a simple first order absorption model will be developed. Full PBPK and minimal PBPK distribution models will be evaluated, both of which consider liver and intestinal metabolism. The fraction of mitapivat sulfate absorbed will be estimated from mass balance data from the [14C] human ADME study (Clinical Study AG348-C-009). In-vitro data will be used to assign the relative contribution of CYP3A4 to the clearance of mitapivat sulfate.  $K_I$  and  $k_{inact}$  values for mechanism based inhibition of CYP3A4 and CYP3A4 induction ( $E_{max}$  and  $EC_{50}$ ) data by mitapivat sulfate will be incorporated within the PBPK model to assess the net DDI effects at steady state. Once an mitapivat sulfate PBPK model has been verified against the available clinical data, a series of DDI simulations assessing mitapivat sulfate as a victim or perpetrator of DDI will be performed in healthy volunteers.

#### **Experiments with mitapivat sulfate as a victim**

**[00155]**— **[00175]** The impact of the co-administration of the strong CYP3A4 inhibitor, itraconazole, on the PK of mitapivat sulfate will be determined based on the following single and multiple-dose administration of 5, 20 and 50 mg BID.

**[00156]**— **[00176]** The impact of the co-administration of the strong CYP3A4 inhibitor, ketoconazole, on the PK of mitapivat sulfate will be determined based following single and multiple-dose administration of 5, 20 and 50 mg BID.

**[00157]**— **[00177]** The impact of the co-administration of the moderate CYP3A4 inhibitor fluconazole on the PK of mitapivat sulfate will be determined based on the following single- and multiple dose administration of 5, 20 and 50 mg BID.

**[00158]**— **[00178]** The impact of the co-administration of the mild CYP3A4 inhibitor cimetidine on the PK of mitapivat sulfate will be determined based on the following single- and multiple dose administration of 5, 20 and 50 mg BID.

**[00159]**— **[00179]** The impact of the co-administration of the strong CYP3A4 inducer, rifampin, on the PK of mitapivat sulfate will be determined based on the following single- and multiple dose administration of 5, 20 and 50 mg BID.

**[00160]**— **[00180]** The impact of the co-administration of the moderate CYP3A4 inducer efavirenz on the PK of mitapivat sulfate will be determined following single- and multiple dose administration of 5, 20 and 50 mg BID.

#### **Experiments with mitapivat sulfate as perpetrator**

**[00161]**— **[00181]** The extent to which 5 to 50 mg BID mitapivat sulfate affects the systemic exposure of CYP3A4 substrate midazolam when co-administered with mitapivat sulfate will be determined.

**[00162]**— **[00182]** The effect of 5 to 50 mg mitapivat sulfate on the systemic exposures of CYP2B6 substrate bupropion when co-administered will be determined. CYP2B6 induction (Emax and EC50[ ]) data by mitapivat sulfate will be incorporated within the PBPK model. Sensitivity analysis will be conducted to gauge the impact of the uncertainty around Emax and EC50 on the predicted DDIs.

**[00163]**— **[00183]** The effect of 5 to 50 mg AG mitapivat sulfate BID on the systemic exposures of CYP2C9 substrate warfarin when co-administered will be determined. CYP2C9 induction (Emax and EC50) data by mitapivat sulfate will be incorporated within the PBPK model. Sensitivity analysis will be conducted to gauge the impact of the uncertainty around Emax and EC50 on the predicted DDIs.

**[00164]**— **[00184]** The effect of 5 to 50 mg mitapivat sulfate BID on the systemic exposures of CYP2C8/CYP3A4 substrate repaglinide when co-administered will be determined. CYP2C8 induction (Emax and EC50) data by mitapivat sulfate will be incorporated within the PBPK model. Sensitivity analysis will be conducted to gauge the impact of the uncertainty around Emax and EC50 on the predicted DDIs.

**[00165]**— **[00185]** The effect of 5 to 50 mg mitapivat sulfate BID on the systemic exposures of CYP2C19 substrate omeprazole when co-administered will be determined. CYP2C19 induction (Emax and EC50) data by mitapivat sulfate will be incorporated within

the PBPK model. Sensitivity analysis will be conducted to gauge the impact of the uncertainty around Emax and EC50 on the predicted DDIs.

~~[00166]~~— [00186] The effect of mitapivat sulfate (5 to 50 mg BID) on the systemic exposures of OATP1B1/OATP1B3 (rosuvastatin), P-gp (digoxin), metformin (MATE1 and OCT2), OAT3 (methotrexate) substrate drugs when co-administered will be determined. The respective IC50 values for inhibition of OATP1B1, P-gp, MATE1, OCT2 and OAT3 by mitapivat sulfate will be incorporated within the PBPK model. Sensitivity analysis will be performed to assess the impact of these parameters on the DDI liability of mitapivat sulfate.

## **Part II**

~~[00167]~~— [00187] The first order absorption component of the previously developed PBPK model will be extended using available pH solubility data to include the ADAM model. The recovery of mitapivat sulfate plasma concentration profiles after single and multiple dosing (up to 50 mg BID) to healthy volunteer subjects will be demonstrated. Once verified against the available clinical data, the modified PBPK model will be used to assess the impact of an increase in gastric pH on the pharmacokinetics of mitapivat sulfate via manipulation of system parameters (gastric pH).

~~[00168]~~— [00188] While a number of embodiments have been described, the scope of this disclosure is to be defined by the appended claims, and not by the specific embodiments that have been represented by way of example. The contents of all references (including literature references, issued patents, published patent applications, and co-pending patent applications) cited throughout this application are hereby expressly incorporated herein in their entireties by reference. Unless otherwise defined, all technical and scientific terms used herein are accorded the meaning commonly known to one with ordinary skill in the art.

**MITAPIVAT THERAPY AND MODULATORS OF CYTOCHROME P450**

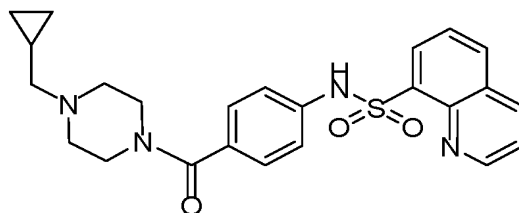
**RELATED APPLICATIONS**

The application claims the benefit of U.S. Provisional Application Serial No. 62/861,600, filed June 14, 2019, the entire teachings of which are incorporated herein by reference.

**BACKGROUND**

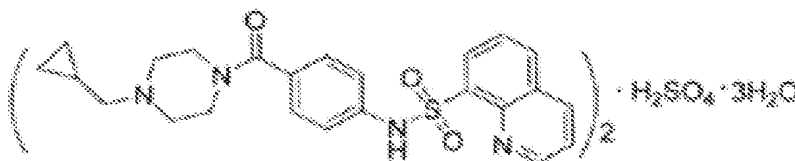
[0001] Pyruvate kinase deficiency (PKD) is a disease of the red blood cells caused by a deficiency of the pyruvate kinase R (PKR) enzyme due to recessive mutations of PKLR gene. PKR activators can be beneficial to treat PKD, thalassemia (*e.g.*, beta-thalassemia), abetalipoproteinemia or Bassen-Kornzweig syndrome, sickle cell disease, paroxysmal nocturnal hemoglobinuria, anemia (*e.g.*, congenital anemias) (*e.g.*, enzymopathies), hemolytic anemia (*e.g.*, hereditary and/or congenital hemolytic anemia, acquired hemolytic anemia, chronic hemolytic anemia caused by phosphoglycerate kinase deficiency, anemia of chronic diseases, non-spherocytic hemolytic anemia or hereditary spherocytosis). Treatment of PKD is supportive, including blood transfusions, splenectomy, chelation therapy to address iron overload, and/or interventions for other disease-related morbidity. Currently, however, there is no approved medicine that treats the underlying cause of PKD, and thus the etiology of life-long hemolytic anemia.

[0002] Mitapivat, also known as AG-348 or by its chemical name *N*-(4-(4-(cyclopropylmethyl)piperazine-1-carbonyl)phenyl)quinoline-8-sulfonamide, and is represented by the following structural formula:



is an allosteric activator of pyruvate kinase R (PKR). See, *e.g.*, International Patent Application Publication Nos. WO 2011/002817 and WO 2016/201227.

**[0003]** Mitapivat sulfate is represented by the following structural formula:



and is currently in Phase 2 and Phase 3 clinical trials as a disease-altering therapy in patients with PKD, hemolytic anemia and thalassemia. See, *e.g.*, U.S. Clinical Trials Identifier Nos. NCT03692052, NCT03559699, NCT03548220, NCT03853798, and NCT02476916.

### SUMMARY

**[0004]** Non-clinical studies described in Example 1 show that the PKR activator, mitapivat, is primarily metabolized by the cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5) enzymes (*i.e.*, >90%), with minor contributions from other detoxification enzymes, namely CYP2C8, CYP2C9, and CYP1A2. It has also been found that in a clinical trial, total exposure of mitapivat increased in the presence of itraconazole, a strong CYP3A4A and p-gp inhibitor, compared with dosing of mitapivat sulfate alone. It has further been found that total exposure of mitapivat decreased in the presence of rifampin, a strong CYP3A4A and p-gp inducer, compared with dosing of mitapivat sulfate alone.

**[0005]** Accordingly, the present disclosure provides methods of treating a disease, disorder or a condition with mitapivat or a pharmaceutically acceptable salt thereof and a CYP3A4/5 inducer or a CYP3A4/5 inhibitor. In another aspect, the present disclosure provides methods of treating a disease, disorder or a condition with mitapivat or a pharmaceutically acceptable salt thereof and a p-glycoprotein (p-gp) inhibitor. As used herein, “CYP3A4/5” means cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5). In some embodiments, the pharmaceutically acceptable salt is mitapivat sulfate.

**[0006]** Specifically, in one embodiment, the present disclosure relates to a method of treating pyruvate kinase deficiency (PKD) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inhibitor of cytochrome P450 3A4/5 (CYP3A4/5). In another embodiment, the present disclosure relates to a method of treating sickle cell disease in a subject, comprising administering to the subject an effective amount of mitapivat or a

pharmaceutically acceptable salt thereof and an effective amount of an inhibitor of cytochrome P450 3A4/5 (CYP3A4/5). In another embodiment, the present disclosure relates to a method of treating thalassemia (*e.g.*, alpha-thalassemia and beta-thalassemia) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inhibitor of cytochrome P450 3A4/5 (CYP3A4/5). In another embodiment, the present disclosure relates to a method of treating hemolytic anemia in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inhibitor of cytochrome P450 3A4/5 (CYP3A4/5). In some embodiments, the inhibitor of CYP3A4/5 is a moderate CYP3A4/5 inhibitor. In some embodiments, the inhibitor of CYP3A4/5 is a mild CYP3A4/5 inhibitor.

**[0007]** In another embodiment, the present disclosure relates to a method of treating pyruvate kinase deficiency (PKD) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inducer of cytochrome P450 3A4/5 (CYP3A4/5). In another embodiment, the present disclosure relates to a method of treating sickle cell disease in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inducer of cytochrome P450 3A4/5 (CYP3A4/5). In another embodiment, the present disclosure relates to a method of treating thalassemia (*e.g.*, alpha-thalassemia and/or beta-thalassemia) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inducer of cytochrome P450 3A4/5 (CYP3A4/5). In another embodiment, the present disclosure relates to a method of treating hemolytic anemia in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inducer of cytochrome P450 3A4/5 (CYP3A4/5). In some embodiments, the inducer of CYP3A4/5 is a moderate CYP3A4/5 inducer. In some embodiments, the inhibitor of CYP3A4/5 is a mild CYP3A4/5 inducer.

**[0008]** Specifically, in one embodiment, the present disclosure relates to a method of treating pyruvate kinase deficiency (PKD) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of a p-glycoprotein inhibitor. In another embodiment, the present disclosure

relates to a method of treating sickle cell disease in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of a p-glycoprotein inhibitor. In another embodiment, the present disclosure relates to a method of treating thalassemia (*e.g.*, alpha-thalassemia and beta-thalassemia) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of a p-glycoprotein inhibitor. In another embodiment, the present disclosure relates to a method of treating hemolytic anemia in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of a p-glycoprotein inhibitor. In some embodiments, the p-glycoprotein inhibitor is a moderate p-glycoprotein inhibitor. In some embodiments, the p-glycoprotein inhibitor is a mild p-glycoprotein inhibitor.

**[0009]** The present disclosure further provides methods of treating a disease, disorder or a condition with mitapivat or a pharmaceutically acceptable salt thereof in the absence of a CYP3A4/5 inducer or a CYP3A4/5 inhibitor or p-glycoprotein inhibitor.

**[0010]** Specifically, in one embodiment, the present disclosure relates to a method of treating pyruvate kinase deficiency (PKD) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of an inducer or an inhibitor of cytochrome P450 3A4/5 (CYP3A4/5), respectively. In another embodiment, the present disclosure relates to a method of treating sickle cell disease in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of an inducer or an inhibitor of cytochrome P450 3A4/5 (CYP3A4/5), respectively. In yet another embodiment, the present disclosure relates to a method of treating thalassemia (*e.g.*, alpha-thalassemia and/or beta-thalassemia) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of an inducer or an inhibitor of cytochrome P450 3A4/5 (CYP3A4/5), respectively. In yet another embodiment, the present disclosure relates to a method of treating hemolytic anemia in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of an inducer or an inhibitor of cytochrome P450 3A4/5 (CYP3A4/5), respectively. In some embodiments, the inducer of CYP3A4/5 is a strong inducer of CYP3A4/5. In some embodiments, the inducer of

CYP3A4/5 is a moderate inducer of CYP3A4/5. In some embodiments, the inhibitor of CYP3A4/5 is a strong inhibitor of CYP3A4/5. In some embodiments, the inhibitor of CYP3A4/5 is a moderate inhibitor of CYP3A4/5.

**[0011]** In another embodiment, the present disclosure relates to a method of treating pyruvate kinase deficiency (PKD) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of a p-glycoprotein inhibitor. In another embodiment, the present disclosure relates to a method of treating sickle cell disease in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of a p-glycoprotein inhibitor. In yet another embodiment, the present disclosure relates to a method of treating thalassemia (*e.g.*, alpha-thalassemia and/or beta-thalassemia) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of a p-glycoprotein inhibitor. In yet another embodiment, the present disclosure relates to a method of treating hemolytic anemia in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of a p-glycoprotein inhibitor. In some embodiments, the p-glycoprotein inhibitor is a strong p-glycoprotein inhibitor. In some embodiments, the p-glycoprotein inhibitor is a mild p-glycoprotein inhibitor.

#### **BRIEF DESCRIPTION OF THE FIGURES**

Figure 1 is a graph showing Arithmetic Mean (SD) Plasma Mitapivat and Metabolite Concentration-Time Profiles following administration of 20 mg mitapivat sulfate alone (Treatment Period 1) and co-administered with multiple doses of 200 mg itraconazole (Treatment Period 2) on linear scale (Part 1). “Metabolite” refers to the CYP3A4 metabolite of mitapivat (see Example 2).

Figure 2 is a graph showing Arithmetic Mean Plasma Mitapivat and Metabolite Concentration-Time Profiles following administration of 20 mg mitapivat sulfate alone (Treatment Period 1) and co-administered with multiple doses of 200 mg itraconazole (Treatment Period 2) on semi-log scale (Part 1) (see Example 2).

Figure 3 is a graph showing Arithmetic Mean (SD) Plasma Mitapivat and Metabolite Concentration-Time Profiles following administration of 50 mg mitapivat sulfate alone

(Treatment Period 1) and co-administered with multiple doses of 600 mg rifampin on linear scale (Treatment Period 2, Part 2, see Example 2).

Figure 4 is a graph showing Arithmetic Mean Plasma Mitapivat and Metabolite Concentration-Time Profiles following administration of 50 mg mitapivat sulfate alone (Treatment Period 1) and co-administered with multiple doses of 600 mg rifampin on semi-log scale (Treatment Period 2, Part 2, see Example 2).

## DETAILED DESCRIPTION

### Definitions

**[0012]** A “CYP3A4/5 modulator” refers to any substance, including, but not limited to a small molecule (with a molecular weight of  $\leq 1000$  Da, or  $\leq 750$  Da, or  $\leq 500$  Da), a nucleoside, a nucleotide, a nucleobase, a sugar, a nucleic acid, an amino acid, a polysaccharide, a peptide, a polypeptide, a protein, an antibody, an aptamer, a natural or synthetic plant-based substance, or a complex or a composition comprising any combination of the aforementioned substances, that can modify or alter the activity and/or expression levels of the enzyme cytochrome P450 3A4 (CYP3A4; EC 1.14.13.97) or 3A5 (CYP3A5; EC1.14.14.1). Briefly, the cytochrome P450 (CYP) is a superfamily of detoxification enzymes that are responsible for the oxidative and reductive metabolic transformation of drug medications and the ultimate “clearance”, which is the elimination of the drug from the body as determined by the measured plasma levels of the drug and optionally, the drug metabolites, such as the metabolite known to be formed by CYP3A4/5. CYP3A4 and CYP3A5 are two of such enzymes and are largely prevalent in the liver.

**[0013]** A CYP3A4/5 modulator may be a CYP3A4/5 inducer or a CYP3A4/5 inhibitor. A “CYP3A4/5 inducer” or “inducer of CYP3A4/5” is a CYP3A4/5 modulator as defined above that can increase, enhance, stimulate, induce, accelerate or augment the CYP3A4/5 activity from its existing state or native state, such as by at least about 0.1%, 0.2%, 0.5%, 1%, 2%, 3%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% and 100% or more relative to a reference level, *e.g.*, the level of the CYP3A4/5 activity and/or expression prior to the exposure or contact with the CYP3A4/5 inducer, including all ranges and values therebetween not expressly mentioned above. The CYP3A4/5 inducer activity may cause mitapivat or a pharmaceutically acceptable salt thereof that is co-administered with the CYP3A4/5 inducer to be metabolized and

eliminated more quickly from the body, as determined by the measured plasma levels of the compound and its metabolites.

**[0014]** A “CYP3A4/5 inhibitor” or “inhibitor of CYP3A4/5” is a CYP3A4/5 modulator as defined above that can decrease, reduce, inhibit, decelerate, attenuate, or suppress the CYP3A4/5 activity and/or expression from its existing state or native state, such as by at least about 0.1%, 0.2%, 0.5%, 1%, 2%, 3%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% and 100% or more relative to a reference level, *e.g.*, the level of the CYP3A4/5 activity and/or expression prior to the exposure or contact with the CYP3A4/5 inhibitor, including all ranges and values therebetween not expressly mentioned above. The CYP3A4/5 inhibitor may cause mitapivat or a pharmaceutically acceptable salt thereof that is co-administered with the CYP3A4/5 inhibitor to be metabolized and eliminated more slowly from the body, as determined by the measured plasma levels of the compound and its metabolites.

**[0015]** If an investigational drug is a CYP inducer, it can be classified as a strong, moderate, or mild inducer based on its effect on an index CYP substrate. The convention is to categorize CYP induction in the following ways:

**[0016]** A strong inducer decreases the AUC of a sensitive index CYP by  $\geq 80$  percent.

**[0017]** A moderate inducer decreases the AUC of a sensitive index CYP substrate by  $\geq 50$  to  $< 80$  percent.

**[0018]** A mild inducer decreases the AUC of a sensitive index CYP substrate by  $\geq 20$  to  $< 50$  percent.

**[0019]** A CYP inhibitor can be classified as a strong, moderate, or mild inhibitor based on its effect on an index CYP substrate. A strong inhibitor increases the AUC of a sensitive index CYP substrate  $\geq 5$ -fold. A moderate inhibitor increases the AUC of a sensitive index CYP substrate by  $\geq 2$ - to  $< 5$ -fold. A mild inhibitor increases the AUC of a sensitive index CYP substrate by  $\geq 1.25$ - to  $< 2$ - fold.

**[0020]** A p-glycoprotein inhibitor refers to any substance, including, but not limited to a small molecule (with a molecular weight of  $\leq 1000$  Da, or  $\leq 750$  Da, or  $\leq 500$  Da), a nucleoside, a nucleotide, a nucleobase, a sugar, a nucleic acid, an amino acid, a

polysaccharide, a peptide, a polypeptide, a protein, an antibody, an aptamer, a natural or synthetic plant-based substance, or a complex or a composition comprising any combination of the aforementioned substances, that can decrease, reduce, inhibit, decelerate, attenuate, or suppress the p-glycoprotein activity and/or expression from its existing state or native state, such as by at least about 0.1%, 0.2%, 0.5%, 1%, 2%, 3%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, 99% and 100% or more relative to a reference level, *e.g.*, the level of the p-glycoprotein. In some embodiments, the p-glycoprotein inhibitor is a strong p-glycoprotein inhibitor, selected from the group consisting of amiodarone, azithromycin, captopril, carvedilol, clarithromycin, conivaptan, cyclosporine, diltiazem, dronedarone, erythromycin, felodipine, itraconazole, ketoconazole, lopinavir, ritonavir, quercetin, quinidine, ranolazine, ticagrelor, and erapamil.

**[0021]** The terms “effective amount” and “therapeutically effective amount” are used interchangeably throughout the present disclosure, whether referring to mitapivat or a pharmaceutically acceptable salt thereof, a CYP3A4/5 inducer, or a CYP3A4/5 inhibitor.

**[0022]** An “effective amount” of mitapivat or a pharmaceutically acceptable salt thereof is an amount sufficient to provide a therapeutic benefit in the treatment of pyruvate kinase deficiency (PKD), thalassemia (*e.g.*, alfa-thalassemia, beta-thalassemia or non-transfusion-dependent thalassemia), abetalipoproteinemia or Bassen-Kornzweig syndrome, sickle cell disease, paroxysmal nocturnal hemoglobinuria, anemia (*e.g.*, congenital anemias (*e.g.*, enzymopathies), hemolytic anemia (*e.g.*, hereditary and/or congenital hemolytic anemia, acquired hemolytic anemia, chronic hemolytic anemia, chronic hemolytic anemia caused by phosphoglycerate kinase deficiency, anemia of chronic diseases, non-spherocytic hemolytic anemia or hereditary spherocytosis), as well as all other conditions disclosed in the section “Diseases, Disorders and Conditions” in the present disclosure, *i.e.*, conditions that are responsive to treatment with a PKR activator such as mitapivat or a pharmaceutically acceptable salt thereof. Such diseases, disorders and conditions are hereinafter referred as “a PKR activator-responsive condition” when referring to any one of these conditions or “PKR activator-responsive conditions” when referring to these conditions collectively. Additionally or alternatively, an “effective amount” of mitapivat or a pharmaceutically acceptable salt thereof is an amount sufficient to delay or minimize one or more effects or symptoms associated with these conditions. In one aspect, an “effective amount” of mitapivat or a pharmaceutically acceptable salt thereof means an amount of mitapivat or a pharmaceutically

acceptable salt thereof, alone or in combination with other therapies, which provides a therapeutic benefit in the treatment of the condition. The term “effective amount” can encompass an amount that improves overall therapy, reduces or avoids effects, symptoms, signs, or causes of the condition, and/or enhances the therapeutic efficacy of another therapeutic agent. In certain embodiments, an “effective amount” of mitapivat or a pharmaceutically acceptable salt thereof is an amount sufficient for eliciting measurable activation of wild-type or mutant PKR. In certain embodiments, an “effective amount” of mitapivat or a pharmaceutically acceptable salt thereof is an amount sufficient for regulating 2,3-diphosphoglycerate levels in blood in need thereof or for treating PKR activator-responsive conditions or treating diseases or conditions that are associated with increased 2,3-diphosphoglycerate levels (*e.g.*, liver diseases). In certain embodiments, an effective amount of mitapivat or a pharmaceutically acceptable salt thereof is an amount sufficient for eliciting measurable activation of wild-type or mutant PKR and for regulating 2,3-diphosphoglycerate levels in blood in need thereof or for treating PKR activator-responsive conditions or treating diseases or conditions that are associated with increased 2,3-diphosphoglycerate levels (*e.g.*, liver diseases). In one aspect, the effective amount is the amount required to generate a subject’s hemoglobin response of  $\geq 1.0$  g/dL (such as  $\geq 1.5$  g/dL or  $\geq 2.0$  g/dL) increase in Hb concentration from baseline. In one aspect, the subject’s baseline Hb concentration is the average of all available Hb concentrations before treatment with a compound described herein. In certain aspects, the effective amount is the amount required to reduce the patient’s transfusion burden. In one aspect, the effective amount of mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) is between about 0.01-100 mg/kg body weight/day of mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate), *e.g.*, about 0.1-100 mg/kg body weight/day.

**[0023]** Concomitant medications will be commonly used with mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate). An “effective amount” of the drug (*e.g.*, an effective amount of a CYP3A4/5 inducer or an effective amount of a CYP3A4/5 inhibitor) being co-administered with mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) is an amount sufficient to provide a desired therapeutic benefit. Drugs that are being co-administered with mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate), including some of the current antiarrhythmics, antibiotics, antidepressants, antifungals, calcium channel blockers, H<sub>2</sub> receptor antagonists, non-nucleoside reverse transcriptase inhibitors (NNRTI), protease inhibitors, etc., will

sometimes be CYP3A4/5 inducers or CYP3A4/5 inhibitors. In such instances, the amount of mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) or the amount of the drug being co-administered should be adjusted to reduce effects of a drug-drug interaction between mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) and the co-administered drug.

**[0024]** In one aspect, an effective amount of a CYP3A4/5 inducer is between about 0.1-1000 mg/kg/day of a CYP3A4/5 inducer, *e.g.*, about 1.0-1000 mg/kg/day, depending on the drug that is co-administered with.

**[0025]** In one aspect, an effective amount of a CYP3A4/5 inhibitor is between about 0.1-1000 mg/kg/day of a CYP3A4/5 inhibitor, *e.g.*, about 1.0-1000 mg/kg/day, depending on the drug that is co-administered with.

**[0026]** In one aspect, an effective amount of a p-gp inhibitor is between about 0.1-1000 mg/kg/day of a p-gp inhibitor, *e.g.*, about 1.0-1000 mg/kg/day, depending on the drug that is co-administered with.

**[0027]** As used herein, reduction in transfusion burden means at least 20% reduction in the number of RBC units transfused within at least 5 weeks of treatment. In certain embodiments, the reduction in transfusion burden is  $\geq 33\%$  reduction in the number of RBC units transfused within at least 5 weeks of treatment. In certain embodiments, reduction of transfusion burden is  $\geq 33\%$  reduction in the number of RBC units transfused within at least 10 weeks (*e.g.*, at least 20 weeks or at least 24 weeks) of treatment.

**[0028]** The term “activating” as used herein means an agent that (measurably) increases the activity of wild type pyruvate kinase R (wt PKR) or causes wild type pyruvate kinase R (wt PKR) activity to increase to a level that is greater than wt PKR’s basal levels of activity or an agent that (measurably) increases the activity of a mutant pyruvate kinase R (mPKR) or causes mutant pyruvate kinase R (mPKR) activity to increase to a level that is greater than that mutant PKR’s basal levels of activity, for examples, to a level that is 20%, 40%, 50%, 60%, 70%, 80%, 90% or 100% of the activity of wild type PKR.

**[0029]** The terms “treatment,” “treat,” and “treating” refer to reversing, alleviating, reducing the likelihood of developing, or inhibiting the progress of a disease or disorder, or one or more effects or symptoms thereof, as described herein. In some embodiments,

treatment may be administered after one or more symptoms have developed, *i.e.*, therapeutic treatment. In other embodiments, treatment may be administered in the absence of symptoms. For example, treatment may be administered to a susceptible individual prior to the onset of symptoms (*e.g.*, in light of a history of symptoms and/or in light of genetic or other susceptibility factors), *i.e.*, prophylactic treatment. Treatment may also be continued after symptoms have resolved, for example to reduce the likelihood of or delay their recurrence.

**[0030]** As used herein the terms “subject” and “patient” may be used interchangeably, and means a mammal in need of treatment with mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate).

**[0031]** The term “pharmaceutically acceptable salt” when referring to a pharmaceutically acceptable salt of mitapivat, refers to those salts which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of humans and lower animals without undue toxicity, irritation, allergic response, and the like, and are commensurate with a reasonable benefit/risk ratio. Pharmaceutically acceptable salts are well known in the art, for example, Berge et al. describe pharmaceutically acceptable salts in detail in J. Pharmaceutical Sciences, 1977, 66, 1-19. Pharmaceutically acceptable salts of mitapivat include those derived from suitable inorganic and organic acids. Examples of pharmaceutically acceptable acid addition salts are salts of an amino group formed with inorganic acids, such as hydrochloric acid, hydrobromic acid, phosphoric acid, sulfuric acid, and perchloric acid or with organic acids, such as acetic acid, oxalic acid, maleic acid, tartaric acid, citric acid, succinic acid, or malonic acid or by using other methods known in the art such as ion exchange. Other pharmaceutically acceptable salts include adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, besylate bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, formate, fumarate, glucoheptonate, glycerophosphate, gluconate, gentisate, hemisulfate, heptanoate, hexanoate, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, tosylate p-toluenesulfonate, undecanoate, valerate salts, and the like. Preferably, a pharmaceutically acceptable salt of mitapivat is a sulfate salt.

[0032] As used herein, the terms “about” and “approximately” are used herein to mean within the typical ranges of tolerance in the art. In one embodiment, “about” means within 2 standard deviations from the mean value. In one embodiment, “about” means  $\pm 10\%$ . In one embodiment, “about” means  $\pm 5\%$ . When “about” is present before a series of numbers or number ranges, it is understood that the term can apply to any and each of the numbers and ranges recited in the series.

### **Compositions and Administration**

[0033] The effective amount of mitapivat, in accordance with a method of the invention, may be administered in the form of a pharmaceutical composition comprising mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate), together with a pharmaceutically acceptable carrier.

[0034] Pharmaceutical compositions of mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) can be prepared by any method known in the art of pharmacology. In general, such preparatory methods include the steps of bringing of mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) into association with a carrier and/or one or more other accessory ingredients, and then, if necessary and/or desirable, shaping and/or packaging the product into a desired single- or multi-dose unit.

[0035] Pharmaceutically acceptable carriers used in the manufacture of provided pharmaceutical compositions include inert diluents, dispersing and/or granulating agents, surface active agents and/or emulsifiers, disintegrating agents, binding agents, preservatives, buffering agents, lubricating agents, and/or oils. Exemplary carriers, diluents, granulating and/or dispersing agents, surface active agents and/or emulsifiers, binding agents, preservatives, antioxidants, chelating agent, antifungal preservatives, alcohol preservatives, acidic preservatives, other types of preservatives, buffering agents, lubricating agents, and natural oils that can be included in a pharmaceutical composition of mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) as disclosed in International Patent Application No. WO2019/104134.

[0036] Pharmaceutical compositions of mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) may be administered orally, parenterally, by inhalation spray, topically, rectally, nasally, buccally, transmucosally, or in an ophthalmic preparation. The

term “parenteral” as used herein includes subcutaneous, intravenous, intramuscular, intra-articular, intra-synovial, intrasternal, intrathecal, intrahepatic, intralesional and intracranial injection or infusion techniques. In one aspect, the pharmaceutical compositions provided herewith are orally administered in an orally acceptable dosage form including, but not limited to, capsules, tablets, emulsions and aqueous suspensions, dispersions and solutions. In the case of tablets for oral use, carriers which are commonly used include one or more substances selected from microcrystalline cellulose, mannitol, croscarmellose sodium, and sodium stearyl fumarate. Lubricating agents, such as magnesium stearate, are also typically added. For oral administration in a capsule form, useful diluents include lactose and dried corn starch. When aqueous suspensions and/or emulsions are administered orally, the active ingredient may be suspended or dissolved in an oily phase is combined with emulsifying and/or suspending agents. If desired, certain sweetening and/or flavoring and/or coloring agents may be added.

**[0037]** A secondary drug can be co-administered with mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate). The effective amount of a secondary drug that is co-administered with mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate), such as a CYP3A4/5 inducer or a CYP3A4/5 inhibitor or a p-gp inhibitor, if used in a method of the invention, may also be administered in the form of a pharmaceutical composition comprising the CYP3A4/5 inducer or the CYP3A4/5 inhibitor or the p-gp inhibitor, together with a pharmaceutically acceptable carrier. Pharmaceutical compositions of the secondary drug may be prepared and administered as described above for pharmaceutical compositions of mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate).

**[0038]** In one aspect, mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) is formulated as a tablet composition together with a pharmaceutically acceptable carrier, in accordance with the disclosures of International Patent Application No. WO2019/104134.

**[0039]** In one embodiment, in therapies comprising mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) and a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor), mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) and the secondary drug are administered “concurrently” to the subject, which means that the subject is administered both drugs on the same day on all days

of the treatment period. In one embodiment, mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) and the secondary drug are administered “sequentially” to the subject, which means that the subject is administered either of the two drugs on all days of the treatment period, but not both drugs. In another embodiment, mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) and the secondary drug are administered “sequentially and concurrently” to the subject, which means that the subject is administered either of the two drugs on certain days of the treatment period, but not both drugs; and the subject is administered both drugs on the same day on other days of the treatment period.

### **Doses and Dosing Regimens**

**[0040]** The amount of mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) that may be combined with carrier materials to produce a composition in a single dosage form will vary depending upon the subject to be treated and the particular mode of administration. For example, a specific dosage and treatment regimen for any particular subject will depend upon a variety of factors, including age, body weight, general health, sex, diet, time of administration, rate of excretion, drug combination, the judgment of the treating physician, and the severity of the particular disease being treated. The amount of mitapivat or a pharmaceutically acceptable salt thereof will also depend upon the particular form (*e.g.*, free base, salt form, crystalline form, etc.) in the composition. In one aspect, a provided composition may be formulated such that a dosage equivalent to about 0.001 to about 100 mg/kg body weight/day of mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, about 0.5 to about 100 mg/kg of mitapivat or a pharmaceutically acceptable salt thereof) can be administered to a subject receiving these compositions. Alternatively, dosages equivalent to 1 mg/kg and 1000 mg/kg of mitapivat or a pharmaceutically acceptable salt thereof every 4 to 120 hours is also acceptable.

**[0041]** In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose of equivalent to about 2 mg to about 3000 mg of mitapivat. In certain embodiments, the dose is an oral dose. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 2 mg to about 3000 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 5 mg to about 350 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated

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mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 120 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 130 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 140 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 150 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 160 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 170 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 180 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 190 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 200 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 210 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 220 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 230 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 240 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 250 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 260 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 270 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 280 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 290 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 300 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 300 to 360 mg of mitapivat. In certain embodiment, the pharmaceutically acceptable salt of mitapivat is mitapivat sulfate.

**[0042]** In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 2 mg to about 3000 mg of

mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 5 mg to about 500 mg of mitapivat of per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 5 mg to about 200 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 5 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 5 mg to about 10 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose of about 15 mg equivalent to mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 20 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 25 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 30 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 35 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 40 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 45 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 50 mg of mitapivat thereof per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 55 mg of mitapivat thereof per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 60 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 70 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 80 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 90 mg of mitapivat per day. In certain

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embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 270 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 280 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 290 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 300 mg of mitapivat per day. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 300-360 mg of mitapivat per day. Dosing can be once, twice, or three times daily. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 5 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 10 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 15 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 20 mg of mitapivat twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 25 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 30 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 35 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 40 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 45 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 50 mg of mitapivat twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 55 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 60 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is

formulated for administration at a dose equivalent to about 65 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 70 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 75 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 80 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 85 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 90 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 95 mg of mitapivat thereof twice per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 100 mg of mitapivat twice per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 5 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 10 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 15 mg of mitapivat thereof once per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 20 mg of mitapivat once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 25 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 30 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 35 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 40 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 45 mg of mitapivat thereof once per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for

administration at a dose equivalent to about 50 mg of mitapivat once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 55 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 60 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 65 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 70 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 75 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 80 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 85 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 95 mg of mitapivat thereof once per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 100 mg of mitapivat once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 110 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 120 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 130 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 140 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 150 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 160 mg to about 200 mg of mitapivat thereof once per day. In another aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 200 mg to about 360 mg of mitapivat thereof once per day. In one aspect, mitapivat or a pharmaceutically acceptable salt

thereof is formulated for administration at a dose equivalent to about 5 mg of mitapivat once every other day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 20 mg of mitapivat once every other day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 50 mg of mitapivat once every other day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 100 mg of mitapivat once every other day. In certain embodiment, the pharmaceutically acceptable salt of mitapivat is mitapivat sulfate.

**[0043]** In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof by about 70% to about 80%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof by about 60% to about 70%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof by about 50% to about 60%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof by about 40% to about 50%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof by about 30% to about 40%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof by about 20% to about 30%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof by about 10% to about 20%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof by about 50%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 400 mg of mitapivat to the dose equivalent to about 200 mg. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 300 mg of mitapivat to the dose equivalent to about 150 mg. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 200 mg of

mitapivat to the dose equivalent to about 100 mg. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 100 mg of mitapivat to the dose equivalent to about 50 mg. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 50 mg of mitapivat to the dose equivalent to about 25 mg. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 20 mg of mitapivat to the dose equivalent to about 10 mg. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 10 mg of mitapivat to the dose equivalent to about 5 mg. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 5 mg of mitapivat to the dose equivalent to about 2.5 mg.

**[0044]** In certain embodiments, for any of the methods presented herein, further comprising reducing the dose frequency of mitapivat or a pharmaceutically acceptable salt thereof by about 70% to about 80%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose frequency of mitapivat or a pharmaceutically acceptable salt thereof by about 60% to about 70%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose frequency of mitapivat or a pharmaceutically acceptable salt thereof by about 50% to about 60%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose frequency of mitapivat or a pharmaceutically acceptable salt thereof by about 40% to about 50%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose frequency of mitapivat or a pharmaceutically acceptable salt thereof by about 30% to about 40%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose frequency of mitapivat or a pharmaceutically acceptable salt thereof by about 20% to about 30%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose frequency of mitapivat or a pharmaceutically acceptable salt thereof by about 10% to about 20%. In certain embodiments, for any of the methods presented herein, further comprising

reducing the dose frequency of mitapivat or a pharmaceutically acceptable salt thereof by about 50%. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose frequency of mitapivat or a pharmaceutically acceptable salt thereof from twice daily to once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose frequency of mitapivat or a pharmaceutically acceptable salt thereof from once daily to once every other day.

**[0045]** In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 400 mg of mitapivat twice daily to the dose equivalent to about 200 mg twice daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 300 mg of mitapivat twice daily to the dose equivalent to about 150 mg twice daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 200 mg of mitapivat twice daily to the dose equivalent to about 100 mg twice daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 100 mg of mitapivat twice daily to the dose equivalent to about 50 mg twice daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 50 mg of mitapivat twice daily to the dose equivalent to about 25 mg twice daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 20 mg of mitapivat twice daily to the dose equivalent to about 10 mg twice daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 10 mg of mitapivat twice daily to the dose equivalent to about 5 mg twice daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 5 mg twice daily of mitapivat to the dose equivalent to about 2.5 mg twice daily.

**[0046]** In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 400 mg of mitapivat once daily to the dose equivalent to about 200 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 300 mg of mitapivat once daily to the dose equivalent to about 150 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 200 mg of mitapivat once daily to the dose equivalent to about 100 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 100 mg of mitapivat once daily to the dose equivalent to about 50 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 50 mg of mitapivat once daily to the dose equivalent to about 25 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 20 mg of mitapivat once daily to the dose equivalent to about 10 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 10 mg of mitapivat once daily to the dose equivalent to about 5 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 5 mg once daily of mitapivat to the dose equivalent to about 2.5 mg once daily.

**[0047]** In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 400 mg of mitapivat twice daily to the dose equivalent to about 200 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 300 mg of mitapivat twice daily to the dose equivalent to about 150 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from

the dose equivalent to about 200 mg of mitapivat twice daily to the dose equivalent to about 100 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 100 mg of mitapivat twice daily to the dose equivalent to about 50 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 50 mg of mitapivat twice daily to the dose equivalent to about 25 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 20 mg of mitapivat twice daily to the dose equivalent to about 10 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 10 mg of mitapivat twice daily to the dose equivalent to about 5 mg once daily. In certain embodiments, for any of the methods presented herein, further comprising reducing the dose of mitapivat or a pharmaceutically acceptable salt thereof from the dose equivalent to about 5 mg twice daily of mitapivat to the dose equivalent to about 2.5 mg once daily.

**[0048]** In some embodiments, mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) is present in the tablet composition in an amount equivalent to about 1 to about 200 mg of mitapivat. In some embodiments, mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) is present in the tablet composition in an amount equivalent to about 1 to about 150 mg of mitapivat. In some embodiments, mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) is present in the tablet composition in an amount equivalent to about 1 to about 100 mg of mitapivat. In some embodiments, mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) is present in the tablet composition in an amount equivalent to about 5 mg of mitapivat. In some embodiments, mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) is present in the tablet composition in an amount equivalent to about 20 mg of mitapivat. In some embodiments, mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) is present in the tablet composition in an amount equivalent to about 50 mg of mitapivat. In some embodiments, mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) is present in the tablet composition in an amount equivalent to about 75 mg of mitapivat. In some embodiments, mitapivat or a pharmaceutically acceptable

salt thereof (*e.g.*, mitapivat sulfate) is present in a tablet composition in an amount equivalent to about 100 mg of mitapivat. In certain embodiment, the pharmaceutically acceptable salt of mitapivat is mitapivat sulfate.

**[0049]** As used herein, the dose amount of mitapivat or a pharmaceutically acceptable salt thereof (*e.g.*, mitapivat sulfate) is based on the equivalence to the free-base form of mitapivat. For example, “mitapivat or a pharmaceutically acceptable salt thereof present in the composition in an amount equivalent to about 2.0 mg of mitapivat” means about 2.0 mg of free-base mitapivat or about 2.33 mg of mitapivat sulfate having the structural formula as depicted in the background of the present disclosure.

**Methods of Treatment (Mitapivat Monotherapy and Concomitant Medications)**

**[0050]** In one aspect, the present disclosure provides methods of treating a disease, disorder or condition with mitapivat or a pharmaceutically acceptable salt thereof and a CYP3A4/5 inducer or a CYP3A4/5 inhibitor or a p-gp inhibitor; use of mitapivat and a CYP3A4/5 inducer or a CYP3A4/5 inhibitor or a p-gp inhibitor for the manufacture of a medicament for treating a disease, disorder or condition; and mitapivat or a pharmaceutically acceptable salt thereof and a CYP3A4/5 inducer or a CYP3A4/5 inhibitor or a p-gp inhibitor for use in treating a disease, disorder or condition. The disease, disorder or condition treated is pyruvate kinase deficiency (PKD), sickle cell disease, or thalassemia as further discussed in the following embodiments, and any one disease, disorder or condition described in the section “Diseases, Disorders and Conditions” provided in the present disclosure.

**[0051]** In one embodiment, the present disclosure relates to a method of treating pyruvate kinase deficiency (PKD) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inducer of cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5).

**[0052]** In another embodiment, the present disclosure relates to a method of treating sickle cell disease in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inducer of cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5).

**[0053]** In yet another embodiment, the present disclosure relates to a method of treating thalassemia in a subject, comprising administering to the subject an effective amount of

mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inducer of cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5). In certain embodiments, the thalassemia is alpha thalassemia. In certain embodiments, the thalassemia is beta thalassemia.

**[0054]** In one embodiment, the method of any one of the preceding embodiments further comprises:

- (a) monitoring the subject for an effect of a drug-drug interaction between mitapivat or a pharmaceutically acceptable salt thereof and the CYP3A4/5 inducer; and
- (b) in the event that an effect of the drug-drug interaction is present, adjusting the effective amount of mitapivat or a pharmaceutically acceptable salt thereof and/or the effective amount of the CYP3A4/5 inducer being administered to the subject to reduce the effect.

**[0055]** In some embodiments, the adjustment is to reduce the dose of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the adjustment is to reduce the dose frequency of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the adjustment is to reduce the dose and dose frequency of mitapivat or a pharmaceutically acceptable salt thereof. Details of the dose and/or dose frequency adjustment are shown in the dose and dose regime section.

**[0056]** In one embodiment, the method of the preceding embodiment further comprises:

- (c) repeating (a) and (b) until there are no further detectable symptoms of the drug-drug interaction.

Steps (a) and (b) can be repeated as many times as necessary until there are no further detectable symptoms of the drug-drug interaction. In one embodiment, step (a) comprises measuring and analyzing the pharmacokinetic (PK) such as AUC (*i.e.*, area under the plasma concentration-time curve, such as AUC<sub>0-24</sub>, AUC<sub>0-t</sub>, AUC<sub>0-inf</sub>, AUC<sub>%estrap</sub>) and C<sub>max</sub> values (*i.e.*, the maximum (or peak) serum concentration that a drug achieves in a specified compartment or test area of the body after the drug has been administered and before the administration of a second dose). In one embodiment, step (a) comprises measuring and analyzing other PK parameters such as but not limited to C<sub>L</sub>/F (*i.e.*, apparent total body clearance of the drug from plasma), V<sub>Z</sub>/F (*i.e.*, apparent volume of distribution during

terminal phase after non-intravenous administration),  $t_{1/2}$  (elimination half-life),  $t_{last}$ ,  $t_{max}$  (time to reach maximum (peak) plasma concentration following drug administration), etc.

**[0057]** A “drug-drug interaction” as used herein refers to a change in concentration in a patient of a first drug (*e.g.*, mitapivat) and consequential change in the effect of the first drug on the subject/patient resulting from administration of a second drug (*e.g.*, a CYP3A4/5 inducer, a CYP3A4/5 inhibitor). The change in concentration and consequential change in effect of the first drug resulting from the second drug typically occurs because of a change in activity or expression level of a detoxification enzyme that acts to clear the first drug from the patient (such as CYP3A4/5), wherein the change in activity or expression level of the detoxification enzyme is caused by the second drug. An increase in activity or expression level of the detoxification enzyme will tend to decrease the concentration of the first drug in the patient and its consequential effect on the patient, whereas a decrease in activity or expression level of the detoxification enzyme will tend to increase the concentration of the first drug in the patient and its consequential effect on the patient.

**[0058]** An “effect of a drug-drug interaction” as used herein refers to a detectable and measurable effect, such as a detectable symptom, on the subject/patient caused by the drug-drug interaction. Where the concentration of the first drug is increased, such as when mitapivat is co-administered with a CYP3A4/5 inhibitor, the “effect” typically refers to undesired phenomena such as an overdose of the first drug, liver failure, kidney failure, and the prevalence of side effects and adverse reactions. Where the concentration of the first drug is decreased, such as when mitapivat is co-administered with a CYP3A4/5 inducer, the “effect” typically refers to a decrease in the therapeutic efficacy of the first drug (*e.g.*, mitapivat). Exemplary effects of a drug-drug interaction as used herein further include headache, insomnia, nausea, viral upper respiratory tract infection, arthralgia, diarrhoea, fatigue, hot flush, influenza, vomiting, cough, dizziness, oropharyngeal pain, pyrexia, back pain, dysmenorrhoea, and gastroenteritis, and dyspepsia, hypertriglyceridemia, hemolytic anemia, hemolysis, dizziness, etc.

**[0059]** In one embodiment, the present disclosure relates to a method of treating pyruvate kinase deficiency (PKD) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inhibitor of cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5).

**[0060]** In another embodiment, the present disclosure relates to a method of treating sickle cell disease in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inhibitor of cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5).

**[0061]** In yet another embodiment, the present disclosure relates to a method of treating thalassemia in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof and an effective amount of an inhibitor of cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5) or a p-gp inhibitor. In certain embodiments, the thalassemia is alpha thalassemia. In certain embodiments, the thalassemia is beta thalassemia.

**[0062]** In one embodiment, the method of any one of the suitable preceding embodiments further comprises:

(a) monitoring the subject for a symptom of a drug-drug interaction between mitapivat or a pharmaceutically acceptable salt thereof and the CYP3A4/5 inhibitor; and

(b) in the event that a symptom of the drug-drug interaction is present, adjusting the effective amount of mitapivat or a pharmaceutically acceptable salt thereof and/or the effective amount of the CYP3A4/5 inhibitor being administered to the subject to reduce the effect.

**[0063]** In some embodiments, the adjustment is to reduce the dose of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the adjustment is to reduce the dose frequency of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the adjustment is to reduce the dose and dose frequency of mitapivat or a pharmaceutically acceptable salt thereof. Details of the dose and/or dose frequency adjustment are shown in the dose and dose regime section.

**[0064]** In one embodiment, the method of the preceding embodiment further comprises:

(c) repeating (a) and (b) until there are no further detectable symptoms of the drug-drug interaction.

Likewise, steps (a) and (b) can be repeated as many times as necessary until there are no further detectable symptoms of the drug-drug interaction. Ways of monitoring the subject for

a symptom of a drug-drug interaction between mitapivat or a pharmaceutically acceptable salt thereof and the CYP3A4/5 inhibitor as recited in step (a) are as described above.

**[0065]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject the effective amount of mitapivat or a pharmaceutically acceptable salt thereof and the effective amount of the CYP3A4/5 inducer (or inhibitor) sequentially.

**[0066]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject the effective amount of mitapivat or a pharmaceutically acceptable salt thereof and the effective amount of the CYP3A4/5 inducer (or inhibitor) concurrently.

**[0067]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject the effective amount of mitapivat or a pharmaceutically acceptable salt thereof and the effective amount of the CYP3A4/5 inducer (or inhibitor) sequentially and concurrently. As used herein, “co-administered” or “in combination with” encompasses administration of mitapivat or a pharmaceutically acceptable salt thereof and the CYP3A4/5 inducer (or inhibitor) sequentially and concurrently, or at different intervals. In another embodiment, for the method of any one of the embodiments, the CYP3A4/5 inducer is a mild CYP3A4/5 inducer. In another embodiment, for the method of any one of the embodiments, the CYP3A4/5 inhibitor is a mild CYP3A4/5 inhibitor. In another embodiment, for the methods of any one of the embodiments, the CYP3A4/5 inhibitor is a moderate CYP3A4/5 inhibitor.

**[0068]** In another aspect, the present disclosure provides methods of treating a disease, disorder or a condition with mitapivat in the absence of a CYP3A4/5 inducer or a CYP3A4/5 inhibitor or a p-gp inhibitor; use of mitapivat in the absence of a CYP3A4/5 inducer or a CYP3A4/5 inhibitor or a p-gp inhibitor for the manufacture of a medicament for treating disease, disorder or a condition; and mitapivat in the absence of a CYP3A4/5 inducer or a CYP3A4/5 inhibitor or a p-gp inhibitor for use in treating a disease, disorder or a condition.

**[0069]** As used herein, “in the absence of” when referring to a secondary drug co-administered with mitapivat (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor or a p-gp inhibitor), refers to levels of the secondary drug in the subject/patient that are sufficiently low that such that any change in the levels of the detoxification enzymes for mitapivat and the

secondary drug results in no detectable symptoms of a drug-drug interaction between mitapivat and the secondary drug.

**[0070]** In one embodiment, the present disclosure relates to a method of treating pyruvate kinase deficiency (PKD) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of an inducer of cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5).

**[0071]** In another embodiment, the present disclosure relates to a method of treating sickle cell disease in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of an inducer of cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5).

**[0072]** In yet another embodiment, the present disclosure relates to a method of treating thalassemia in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of an inducer of cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5). In some embodiments, the thalassemia is alpha thalassemia. In some embodiments, the thalassemia is beta thalassemia.

**[0073]** In one embodiment, in accordance with the method of any one of the suitable preceding embodiments, administration of a CYP3A4/5 inducer, if any, is terminated at a sufficient time interval prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof such that there is no detectable symptom of a drug-drug interaction between mitapivat or a pharmaceutically acceptable salt thereof and the CYP3A4/5 inducer. In certain embodiments, the CYP3A4/5 inducer is a strong CYP3A4/5 inducer. In certain embodiments, the CYP3A4/5 inducer is a moderate CYP3A4/5 inducer. In some embodiments, the CYP3A4/5 inducer is terminated at least 30 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inducer is terminated at least 28 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inducer is terminated at least 25 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inducer is terminated at least 21 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inducer is terminated at least 15 days prior to

the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inducer is terminated at least 14 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inducer is terminated at least 10 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inducer is terminated at least 7 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inducer is terminated at least 5 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof.

**[0074]** In one embodiment, the present disclosure relates to a method of treating pyruvate kinase deficiency (PKD) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of an inhibitor of cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5).

**[0075]** In another embodiment, the present disclosure relates to a method of treating sickle cell disease in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of an inhibitor of cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5).

**[0076]** In yet another embodiment, the present disclosure relates to a method of treating thalassemia in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of an inhibitor of cytochrome P450 3A4 (CYP3A4) or 3A5 (CYP3A5). In certain embodiment, the thalassemia is alpha-thalassemia. In certain embodiment, the thalassemia is beta-thalassemia.

**[0077]** In one embodiment, in accordance with the method of any one of the suitable preceding embodiments, administration of a CYP3A4/5 inhibitor, if any, is terminated at a sufficient time interval prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof such that there is no detectable symptom of a drug-drug interaction between mitapivat or a pharmaceutically acceptable salt thereof and the CYP3A4/5 inhibitor. In certain embodiments, the CYP3A4/5 inhibitor is a strong CYP3A4/5 inhibitor. In certain embodiments, the CYP3A4/5 inhibitor is a moderate CYP3A4/5 inhibitor. In some embodiments, the CYP3A4/5 inhibitor is terminated at least 21 days prior

to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inhibitor is terminated at least 15 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inhibitor is terminated at least 14 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inhibitor is terminated at least 10 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inhibitor is terminated at least 7 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inhibitor is terminated at least 5 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inhibitor is terminated at least 3 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inhibitor is terminated at least 2 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the CYP3A4/5 inhibitor is terminated at least 1 day prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof.

**[0078]** In one embodiment, the present disclosure relates to a method of treating pyruvate kinase deficiency (PKD) in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of a p-gp inhibitor.

**[0079]** In another embodiment, the present disclosure relates to a method of treating sickle cell disease in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of a p-gp inhibitor.

**[0080]** In yet another embodiment, the present disclosure relates to a method of treating thalassemia in a subject, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof in the absence of a p-gp inhibitor. In certain embodiment, the thalassemia is alpha-thalassemia. In certain embodiment, the thalassemia is beta-thalassemia.

**[0081]** In one embodiment, in accordance with the method of any one of the suitable preceding embodiments, administration of a p-gp inhibitor, if any, is terminated at a sufficient time interval prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof such that there is no detectable symptom of a drug-drug interaction between mitapivat or a pharmaceutically acceptable salt thereof and the p-gp inhibitor. In certain embodiments, the p-gp inhibitor is a strong p-gp inhibitor (*e.g.*, amiodarone, azithromycin, captopril, carvedilol, clarithromycin, conivaptan, cyclosporine, diltiazem, dronedarone, erythromycin, felodipine, itraconazole, ketoconazole, lopinavir, ritonavir, quercetin, quinidine, ranolazine, ticagrelor, and erapamil). In some embodiments, the p-gp inhibitor is terminated at least 21 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the p-gp inhibitor is terminated at least 15 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the p-gp inhibitor is terminated at least 14 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the p-gp inhibitor is terminated at least 10 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the p-gp inhibitor is terminated at least 7 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the p-gp inhibitor is terminated at least 5 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the p-gp inhibitor is terminated at least 3 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the p-gp inhibitor is terminated at least 2 days prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof. In some embodiments, the p-gp inhibitor is terminated at least 1 day prior to the administration of the effective amount of mitapivat or a pharmaceutically acceptable salt thereof.

**[0082]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject about 1 mg to about 2500 mg, or about 5 mg to 2500 mg, or about 30 mg to 2500 mg, or about 5 mg to about 700 mg, or about 15 mg to about 700 mg, about 5 mg to about 360 mg, about 5 mg to about 300 mg, about 50 mg to about 300 mg, about 120 mg to about 360 mg, about 120 mg to about 300 mg, about 5 mg to about 120 mg,

about 5 mg, about 20 mg, about 50 mg, about 100 mg, about 120 mg , about 200 mg, about 300 mg, or about 360 mg of mitapivat or a pharmaceutically acceptable salt thereof.

**[0083]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject about 1 mg to about 2500 mg of mitapivat or a pharmaceutically acceptable salt thereof.

**[0084]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject about 5 mg to 2500 mg of mitapivat or a pharmaceutically acceptable salt thereof.

**[0085]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject about 5 mg to about 360 mg of mitapivat or a pharmaceutically acceptable salt thereof.

**[0086]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject about 5 mg to about 300 mg of mitapivat or a pharmaceutically acceptable salt thereof.

**[0087]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject about 50 mg to about 300 mg of mitapivat or a pharmaceutically acceptable salt thereof.

**[0088]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject about 5 mg of mitapivat or a pharmaceutically acceptable salt thereof.

**[0089]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject about 20 mg of mitapivat or a pharmaceutically acceptable salt thereof.

**[0090]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject about 50 mg of mitapivat or a pharmaceutically acceptable salt thereof.

**[0091]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject about 100 mg of mitapivat or a pharmaceutically acceptable salt thereof.

**[0092]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject the effective amount of mitapivat or a pharmaceutically acceptable salt thereof once daily.

**[0093]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject the effective amount of mitapivat or a pharmaceutically acceptable salt thereof twice daily.

**[0094]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject a sulfate salt of mitapivat, such as a sulfate salt of mitapivat having the structural formula depicted in the background of the present disclosure.

**[0095]** In one embodiment, in the method of any one of the suitable preceding embodiments, the CYP3A4/5 inducer is a strong inducer of CYP3A4/5.

**[0096]** In one embodiment, in the method of any one of the suitable preceding embodiments, the CYP3A4/5 inducer is a moderate inducer of CYP3A4/5.

**[0097]** In one embodiment, in the method of any one of the suitable preceding embodiments, the CYP3A4/5 inducer is a mild inducer of CYP3A4/5.

**[0098]** In one embodiment, in the method of any one of the suitable preceding embodiments, the inducer of CYP3A4/5 is a strong CYP3A4/5 inducer selected from a group consisting of apalutamide, enzalutamide, mitotane, phenytoin, efavirenz, nevirapine, carbamazepine, glucocorticoids, modafinil, oxcarbazepine, phenobarbital, phenytoin, pioglitazone, rifabutin, rifampin, St. John's Wort, and troglitazone.

**[0099]** In one embodiment, in the method of any one of the suitable preceding embodiments, the inducer of CYP3A4/5 is a moderate CYP3A4/5 inducer selected from a group consisting of bosentan, etravirine, phenobarbital, and primidone.

**[00100]** In one embodiment, in the method of any one of the suitable preceding embodiments, the inducer of CYP3A4/5 is a mild CYP3A4/5 inducer selected from a group consisting of armodafinil, modafinil, and rufinamide.

**[00101]** In one embodiment, in the method of any one of the suitable preceding embodiments, the CYP3A4/5 inducer is selected from the group consisting of aminoglutethimide, bexarotene, bosentan, carbamazepine, dexamethasone, efavirenz, fosphenytoin, griseofulvin, modafinil, nafcillin, nevirapine, oxcarbazepine, phenobarbital, phenytoin, primidone, rifabutin, rifampin, rifapentine, St. John's wort, avasimibe, dabrafenib, mifepristone, etravirine, armodafinil, glycerol phenylbutyrate, asunaprevir, rifunamide, oritavancin, clobazam, felbamate, eslicarbazepine acetate, and genistein.

**[00102]** In one embodiment, in the method of any one of the suitable preceding embodiments, the CYP3A4/5 inducer is rifampin. In one aspect, rifampin is administered to the subject in the form of a capsule composition. In one aspect, about 0.1-1000 mg of rifampin is administered to the subject. In one aspect, about 100-1000 mg of rifampin is administered to the subject.

**[00103]** In one embodiment, the method of any one of the suitable preceding embodiments comprises administering about 600 mg of rifampin to the subject. In one aspect, the method of any one of the suitable preceding embodiments comprises administering about 600 mg of rifampin to the subject once daily.

**[00104]** In one embodiment, in the method of any one of the suitable preceding embodiments, the inhibitor of CYP3A4/5 is a strong inhibitor of CYP3A4/5.

**[00105]** In one embodiment, in the method of any one of the suitable preceding embodiments, the inhibitor of CYP3A4/5 is a moderate inhibitor of CYP3A4/5.

**[00106]** In one embodiment, in the method of any one of the suitable preceding embodiments, the inhibitor of CYP3A4/5 is a mild inhibitor of CYP3A4/5.

**[00107]** In one embodiment, in the method of any one of the suitable preceding embodiments, the inhibitor of CYP3A4/5 is selected from the group consisting of toamiodarone, amprenavir, aprepitant, atazanavir, chloramphenicol, clarithromycin, conivaptan, cyclosporine, darunavir, dasatinib, delavirdine, diltiazem, erythromycin,

fluconazole, fluoxetine, fluvoxamine, fosamprenavir, imatinib, indinavir, isoniazid, itraconazole, ketoconazole, lapatinib, miconazole, nefazodone, nelfinavir, posaconazole, ritonavir, quinupristin, saquinavir, tamoxifen, telithromycin, troleandomycin, verapamil, voriconazole, grapefruit, Seville oranges, limes, pomelos, fruit juices, and vegetables from mustard green family.

**[00108]** In one embodiment, in the method of any one of the suitable preceding embodiments, the inhibitor of CYP3A4/5 is a strong CYP3A4/5 inhibitor selected from a group consisting of boceprevir, cobicistat, danoprevir, elvitegravir, ritonavir, grapefruit juice, indinavir, itraconazole, ketoconazole, lopinavir, paritaprevir, ombitasvir, dasabuvir, posaconazole, saquinavir, telaprevir, tipranavir, telithromycin, troleandomycin, nelfinavir, clarithromycin, nefazodone, saquinavir, suboxone, and voriconazole.

**[00109]** In one embodiment, in the method of any one of the suitable preceding embodiments, the inhibitor of CYP3A4/5 is a moderate CYP3A4/5 inhibitor selected from a group consisting of aprepitant, ciprofloxacin, conivaptan, crizotinib, cyclosporine, diltiazem, dronedarone, erythromycin, fluconazole, fluvoxamine, imatinib, tofisopam, and verapamil.

**[00110]** In one embodiment, in the method of any one of the suitable preceding embodiments, the inhibitor of CYP3A4/5 is a mild CYP3A4/5 inhibitor selected from a group consisting of chlorzoxazone, cilostazol, cimetidine, clotrimazole, fosaprepitant, istradefylline, ivacaftor, lomitapide, ranitidine, ranolazine, ticagrelor.

**[00111]** As used herein, the terms “weak inhibitor” and ‘mild inhibitor” are used interchangeably.

**[00112]** As used herein, the terms “weak inducer” and ‘mild inducer” are used interchangeably.

**[00113]** In one embodiment, in the method of any one of the suitable preceding embodiments, the inhibitor of CYP3A4/5 is itraconazole. In one aspect, about 0.1-1000 mg of itraconazole is administered to the subject. In one aspect, itraconazole is administered in the form of an oral solution. In one aspect, about 50-500 mg of itraconazole is administered to the subject.

**[00114]** In one embodiment, the method of any one of the suitable preceding embodiments comprises administering about 200 mg of itraconazole to the subject. In one aspect, about 0.1-1000 mg of itraconazole is administered to the subject. In one aspect, about 50-500 mg of itraconazole is administered to the subject. In one aspect, about 200 mg of itraconazole is administered to the subject. In one aspect, about 200 mg of itraconazole is administered to the subject once daily. In one aspect, about 100 mg of itraconazole is administered to the subject. In one aspect, about 100 mg of itraconazole is administered to the subject once daily. In one aspect, itraconazole is administered in the form of an oral solution.

**Diseases, Disorders and Conditions**

**[00115]** Pyruvate kinase deficiency (PKD) is a glycolytic enzymopathy that results in life-long hemolytic anemia. In certain embodiments, the subject having PKD is a patient having at least 2 mutant alleles in PKLR gene. In certain embodiments, the subject having PKD is a patient having at least 2 mutant alleles in PKLR gene and at least one is a missense mutation. See Canu et al., Blood Cells, Molecules and Diseases **2016**, 57, pp. 100-109. In certain embodiments, a subject having PKD has an Hb concentration less than or equal to 10.0 g/dL. In certain embodiments, the subject having PKD is an adult not under regular transfusion (*e.g.*, having had no more than 4 transfusion episodes in the 12-month period up to the treatment). In certain embodiments, the subject having PKD is an adult transfusion independent (*e.g.*, having no more than 3 units of RBCs transfused in the 12-month period prior to the treatment). In certain embodiments, the subject having PKD is an adult under regular transfusion (*e.g.*, having had at least 4 transfusion episodes (*e.g.*, at least 6 transfusion episodes) in the 12-month period prior to the treatment). In certain embodiments, the subject having PKD has a total number of at least 5 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 10 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 15 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 20 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 25 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 30 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 40 transfusion episodes during the subject's lifetime. In certain

embodiments, the subject having PKD has a total number of at least 50 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 60 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 70 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD is not homozygous for the R479H mutation or does not have 2 non-missense mutations in the PKLR gene. In certain embodiments, the subject having PKD, under regular transfusion, has hemoglobin (Hb)  $\leq 12.0$  g/dL (if male) or  $\leq 11.0$  g/dL (if female), prior to the treatment. In certain embodiments, the subject having PKD, under regular transfusion, has transfusion occurring on average less than or equal to once every three weeks. In certain embodiments, the subject having PKD has received at least 0.8 mg (*e.g.*, at least 1.0 mg) folic acid daily (*e.g.*, for at least 21 days) prior to the treatment. In certain embodiments, the subject with PKD under regular transfusion achieves a reduction in transfusion burden (*e.g.*, at least 33% reduction in the number of RBC units transfused) during the 5 weeks, 10 weeks, 15 weeks, 20 weeks, or 24 weeks, 28 weeks, or 32 weeks of treatment. In certain embodiments, the subject having PKD, not under regular transfusion (having had no more than 4 transfusion episodes in the 12-month period prior to the treatment and/or no transfusion in the 3 months prior to the treatment), has hemoglobin (Hb)  $\leq 10.0$  g/dL regardless of gender prior to the treatment. In certain embodiments, the subject having PKD has undergone splenectomy. In certain embodiment, the subject having PKD is a child 18 years old or younger. In certain embodiments, the child is transfusion dependent.

**[00116]** Exemplified conditions related to PKD include, but are not limited to, anemias, cholecystolithiasis, gallstones, tachycardia, hemochromatosis, icteric sclera, splenomegaly, leg ulcers, jaundice, fatigue, and shortness of breath. As described herein, PKD is a deficiency of PKR. In certain embodiments, the deficiency of PKR is associated with a PKR mutation.

**[00117]** In certain embodiments, the subject with PKD achieves a hemoglobin response of at least 1.0 g/dL increase in Hb concentration after the treatment compared to the baseline of prior to the treatment. In certain embodiments, the subject with PKD achieves a hemoglobin response of at least 1.5 g/dL increase in Hb concentration from baseline prior to the treatment. In certain embodiments, the subject with PKD achieves a hemoglobin response of at least 2.0 g/dL increase in Hb concentration from baseline prior to the treatment.

**[00118]** In an embodiment, the mutant PKR is selected from the group consisting of A31V, A36G, G37Q, R40W, R40Q, L73P, S80P, P82H, R86P, I90N, T93I, G95R, M107T, G111R, A115P, S120F, H121Q, S130P, S130Y, V134D, R135D, A137T, G143S, I153T, A154T, L155P, G159V, R163C, R163L, T164N, G165V, L167M, G169G, E172Q, W201R, I219T, A221Y, D221N, G222A, I224T, G232C, N253D, G263R, G263W, E266K, V269F, L272V, L272P, G275R, G275R, E277K, V280G, D281N, F287V, F287L, V288L, D293N, D293V, A295I, A295V, I310N, I314T, E315K, N316K, V320L, V320M, S330R, D331N, D331G, D331E, G332S, V335M, A336S, R337W, R337P, R337Q, D339N, D339Q, G341A, G341D, I342F, K348N, A352D, I357T, G358R, G358E, R359C, R359H, C360Y, N361D, G364D, K365M, V368F, T371I, L374P, S376I, T384M, R385W, R385K, E387G, D390N, A392T, N393D, N393S, N393K, A394S, A394D, A394V, V395L, D397V, G398A, M403I, G406R, E407K, E407G, T408P, T408A, T408I, K410E, G411S, G411A, Q421K, A423A, A423A, R426W, R426Q, E427A, E427N, A431T, R449C, I457V, G458D, A459V, V460M, A468V, A468G, A470D, T477A, R479C, R479H, S485F, R486W, R486L, R488Q, R490W, I494T, A495T, A495V, R498C, R498H, A503V, R504L, Q505E, V506I, R510Q, G511R, G511E, R518S, R531C, R532W, R532Q, E538D, G540R, D550V, V552M, G557A, R559G, R559P, N566K, M568V, R569Q, R569L, Q58X, E174X, W201X, E241X, R270X, E440X, R486X, Q501X, L508X, R510X, E538X, R559X. These mutations are described in Canu et al., *Blood Cells, Molecules and Diseases* **2016**, 57, pp. 100-109. In an embodiment, the mutant PKR is selected from G332S, G364D, T384M, K410E, R479H, R479K, R486W, R532W, R510Q, and R490W. In certain embodiments, the mutant PKR is selected from A468V, A495V, I90N, T408I, and Q421K, and R498H. In certain embodiments, the mutant PKR is R532W, K410E, or R510Q. In certain embodiments, the mutant PKR is R510Q, R486W, or R479H.

**[00119]** In other aspects, provided are methods of treating a disease selected from hemolytic anemia, hereditary spherocytosis, hereditary elliptocytosis, abetalipoproteinemia, Bassen-Kornzweig syndrome, and paroxysmal nocturnal hemoglobinuria in a subject in need thereof, comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor). Also provided is mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor), for use in treating disease selected from hemolytic anemia, hereditary

spherocytosis, hereditary elliptocytosis, abetalipoproteinemia, Bassen-Kornzweig syndrome, and paroxysmal nocturnal hemoglobinuria in a subject. Further provided is the use of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor), in the manufacture of a medicament for treating a disease selected from hemolytic anemia, hereditary spherocytosis, hereditary elliptocytosis, abetalipoproteinemia, Bassen-Kornzweig syndrome, and paroxysmal nocturnal hemoglobinuria in a subject in need thereof. In one aspect, the disease to be treated is hemolytic anemia.

**[00120]** Thalassemia is an inherited blood disorder in which the normal ratio of  $\alpha$ - to  $\beta$ -globin production is disrupted due to a disease-causing variant in 1 or more of the globin genes. In certain embodiments, Alpha-globin aggregates (as found in  $\beta$ -thalassemia) readily precipitate, which disrupts the red blood cell (RBC) membrane and results in oxidative stress. In certain embodiments, Beta-globin tetramers (Hb H, found in  $\alpha$ -thalassemia) are generally more soluble, but are still unstable and can form precipitates. The imbalance of the globin chain synthesis can lead to a net reduction in Hb concentrations and has dramatic effects on the survival of RBC precursors, ultimately resulting in their premature destruction in the bone marrow and in extramedullary sites (Cappellini et al., 2014). In certain embodiments, the disorder results in large numbers of red blood cells being destroyed, which leads to anemia. In certain embodiments, the thalassemia is alpha thalassemia. In certain embodiments, the thalassemia is beta thalassemia. In other embodiments, the thalassemia is non-transfusion-dependent thalassemia. In other embodiments, the thalassemia is beta thalassemia intermedia. In other embodiments, the thalassemia is Hb E beta thalassemia. In other embodiments, the thalassemia is beta thalassemia with mutations of 1 or more alpha genes.

**[00121]** In certain embodiments, the subject is an adult subject with thalassemia. In certain embodiments, the subject has thalassemia such as  $\beta$ -thalassemia intermedia, Hb E  $\beta$ -thalassemia,  $\alpha$ -thalassemia (Hb H disease), or  $\beta$ -thalassemia with mutations of 1 or more  $\alpha$  genes. In certain embodiments, the subject has non-transfusion-dependent thalassemia. In certain embodiments, the subject has non-transfusion-dependent alpha thalassemia. In certain embodiments, the subject has beta-thalassemia or non-transfusion-dependent beta-thalassemia. In certain embodiments, the subject is an adult male subject with thalassemia such as beta-thalassemia or non-transfusion-dependent thalassemia. In certain embodiments, the subject is a female subject with thalassemia such as beta-thalassemia or non-transfusion-

dependent thalassemia. In certain embodiments, the subject is an adult female subject with thalassemia such as beta-thalassemia or non-transfusion-dependent thalassemia. In certain embodiments, the subject has a hemoglobin concentration of less than or equal to 6.0 g/dL. In certain embodiments, the subject has a hemoglobin concentration of less than or equal to 7.0 g/dL. In certain embodiments, the subject has a hemoglobin concentration of less than or equal to 8.0 g/dL. In certain embodiments, the subject has a hemoglobin concentration of less than or equal to 9.0 g/dL. In certain aspects, the subject having non-transfusion-dependent thalassemia does not have a known history (*e.g.*, has been diagnosed in the past) of Hb S or Hb C forms of thalassemia. In certain embodiments, the term “non-transfusion dependent” thalassemia refers to subjects with thalassemia having no more than 4 (*e.g.*, five) units of RBCs transfused during a 24-week period up to the first day of administration of mitapivat and/or no RBC transfusions in the 8 weeks prior to the first day of administration of mitapivat. In certain embodiment, the subject is an adult over 18 years old. In certain embodiment, the subject is a child 18 years old or younger.

**[00122]** As used herein, sickle cell disease (SCD), Hemoglobin SS disease, and sickle cell anemia are used interchangeably. Sickle cell disease (SCD) is an inherited blood disorder caused by the presence of sickle hemoglobin (HbS). In certain embodiments, subjects with SCD have abnormal hemoglobin, called hemoglobin S or sickle hemoglobin, in their red blood cells. In certain embodiments, people having SCD have at least one abnormal gene causing the body to make hemoglobin S. In certain embodiments, people having SCD have two hemoglobin S genes, Hemoglobin SS.

**[00123]** In other aspects, provided herein are methods for treating anemia in a subject in need thereof comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor). Also provided is mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor), for use in treating anemia in a subject in need thereof. Further provided is the use of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor), in the manufacture of a medicament for treating anemia. In one aspect, the anemia to be treated is dyserythropoietic anemia.

**[00124]** In certain embodiments, the anemia is a dyserythropoietic anemia such as congenital dyserythropoietic anemia type I, II, III, or IV. In certain embodiments, the anemia is hemolytic anemia. In certain embodiments, the hemolytic anemia is a congenital and/or hereditary form of hemolytic anemia such as PKD, sickle cell disease, thalassemias (*e.g.*, alpha or beta or non-transfusion-dependent thalassemia), hereditary spherocytosis, hereditary elliptocytosis), paroxysmal nocturnal hemoglobinuria, abeta-lipoproteinemia (Bassen-Kornzweig syndrome). In certain embodiments, the hemolytic anemia is acquired hemolytic anemia such as autoimmune hemolytic anemia, drug-induced hemolytic anemia. In certain embodiments, the hemolytic anemia is anemia as part of a multi-system disease, such as the anemia of Congenital Erythropoietic Purpura, Fanconi, Diamond-Blackfan.

**[00125]** As used herein, the term “anemia” refers to a deficiency of red blood cells (RBCs) and/or hemoglobin. As used herein, anemia includes all types of clinical anemia, for example (but not limited to): microcytic anemia, iron deficiency anemia, hemoglobinopathies, heme synthesis defect, globin synthesis defect, sideroblastic defect, normocytic anemia, anemia of chronic disease, aplastic anemia, hemolytic anemia, macrocytic anemia, megaloblastic anemia, pernicious anemia, dimorphic anemia, anemia of prematurity, Fanconi anemia, hereditary spherocytosis, sickle cell disease, warm autoimmune hemolytic anemia, cold agglutinin hemolytic anemia, osteopetrosis, thalassemia, and myelodysplastic syndrome.

**[00126]** In certain embodiments, anemia can be diagnosed on a complete blood count. In certain embodiments, anemia can be diagnosed based on the measurement of one or more markers of hemolysis (*e.g.*, RBC count, hemoglobin, reticulocytes, schistocytes, lactate Dehydrogenase (LDH), haptoglobin, bilirubin, and ferritin) and/or hemosiderinuria mean corpuscular volume (MCV) and/or red cell distribution width (RDW). In the context of the present invention, anemia is present if an individual has a hemoglobin (Hb) less than the desired level, for example, the Hb concentration of less than 14 g/dL, more preferably of less than 13 g/dL, more preferably of less than 12 g/dL, more preferably of less than 11 g/dL, or most preferably of less than 10 g/dL.

**[00127]** In certain embodiments, provided herein is a method of increasing the amount of hemoglobin in a subject by administering an effective amount of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor). In certain embodiments, also provided herein is a method of increasing the amount of

hemoglobin in a subject having thalassemia comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor). Further provided is a method of increasing the amount of hemoglobin in subjects having non-transfusion-dependent thalassemia comprising administering an effective amount of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor) to the subject. In certain embodiments, the provided methods increase hemoglobin concentration in the subject. In certain embodiments, the provided methods increase Hb concentration to a desired level, for example, above 10 g/dL, more preferably above 11 g/dL, more preferably above 12 g/dL, more preferably above 13 g/dL, or most preferably above 14 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 0.5 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 1.0 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 1.5 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 2.0 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 2.5 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 3.0 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 3.5 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 4.0 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 4.5 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 5.0 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 5.5 g/dL. In certain embodiments, the provided methods increase Hb concentration by at least about 6.0 g/dL. In certain embodiments, the increase in Hb concentration is determined from baseline at one or more assessment between week 1 and week 20 (*e.g.*, between week 2 and week 15, between week 3 and week 15, and between week 4 and week 12) of treatment with an effective amount of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor). In certain embodiments, the provided methods increase Hb concentration as described above in female subjects having thalassemia (*e.g.*, beta-thalassemia or non-transfusion-dependent thalassemia). In certain embodiments, the provided methods increase Hb concentration from baseline to about 12 g/dL in female subjects having

thalassemia (*e.g.*, beta-thalassemia or non-transfusion-dependent thalassemia). In certain embodiments, the provided methods increase Hb concentration as described above in male subjects having thalassemia (*e.g.*, beta-thalassemia or non-transfusion-dependent thalassemia). In certain embodiments, the provided methods increase Hb concentration from baseline to about 13 g/dL in male subjects having thalassemia (*e.g.*, beta-thalassemia or non-transfusion-dependent thalassemia).

**[00128]** In some aspects, provided herein are methods for treating hemolytic anemia in a subject in need thereof comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor). Also provided is mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor), for use in treating hemolytic anemia in a subject in need thereof. Further provided is the use of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor), in the manufacture of a medicament for treating hemolytic anemia. In one aspect, the hemolytic anemia to be treated is hereditary and/or congenital hemolytic anemia, acquired hemolytic anemia, or anemia as part of a multi-system disease.

**[00129]** In some aspects, provided herein are methods for activating wild-type or mutant PKR in red blood cells in a subject in need thereof comprising administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor). Also provided is mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor), for use in activating wild-type or mutant PKR in red blood cells in a subject in need thereof. Further provided is the use of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor), in the manufacture of a medicament for activating wild-type or mutant PKR in red blood cells.

**[00130]** Mitapivat is an activator of PKR mutants having lower activities compared to the wild type, thus are useful for methods of the present disclosure. Such mutations in PKR can affect enzyme activity (catalytic efficiency), regulatory properties (modulation by fructose biphosphate (FBP)/ATP), and/or thermostability of the enzyme. Examples of such mutations are described in Valentini et al., JBC 2002. Some examples of the mutants that are activated by the compounds described herein include G332S, G364D, T384M, R479H, R479K, R486W, R532W, R510Q, and R490W. Without being bound by theory, in certain embodiments, mitapivat affects the activities of PKR mutants by activating FBP non-responsive PKR mutants, restoring thermostability to mutants with decreased stability, or restoring catalytic efficiency to impaired mutants.

**[00131]** In certain embodiments, mitapivat increases the affinity of PKR to phosphoenolpyruvate (PEP). In certain embodiments, mitapivat restores the ability of RBCs to convert PEP and ADP to pyruvate and ATP.

**[00132]** In certain embodiments, provided herein are methods of reducing transfusion frequency of a subject with PKD comprising administering to the subject mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor). In certain embodiments, the transfusion frequency is reduced by at least 5% in the number of RBC units transfused over at least 15 weeks. In certain embodiments, the transfusion frequency is reduced by at least 10% in the number of RBC units transfused over at least 15 weeks. In certain embodiments, the transfusion frequency is reduced by at least 15% in the number of RBC units transfused over at least 15 weeks. In certain embodiments, the transfusion frequency is reduced by at least 20% in the number of RBC units transfused over at least 15 weeks. In certain embodiments, the transfusion frequency is reduced by at least 25% in the number of RBC units transfused over at least 15 weeks. In certain embodiments, the transfusion frequency is reduced by at least 30% in the number of RBC units transfused over at least 15 weeks. In certain embodiments, the transfusion frequency is reduced by at least 35% in the number of RBC units transfused over at least 15 weeks. In certain embodiments, the transfusion frequency is reduced by at least 40% in the number of RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 5% in the number of RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 10% in the number of

RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 15% in the number of RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 20% in the number of RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 25% in the number of RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 30% in the number of RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 35% in the number of RBC units transfused over at least 20 weeks. In certain embodiments, the transfusion frequency is reduced by at least 40% in the number of RBC units transfused over at least 20 weeks.

**[00133]** In some aspects, provided herein are methods of evaluating the efficacy of mitapivat therapy (monotherapy or concomitant medications with a CYP3A4/5 inducer or a CYP3A4/5 inhibitor) in a subject, the method comprising: administering to the subject mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor); and acquiring a value for the level of mitapivat, the level of 2,3-diphosphoglycerate (2,3-DPG), the level of adenosine triphosphate (ATP), or the activity of PKR in the subject, to thereby evaluate the efficacy of the mitapivat therapy. In some aspects, the value for the level is acquired by analyzing the plasma concentration of mitapivat and optionally, the metabolite known to be formed by CYP3A4/5. In some aspects, the level of 2,3-DPG is acquired by analyzing the blood concentration of 2,3-DPG. In some aspects, the level of ATP is acquired by analyzing the blood concentration of ATP. In some aspects, the activity of PKR is acquired by analyzing the blood concentration of a <sup>13</sup>C-label in the blood. In some aspects, the analysis is performed by sample analysis of bodily fluid. In some aspects, the bodily fluid is blood. In some aspects, the analysis is performed by mass spectroscopy. In some aspects, the analysis is performed by LC-MS.

**[00134]** In some aspects, provided herein are methods of treating a subject, the method comprising: administering to the subject an effective amount of mitapivat or a pharmaceutically acceptable salt thereof, in combination with or in the absence of an effective amount of a secondary drug (*e.g.*, a CYP3A4/5 inducer or a CYP3A4/5 inhibitor); and acquiring a value for the level of mitapivat and optionally its metabolite known to be

formed by CYP3A4/5, the level of 2,3-diphosphoglycerate (2,3-DPG), the level of adenosine triphosphate (ATP), or the activity of PKR in the subject, to thereby treat the subject.

## EXEMPLIFICATIONS

**[00135]** As depicted in the Examples below, the drug-drug interactions between mitapivat sulfate and a CYP3A4/5 inducer (*e.g.*, rifampin) or a CYP3A4/5 inhibitor (*e.g.*, itraconazole) are studied. Mitapivat can be synthesized using the procedures described in International Patent Application Publication Nos. WO 2011/002817 and WO 2016/201227.

### **Example 1: Mitapivat sulfate is primarily metabolized by CYP3A4**

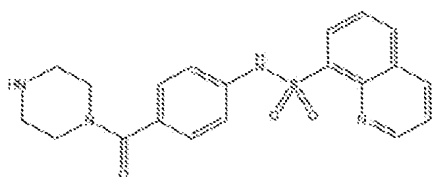
**[00136]** CYP-enzyme phenotyping using human liver microsomes and recombinant CYP enzymes revealed that mitapivat sulfate was primarily metabolized by CYP3A4/5 (>90%), with minor contributions from CYP2C9, CYP2C8, and CYP1A2. There was evidence of metabolism-dependent inhibition of CYP2C19 (largely reversible) and CYP3A4 (largely irreversible). Mitapivat sulfate was found to be a substrate and inhibitor for P-gp but not for breast cancer resistance protein (BCRP) and was found to be a potential inducer of human CYP2B6 and CYP3A4. Mitapivat sulfate appeared to be a mild inhibitor of CYP2C8, CYP2C9, xYP2C19, CYP2D6, and CYP3A4/5 enzymes (testosterone 6 $\beta$ -hydroxylation), bile salts export pump (BSEP), organic anion transporting polypeptide (OATP)1B1, organic anion transporter (OAT)3, and organic cation transporter (OCT)2, and of uridine-5'-diphospho-glucuronosyltransferase (UGT) 1A3, 1A4, and 1A9. Mitapivat sulfate does not appear to be an inhibitor of multidrug resistance-associated protein (MRP)2, MRP3, OATP1B3, and OAT1. The metabolite was found to be a mild inhibitor of CYP2C9 and CYP2C19 and was not an inhibitor of P-gp or BCRP under tested concentrations.

### **Example 2: Drug-drug Interaction (DDI) Studies of Mitapivat Sulfate and a CYP3A4 Inducer or a CYP3A4 Inhibitor**

**[00137]** Twenty-eight (28) healthy, adult male and female (non-childbearing potential) subjects were enrolled in the study in total; 14 subjects in each study part (Parts 1 and 2). A minimum of 8 female subjects were enrolled in the study (*i.e.*, a minimum of 4 female subjects per study part). Each subject participated in either Part 1 or Part 2, but not both.

**Part 1**

**[00138]** On Day 1 of Treatment Period 1, a single oral dose of 20 mg mitapivat sulfate was administered. Serial blood samples for plasma assay of mitapivat concentrations and its CYP3A4 metabolite, referred to herein as the “Metabolite” (structure below),



were collected from predose to 120 hours following administration of mitapivat sulfate. In Treatment Period 2, an oral dose of 200 mg itraconazole was administered once daily (QD) for 9 consecutive days (Day 1 through Day 9 of Treatment Period 2) with a single oral dose of 20 mg mitapivat sulfate coadministered on Day 5. Serial blood samples for plasma assay of mitapivat and the Metabolite concentrations were collected from predose to 120 hours following coadministration of mitapivat sulfate and itraconazole on Day 5.

**[00139]** In Treatment Period 1, mitapivat sulfate was administered orally with approximately 240 mL of water. In Treatment Period 2, on Days 1 to 4, itraconazole was administered alone immediately followed by approximately 220 mL of water, and on Day 5, itraconazole was administered first (no water) and was immediately followed by mitapivat sulfate administration with approximately 220 mL of water. Study drugs (mitapivat sulfate and itraconazole) were administered following an overnight fast of at least 10 hours on Day 1 of Treatment Period 1 (mitapivat sulfate only) and Day 5 of Treatment Period 2 (mitapivat sulfate and itraconazole), and subjects remained fasted for 4 hours after dosing. On all other dosing days, itraconazole was administered following a predose fast of at least 4 hours and subjects remained fasted for at least 2 hours after dosing.

**Part 2**

**[00140]** On Day 1 of Treatment Period 1, a single oral dose of 50 mg mitapivat sulfate was administered. Serial blood samples for plasma assay of mitapivat and the Metabolite concentrations were collected from predose to 120 hours following administration of mitapivat sulfate. In Treatment Period 2, an oral dose of 600 mg rifampin was administered QD for 12 consecutive days (Day 1 through Day 12 of Treatment Period 2) with a single oral dose of 50 mg mitapivat sulfate coadministered on Day 8. Serial blood samples for plasma

assay of mitapivat sulfate and the Metabolite concentrations were collected from predose to 120 hours following coadministration of mitapivat and rifampin on Day 8.

**[00141]** In Part 2, study drugs were administered with approximately 240 mL of water on all dosing days including the coadministration of mitapivat sulfate and rifampin on Day 8 of Treatment Period 2. Mitapivat sulfate and rifampin was administered following an overnight fast of at least 10 hours on Day 1 of Treatment Period 1 (mitapivat sulfate only) and Day 8 of Treatment Period 2 (both mitapivat sulfate and rifampin) and subjects remained fasted for 4 hours after dosing. On all other dosing days, rifampin was administered following a predose fast of at least 4 hours and subjects remained fasted for at least 2 hours after dosing. There was a washout period of 7 days between the mitapivat sulfate dose in Treatment Period 1 and the first itraconazole (Part 1) or rifampin (Part 2) dose in Treatment Period 2. All study drugs were consumed within 5 minutes for both Part 1 and Part 2.

### **Test and Reference Products, Dose, Duration and Mode of Administration**

#### **Part 1**

**[00142]** Mitapivat sulfate (20 mg tablets) administered along with Itraconazole (also referred to as Sporanox®, 10 mg/mL oral solution, is considered as the test arm in this study. Mitapivat sulfate administered alone is considered as the reference arm in this study.

In Period 1, a single oral dose of 20 mg mitapivat sulfate was administered orally with approximately 240 mL of water. In Period 2, on Day 5 (coadministration of itraconazole and mitapivat sulfate), 200 mg itraconazole (20 mL of 10 mg/mL oral solution) was administered first (no water) and was immediately followed by 20 mg mitapivat sulfate (1 x 20 mg tablet) administration with approximately 220 mL of water. On all other dosing days, itraconazole was administered alone immediately followed by approximately 220 mL of water.

Each subject received 2 doses of mitapivat sulfate and 9 doses of itraconazole in Part 1.

#### **Part 2**

**[00143]** Mitapivat sulfate (50 mg tablets) administered along with Rifampin (also referred to as Rifadin®, 300 mg capsules) is considered as the test arm in this study. Mitapivat sulfate (50 mg tablets) administered alone is considered as the reference arm in this study.

**[00144]** In Period 1, a single oral dose of 50 mg mitapivat sulfate was administered orally. In Period 2, subjects were administered 600 mg rifampin (2 x 300 mg capsules) QD on

Day 1 to Day 12 inclusive, with 50 mg mitapivat sulfate (1 x 50 mg tablet) coadministered on Day 8. Study drugs were administered with approximately 240 mL of water on all dosing days including the coadministration of mitapivat sulfate and rifampin on Day 8 of Period 2.

**[00145]** Each subject received 2 doses of mitapivat sulfate and 12 doses of rifampin in Part 2.

#### **Duration of Treatment:**

**[00146]** The total duration of participation including the screening period for each subject was approximately 49 to 62 days for Part 1 and approximately 61 or 63 days in Part 2 of the study.

**[00147]** In each study part, Treatment Period 1 was approximately 6 days and Treatment Period 2 was approximately 12 days; a washout phase was 7 days between both treatment periods in each study part.

#### **Criteria for Evaluation of Pharmacokinetics**

**[00148]** In Parts 1 and 2, for mitapivat and the Metabolite, blood samples were obtained for all subjects to determine the plasma concentrations of mitapivat, and the Metabolite, before mitapivat sulfate dosing (0 hour) on Day 1 of Period 1 (Parts 1 and 2) and Day 5 (Part 1) or Day 8 (Part 2) of Period 2 at 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 16, 24, 48, 72, 96, and 120 hours postdose. Plasma concentrations of mitapivat and the Metabolite were determined using methods validated with respect to accuracy, precision, linearity, sensitivity, and specificity, with a lower limit of quantitation (LLOQ) of 0.5 ng/mL for both analytes. A noncompartmental PK approach was used to analyze individual plasma mitapivat and the Metabolite concentration time data (using Phoenix® WinNonlin® Version 7.0). The following PK parameters were calculated:  $AUC_{0-t}$ ,  $AUC_{0-inf}$ ,  $AUC_{0-24}$ ,  $AUC_{\%extrap}$ ,  $C_{max}$ ,  $CL/F$  (mitapivat only),  $T_{last}$ ,  $T_{max}$ ,  $t_{1/2}$ , and  $V_z/F$  (mitapivat only).

#### **Statistical Methods for Pharmacokinetics**

**[00149]** The plasma concentrations of mitapivat and the Metabolite were listed and summarized by treatment and collection time for all subjects in the PK Population. Plasma concentrations were presented with the same level of precision as received from the bioanalytical laboratory including sample size (n), arithmetic mean (Mean), standard

deviation (SD), coefficient of variation (CV%), standard error of the mean (SEM), minimum, maximum, and median. Concentrations from excluded subjects were included in the plasma concentration tables, but were excluded from the summary statistics and noted as such in the tables. All BLQ values were presented as “BLQ” in the plasma concentration table listings and footnoted accordingly.

**[00150]** PK parameters were presented with a minimum of 3 significant figures. PK parameters were listed by subject, tabulated by treatment, and summarized using descriptive statistics (n, mean, SD, CV%, SEM, minimum, median, maximum, geometric mean (Geom. Mean) and geometric mean coefficient of variation (Geom. CV%). Time-based parameters (*i.e.*, Tmax and  $t_{1/2}$ ) and treatment:reference AUC and Cmax ratios were presented to 2 decimals. Excluded subjects were included in the PK parameter table listings, but were excluded from the summary statistics and noted as such in the tables.

**[00151]** To evaluate the effect of itraconazole and rifampin on mitapivat and the Metabolite (primary objective), plasma mitapivat and the Metabolite PK parameters (Cmax, AUC<sub>0-t</sub>, and AUC<sub>0-inf</sub>) were natural log (ln) transformed before analysis. The ln-transformed value of the PK parameters was analyzed using a linear mixed effect model for each study part separately with treatment as a fixed effect and subject as a random effect. The linear mixed effect model included treatment as a fixed effect and subject as a random effect. The inferential results (least-squares means [LSMs], difference between LSMs, and 90% confidence intervals [CIs] of the difference) were back transformed to the original scale. Geometric LSMs, geometric mean ratios (GMRs), and 90% CIs were presented. The GMR of the LSMs were calculated from the back-transformed difference between the treatment LSMs. The GMRs were expressed as a percentage relative to mitapivat sulfate given alone for both Part 1 and Part 2. The 90% CIs for the ratios were derived by back-transformation of the CIs obtained for the difference between the treatment LSMs. The CIs were expressed as a percentage relative to mitapivat sulfate given alone for both Part 1 and Part 2.

**[00152]** The median differences in Tmax for evaluation of the effect of itraconazole and rifampin on mitapivat and the Metabolite along with the range of the difference were calculated using the non parametric Wilcoxon Signed Rank test, and the p-value was presented. Note that Tmax was not ln-transformed for these analyses.

## Pharmacokinetic Results

## **Part 1**

**[00153]** Mean plasma mitapivat and the Metabolite concentration-time profiles following 20 mg mitapivat sulfate administered alone (Period 1) or coadministered with multiple doses of 200 mg itraconazole (Period 2) on linear and semi-log scales are presented in Figures 1 and 2.

### **Mitapivat**

**[00154]** Mitapivat plasma concentrations were detectable in all subjects at 0.5 hour (the first scheduled postdose sample) in both Periods 1 and 2. Mitapivat was still detectable up to the last sample time at 120 hours postdose in the majority of subjects in Period 1 and in all subjects in Period 2.

**[00155]** Measurable predose mitapivat concentrations were present in all but 2 subjects in Period 2 (two subjects were BLQ). As used herein, BLQ refers to below the limit of quantification. These predose concentrations were < 5% of the C<sub>max</sub>, so PK calculations were performed.

**[00156]** Mitapivat concentrations exhibited a characteristic multi-compartmental pharmacokinetic profile. When coadministered with itraconazole, mitapivat concentrations rose to a higher mean peak with a delay in time to peak by approximately 2 hours compared to when the drug was administered alone. The post-peak decline of the multi-compartmental behavior was slower after coadministration with itraconazole when compared to mitapivat administered alone. Overall, higher mitapivat concentrations were experienced for a longer time period when mitapivat was coadministered with itraconazole.

### **The Metabolite**

**[00157]** When mitapivat was administered alone, the Metabolite plasma concentrations were detectable in all but 1 subject at 0.5 hour. The Metabolite remained detectable all but 1 subject up to 48 hours postdose, with quantifiable the Metabolite out to 72 hours in 3 subjects and 96 hours in 1 subject. In Period 2 (coadministration with itraconazole), the Metabolite concentrations were delayed relative to administration of mitapivat alone. Concentrations were not measurable in all subjects until 1.5 hours postdose. Maximum concentrations were also reduced. However, the Metabolite residence was prolonged in the presence of itraconazole, with concentrations still detectable in all but 1 subject throughout the 120-hour sampling interval (in 1 subject the last measurable concentration was at 96 hours postdose).

[00158] The statistical comparisons of mitapivat PK parameters following 20 mg mitapivat sulfate administered alone (Period 1) or coadministered with multiple doses of 200 mg itraconazole (Period 2) PK parameters are summarized below in Table 1.

**Table 1**  
**Summary of Statistical Comparisons of Plasma Mitapivat Pharmacokinetic Parameters Following 20 mg Mitapivat Sulfate Co-Administered With Multiple Doses of 200 mg Itraconazole Versus Administered Alone (Part 1)**

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Mitapivat Sulfate + Itraconazole (Test) |    | Mitapivat sulfate alone (Reference) |    |        |                         |                   |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------|----|-------------------------------------|----|--------|-------------------------|-------------------|
| Parameter (unit)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Geometric LSM                           | n  | Geometric LSM                       | n  | GMR    | 90% Confidence Interval | Intra-subject CV% |
| AUC <sub>0-t</sub> (ng*hr/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | 8860                                    | 14 | 1885                                | 14 | 4.6998 | 4.2519 - 5.1949         | 15.05             |
| AUC <sub>0-inf</sub> (ng*hr/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 9475                                    | 14 | 1942                                | 14 | 4.8780 | 4.4154 - 5.3890         | 14.97             |
| C <sub>max</sub> (ng/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | 704.3                                   | 14 | 412.5                               | 14 | 1.7074 | 1.5815 - 1.8433         | 11.48             |
| Mitapivat sulfate alone: 20 mg mitapivat sulfate (1 x 20 mg tablet) at Hour 0 on Day 1 (Period 1)<br>Mitapivat sulfate + itraconazole: 200 mg itraconazole (20 mL of 10 mg/mL oral solution) QD on Days 1 to Day 9 inclusive (within ± 1 hour of dosing time on Day 1) with 20 mg mitapivat sulfate (1 x 20 mg tablet) coadministered at Hour 0 on Day 5 (Period 2)<br>Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the linear mixed effect model.<br>Geometric Mean Ratio (GMR) = test / reference<br>Intra-subject CV% = 100 x (square root (exp[MSE]-1), where MSE = Residual variance from linear mixed effect model. |                                         |    |                                     |    |        |                         |                   |

[00159] The coadministration of 200 mg itraconazole with 20 mg mitapivat sulfate increased mitapivat exposure relative to when mitapivat sulfate was administered alone. The geometric mean AUC<sub>0-t</sub> and AUC<sub>0-inf</sub> ratios of mitapivat in the presence and absence of itraconazole were 4.7 and 4.9, respectively. The geometric mean Cmax ratio of mitapivat in the presence and absence of itraconazole was 1.7. The intra-subject variability was low at approximately 15% for AUCs and 11% for Cmax.

[00160] The statistical comparisons of the Metabolite PK parameters following 20 mg mitapivat sulfate administered alone (Period 1) or coadministered with multiple doses of 200 mg itraconazole (Period 2) PK parameters are summarized below in Table 2.

**Table 2**  
**Summary of Statistical Comparisons of Plasma Metabolite Pharmacokinetic Parameters Following 20 mg Mitapivat Sulfate Co-Administered With Multiple Doses of 200 mg Itraconazole Versus Administered Alone (Part 1)**

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Mitapivat Sulfate + Itraconazole (Test) |    | Mitapivat sulfate alone (Reference) |    |        |                         |                   |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------|----|-------------------------------------|----|--------|-------------------------|-------------------|
| Parameter (unit)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 | Geometric LSM                           | n  | Geometric LSM                       | n  | GMR    | 90% Confidence Interval | Intra-subject CV% |
| AUC <sub>0-t</sub> (ng*hr/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | 300.0                                   | 14 | 197.1                               | 14 | 1.5218 | 1.4268 - 1.6231         | 9.65              |
| AUC <sub>0-inf</sub> (ng*hr/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | 338.6                                   | 14 | 216.2                               | 14 | 1.5661 | 1.4784 - 1.6590         | 8.63              |
| C <sub>max</sub> (ng/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | 9.110                                   | 14 | 18.79                               | 14 | 0.4849 | 0.4441 - 0.5295         | 13.20             |
| Mitapivat sulfate alone: 20 mg mitapivat sulfate (1 x 20 mg tablet) at Hour 0 on Day 1 (Period 1)<br>Mitapivat sulfate + itraconazole: 200 mg itraconazole (20 mL of 10 mg/mL oral solution) QD on Days 1 to Day 9 inclusive (within ± 1 hour of dosing time on Day 1) with 20 mg mitapivat sulfate (1 x 20 mg tablet) coadministered at Hour 0 on Day 5 (Period 2)<br>Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the linear mixed effect model.<br>Geometric Mean Ratio (GMR) = test / reference<br>Intra-subject CV% = 100 x (square root (exp[MSE]-1), where MSE = Residual variance from linear mixed effect model. |                                         |    |                                     |    |        |                         |                   |

[00161]           The coadministration of 200 mg itraconazole with 20 mg mitapivat sulfate increased total Metabolite exposure relative to when mitapivat sulfate was administered alone. The geometric mean AUC<sub>0-t</sub> and AUC<sub>0-inf</sub> ratios of the Metabolite in the presence and absence of itraconazole were 1.5 and 1.6, respectively. The geometric mean C<sub>max</sub> ratio of the Metabolite in the presence and absence of itraconazole was 0.5. The intra-subject variability was low at approximately 9% to 10% for AUCs and 13% for C<sub>max</sub>.

Part 2

[00162]           Mean plasma mitapivat and metabolite the Metabolite concentration-time profiles following 50 mg mitapivat sulfate administered alone (Period 1) or coadministered with multiple doses of 600 mg rifampin (Period 2) on linear and semi-log scales are presented in Figures 3 and 4.

Mitapivat

[00163]           Mitapivat plasma concentrations were detectable in all subjects at 0.5 hour (the 1st scheduled postdose sample) when given alone or with rifampin. Mitapivat was still detectable up to the last sample time at 120 hours postdose in all but 1 subject when given alone had a BLQ concentration at 120 hours). When coadministered with rifampin, detectable mitapivat concentrations were not present in any sample collections from any subject beyond 24 hours postdose (except 1 subject with detectable mitapivat at 48 hours).

[00164]           Mitapivat concentrations increased to a lower and slightly earlier peak when coadministered with rifampin compared to when administered alone. The peak arithmetic

mean mitapivat concentrations were observed at a nominal time of approximately 1 hour postdose for Period 1 and 0.5 hour postdose for Period 2. The mean concentrations of mitapivat then declined in a multi exponential manner for both periods; however, the post-peak initial decline in mitapivat concentrations was more rapid and to lower levels when coadministered with rifampin compared to dosing alone.

**The Metabolite**

**[00165]** The Metabolite plasma concentrations were detectable at 0.5 hour postdose in all subjects after mitapivat sulfate was given alone and in all but 1 subject (1 subject was reported as BLQ) when mitapivat sulfate was coadministered with rifampin. The Metabolite was still detectable in all but 4 subjects up to the last scheduled sampling time of 120 hours postdose when mitapivat sulfate was given alone. When mitapivat sulfate was coadministered with rifampin, the Metabolite remained detectable in all subjects up to 24 hours postdose, with quantifiable Metabolite out to 48 hours in 6 subjects and 72 hours in 1 subject. No Metabolite concentrations were detectable in samples collected beyond 72 hours.

**[00166]** The peak arithmetic mean Metabolite concentrations were observed at a nominal time of approximately 2 hours postdose with mitapivat sulfate given alone and 1 hour when mitapivat sulfate was coadministered with rifampin. When mitapivat sulfate was given alone, higher mean concentrations of the Metabolite were observed over the initial 1 hour postdose. Metabolite concentrations were higher when mitapivat sulfate was coadministered with rifampin beyond 1 hour. The mean concentrations of the Metabolite then declined in a multi exponential fashion for both treatments. Decline in Metabolite concentrations was more rapid when mitapivat sulfate was coadministered with rifampin.

**[00167]** The statistical comparisons of mitapivat PK parameters following 50 mg mitapivat sulfate administered alone (Period 1) or coadministered with multiple doses of 600 mg rifampin (Period 2) PK parameters are summarized below in Table 3.

**Table 3**  
**Summary of Statistical Comparisons of Plasma Mitapivat Pharmacokinetic Parameters Following 50 mg Mitapivat Sulfate Co-Administered With Multiple Doses of 600 mg Rifampin Versus Administered Alone (Part 2)**

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | Mitapivat sulfate + rifampin (Test) |    | Mitapivat sulfate alone (Reference) |    |        |                         |                   |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|----|-------------------------------------|----|--------|-------------------------|-------------------|
| Parameter (unit)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Geometric LSM                       | n  | Geometric LSM                       | n  | GMR    | 90% Confidence Interval | Intra-subject CV% |
| AUC <sub>0-t</sub> (ng*hr/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 486.4                               | 14 | 5565                                | 14 | 0.0874 | 0.0719 - 0.1062         | 29.73             |
| AUC <sub>0-inf</sub> (ng*hr/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 496.4                               | 14 | 5681                                | 14 | 0.0874 | 0.0719 - 0.1062         | 29.84             |
| C <sub>max</sub> (ng/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 292.4                               | 14 | 1263                                | 14 | 0.2316 | 0.1933 - 0.2773         | 27.45             |
| Mitapivat sulfate alone: 50 mg mitapivat sulfate (1 x 50 mg tablet) at Hour 0 on Day 1 (Period 1)<br>Mitapivat sulfate + rifampin: 600 mg rifampin (2 x 300 mg capsules) QD on Day 1 to Day 12 inclusive (within ± 1 hour of dosing time on Day 1) with 50 mg mitapivat sulfate (1 x 50 mg tablet) coadministered at Hour 0 on Day 8 (Period 2)<br>Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the linear mixed effect model.<br>Geometric Mean Ratio (GMR) = test / reference<br>Intra-subject CV% = 100 x (square root (exp[MSE]-1), where MSE = Residual variance from linear mixed effect model. |                                     |    |                                     |    |        |                         |                   |

[00168]           The coadministration of 600 mg rifampin with 50 mg mitapivat sulfate decreased mitapivat exposure relative to when mitapivat sulfate was administered alone. The geometric mean AUC<sub>0-t</sub> and AUC<sub>0-inf</sub> ratios of mitapivat in the presence and absence of rifampin were 0.09. The geometric mean C<sub>max</sub> ratio of mitapivat in the presence and absence of rifampin was 0.23. The intra-subject variability was low at approximately 30% for AUCs and 27% for C<sub>max</sub>.

[00169]           The statistical comparisons of Metabolite PK parameters following 50 mg mitapivat sulfate administered alone (Period 1) or co-administered with multiple doses of 600 mg rifampin (Period 2) PK parameters are summarized below in Table 4.

**Table 4**  
**Summary of Statistical Comparisons of Plasma Metabolite Pharmacokinetic Parameters Following 50 mg Mitapivat Sulfate Co-Administered With Multiple Doses of 600 mg Rifampin Versus Administered Alone (Part 2)**

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | Mitapivat sulfate + rifampin (Test) |    | Mitapivat sulfate alone (Reference) |    |        |                         |                   |
|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------|----|-------------------------------------|----|--------|-------------------------|-------------------|
| Parameter (unit)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | Geometric LSM                       | n  | Geometric LSM                       | n  | GMR    | 90% Confidence Interval | Intra-subject CV% |
| AUC <sub>0-t</sub> (ng*hr/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                | 230.5                               | 14 | 623.0                               | 14 | 0.3700 | 0.3316 - 0.4129         | 16.50             |
| AUC <sub>0-inf</sub> (ng*hr/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              | 244.9                               | 14 | 662.6                               | 14 | 0.3696 | 0.3321 - 0.4113         | 16.08             |
| C <sub>max</sub> (ng/mL)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | 64.47                               | 14 | 53.80                               | 14 | 1.1983 | 1.0642 - 1.3492         | 17.86             |
| Mitapivat sulfate alone: 50 mg mitapivat sulfate (1 x 50 mg tablet) at Hour 0 on Day 1 (Period 1)<br>Mitapivat sulfate + rifampin: 600 mg rifampin (2 x 300 mg capsules) QD on Day 1 to Day 12 inclusive (within ± 1 hour of dosing time on Day 1) with 50 mg mitapivat sulfate (1 x 50 mg tablet) coadministered at Hour 0 on Day 8 (Period 2)<br>Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the linear mixed effect model.<br>Geometric Mean Ratio (GMR) = test / reference<br>Intra-subject CV% = 100 x (square root (exp[MSE]-1), where MSE = Residual variance from linear mixed effect model. |                                     |    |                                     |    |        |                         |                   |

[00170] The coadministration of 600 mg rifampin with 50 mg mitapivat sulfate decreased the Metabolite exposure relative to when mitapivat sulfate was administered alone. The geometric mean AUC<sub>0-t</sub> and AUC<sub>0-inf</sub> ratios of the Metabolite in the presence versus absence of rifampin were 0.37. The geometric mean C<sub>max</sub> ratio of the Metabolite in the presence versus absence of rifampin was 1.2. The intra-subject variability was low at approximately 16% to 18% for AUCs and C<sub>max</sub>.

### **Conclusions**

[00171] Total exposure of mitapivat increased in the presence of itraconazole compared to dosing of mitapivat sulfate alone.

[00172] Total exposure of mitapivat decreased in the presence of rifampin compared to dosing of mitapivat sulfate alone.

### **Example 3. Development of a PBPK Model for Mitapivat Sulfate to Evaluate the Potential for Drug-Drug Interactions**

#### **Part I**

[00173] A physiologically based pharmacokinetic model (hereinafter “PBPK” model) for mitapivat sulfate based on the currently available in-vitro and clinical PK data is being developed. The developed PBPK model will be used to assess DDI liability with mitapivat sulfate acting as a victim of CYP3A4-mediated metabolism as well as a perpetrator of CYP and transporter mediated DDI.

[00174] Specifically, a PBPK model that includes a simple first order absorption model will be developed. Full PBPK and minimal PBPK distribution models will be evaluated, both of which consider liver and intestinal metabolism. The fraction of mitapivat sulfate absorbed will be estimated from mass balance data from the [14C] human ADME study (Clinical Study AG348-C-009). In-vitro data will be used to assign the relative contribution of CYP3A4 to the clearance of mitapivat sulfate.  $K_I$  and  $k_{inact}$  values for mechanism based inhibition of CYP3A4 and CYP3A4 induction ( $E_{max}$  and  $EC_{50}$ ) data by mitapivat sulfate will be incorporated within the PBPK model to assess the net DDI effects at steady state. Once an mitapivat sulfate PBPK model has been verified against the available clinical data, a series of

DDI simulations assessing mitapivat sulfate as a victim or perpetrator of DDI will be performed in healthy volunteers.

**Experiments with mitapivat sulfate as a victim**

**[00175]** The impact of the co-administration of the strong CYP3A4 inhibitor, itraconazole, on the PK of mitapivat sulfate will be determined based on the following single and multiple-dose administration of 5, 20 and 50 mg BID.

**[00176]** The impact of the co-administration of the strong CYP3A4 inhibitor, ketoconazole, on the PK of mitapivat sulfate will be determined based following single and multiple-dose administration of 5, 20 and 50 mg BID.

**[00177]** The impact of the co-administration of the moderate CYP3A4 inhibitor fluconazole on the PK of mitapivat sulfate will be determined based on the following single- and multiple dose administration of 5, 20 and 50 mg BID.

**[00178]** The impact of the co-administration of the mild CYP3A4 inhibitor cimetidine on the PK of mitapivat sulfate will be determined based on the following single- and multiple dose administration of 5, 20 and 50 mg BID.

**[00179]** The impact of the co-administration of the strong CYP3A4 inducer, rifampin, on the PK of mitapivat sulfate will be determined based on the following single- and multiple dose administration of 5, 20 and 50 mg BID.

**[00180]** The impact of the co-administration of the moderate CYP3A4 inducer efavirenz on the PK of mitapivat sulfate will be determined following single- and multiple dose administration of 5, 20 and 50 mg BID.

**Experiments with mitapivat sulfate as perpetrator**

**[00181]** The extent to which 5 to 50 mg BID mitapivat sulfate affects the systemic exposure of CYP3A4 substrate midazolam when co-administered with mitapivat sulfate will be determined.

**[00182]** The effect of 5 to 50 mg mitapivat sulfate on the systemic exposures of CYP2B6 substrate bupropion when co-administered will be determined. CYP2B6 induction (Emax and EC50) data by mitapivat sulfate will be incorporated within the PBPK model. Sensitivity analysis will be conducted to gauge the impact of the uncertainty around Emax and EC50 on the predicted DDIs.

**[00183]** The effect of 5 to 50 mg AG mitapivat sulfate BID on the systemic exposures of CYP2C9 substrate warfarin when co-administered will be determined. CYP2C9 induction (Emax and EC50) data by mitapivat sulfate will be incorporated within the PBPK model. Sensitivity analysis will be conducted to gauge the impact of the uncertainty around Emax and EC50 on the predicted DDIs.

**[00184]** The effect of 5 to 50 mg mitapivat sulfate BID on the systemic exposures of CYP2C8/CYP3A4 substrate repaglinide when co-administered will be determined. CYP2C8 induction (Emax and EC50) data by mitapivat sulfate will be incorporated within the PBPK model. Sensitivity analysis will be conducted to gauge the impact of the uncertainty around Emax and EC50 on the predicted DDIs.

**[00185]** The effect of 5 to 50 mg mitapivat sulfate BID on the systemic exposures of CYP2C19 substrate omeprazole when co-administered will be determined. CYP2C19 induction (Emax and EC50) data by mitapivat sulfate will be incorporated within the PBPK model. Sensitivity analysis will be conducted to gauge the impact of the uncertainty around Emax and EC50 on the predicted DDIs.

**[00186]** The effect of mitapivat sulfate (5 to 50 mg BID) on the systemic exposures of OATP1B1/OATP1B3 (rosuvastatin), P-gp (digoxin), metformin (MATE1 and OCT2), OAT3 (methotrexate) substrate drugs when co-administered will be determined. The respective IC50 values for inhibition of OATP1B1, P-gp, MATE1, OCT2 and OAT3 by mitapivat sulfate will be incorporated within the PBPK model. Sensitivity analysis will be performed to assess the impact of these parameters on the DDI liability of mitapivat sulfate.

## **Part II**

**[00187]** The first order absorption component of the previously developed PBPK model will be extended using available pH solubility data to include the ADAM model. The recovery of mitapivat sulfate plasma concentration profiles after single and multiple dosing

(up to 50 mg BID) to healthy volunteer subjects will be demonstrated. Once verified against the available clinical data, the modified PBPK model will be used to assess the impact of an increase in gastric pH on the pharmacokinetics of mitapivat sulfate via manipulation of system parameters (gastric pH).

**[00188]** While a number of embodiments have been described, the scope of this disclosure is to be defined by the appended claims, and not by the specific embodiments that have been represented by way of example. The contents of all references (including literature references, issued patents, published patent applications, and co-pending patent applications) cited throughout this application are hereby expressly incorporated herein in their entireties by reference. Unless otherwise defined, all technical and scientific terms used herein are accorded the meaning commonly known to one with ordinary skill in the art.

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |                                                                                                     |                                                                          |  |
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| Doc Code: PET.AUTO<br>Document Description: Petition automatically granted by EFS-Web                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                                                     | PTO/SB/140<br>U.S. Patent and Trademark Office<br>Department of Commerce |  |
| Electronic Petition Request                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    | PETITION TO WITHDRAW AN APPLICATION FROM ISSUE AFTER PAYMENT OF THE ISSUE FEE UNDER 37 CFR 1.313(c) |                                                                          |  |
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| First Named Inventor                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           | Varsha Iyer                                                                                         |                                                                          |  |
| Art Unit                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | 1621                                                                                                |                                                                          |  |
| Examiner Name                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | GEORGE KOSTURKO                                                                                     |                                                                          |  |
| Attorney Docket Number                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         | 128748-01502                                                                                        |                                                                          |  |
| Title                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          | MITAPIVAT THERAPY AND MODULATORS OF CYTOCHROME P450                                                 |                                                                          |  |
| <p>An application may be withdrawn from issue for further action upon petition by the applicant. To request that the Office withdraw an application from issue, applicant must file a petition under this section including the fee set forth in § 1.17(h) and a showing of good and sufficient reasons why withdrawal of the application from issue is necessary.</p> <p>APPLICANT HEREBY PETITIONS TO WITHDRAW THIS APPLICATION FROM ISSUE UNDER 37 CFR 1.313(c).</p> <p>A grantable petition requires the following items:</p> <p>(1) Petition fee; and</p> <p>(2) One of the following reasons:</p> <p>(a) Unpatentability of one or more claims, which must be accompanied by an unequivocal statement that one or more claims are unpatentable, an amendment to such claim or claims, and an explanation as to how the amendment causes such claim or claims to be patentable;</p> <p>(b) Consideration of a request for continued examination in compliance with § 1.114 (for a utility or plant application only); or</p> <p>(c) Express abandonment of the application. Such express abandonment may be in favor of a continuing application, but not a CPA under 37 CFR 1.53(d).</p> |                                                                                                     |                                                                          |  |
| Petition Fee                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                                                                                                     |                                                                          |  |
| <div><div><input type="radio"/> Small Entity</div><div><input type="radio"/> Micro Entity</div><div><input checked="" type="radio"/> Regular Undiscounted</div></div>                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                                                                                                     |                                                                          |  |
| Reason for withdrawal from issue                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                               |                                                                                                     |                                                                          |  |

- ☐ One or more claims are unpatentable
- ☒ Consideration of a request for continued examination (RCE) (List of Required Documents and Fees)
- ☐ Applicant hereby expressly abandons the instant application (any attorney/agent signing for this reason must have power of attorney pursuant to 37 CFR 1.32(b)).

RCE request,submission, and fee.

- ☐ I certify, in accordance with 37 CFR 1.4(d)(4) that :  
The RCE request ,submission, and fee have already been filed in the above-identified application on
- ☒ Are attached.

THIS PORTION MUST BE COMPLETED BY THE SIGNATORY OR SIGNATORIES

I certify, in accordance with 37 CFR 1.4(d)(4) that I am:

- ☐ An attorney or agent registered to practice before the Patent and Trademark Office who has been given power of attorney in this application.
- ☒ An attorney or agent registered to practice before the Patent and Trademark Office, acting in a representative capacity.
- ☐ A sole inventor
- ☐ A joint inventor; I certify that I am authorized to sign this submission on behalf of all of the inventors as evidenced by the power of attorney in the application
- ☐ A joint inventor; all of whom are signing this e-petition

|                     |                  |
|---------------------|------------------|
| Signature           | /Andrew R. Ehle/ |
| Name                | Andrew R. Ehle   |
| Registration Number | 78384            |

| Electronic Patent Application Fee Transmittal |          |                                                     |        |                      |
|-----------------------------------------------|----------|-----------------------------------------------------|--------|----------------------|
| Application Number:                           |          | 16900610                                            |        |                      |
| Filing Date:                                  |          | 12-Jun-2020                                         |        |                      |
| Title of Invention:                           |          | MITAPIVAT THERAPY AND MODULATORS OF CYTOCHROME P450 |        |                      |
| First Named Inventor/Applicant Name:          |          | Varsha Venkatachalam Iyer                           |        |                      |
| Filer:                                        |          | Andrew Ehle                                         |        |                      |
| Attorney Docket Number:                       |          | 128748-01502                                        |        |                      |
| Filed as Large Entity                         |          |                                                     |        |                      |
| Filing Fees for   Utility under 35 USC 111(a) |          |                                                     |        |                      |
| Description                                   | Fee Code | Quantity                                            | Amount | Sub-Total in USD(\$) |
| Basic Filing:                                 |          |                                                     |        |                      |
| PETITION FEE- 37 CFR 1.17(H) (GROUP III)      | 1464     | 1                                                   | 140    | 140                  |
| RCE- 1ST REQUEST                              | 1801     | 1                                                   | 1360   | 1360                 |
| Pages:                                        |          |                                                     |        |                      |
| Claims:                                       |          |                                                     |        |                      |
| Miscellaneous-Filing:                         |          |                                                     |        |                      |
| Petition:                                     |          |                                                     |        |                      |
| Patent-Appeals-and-Interference:              |          |                                                     |        |                      |

| Description                       | Fee Code | Quantity | Amount | Sub-Total in USD(\$) |
|-----------------------------------|----------|----------|--------|----------------------|
| Post-Allowance-and-Post-Issuance: |          |          |        |                      |
| Extension-of-Time:                |          |          |        |                      |
| Miscellaneous:                    |          |          |        |                      |
| Total in USD (\$)                 |          |          |        | 1500                 |



UNITED STATES PATENT AND TRADEMARK OFFICE

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Commissioner for Patents  
United States Patent and Trademark Office  
P.O. Box 1450  
Alexandria, VA 22313-1450  
[www.uspto.gov](http://www.uspto.gov)

Decision Date : August 4, 2023

In re Application of :

Varsha Venkatachalam Iyer

DECISION ON PETITION  
UNDER CFR 1.313(c)(2)

Application No : 16900610

Filed : 12-Jun-2020

Attorney Docket No : 128748-01502

This is an electronic decision on the petition under 37 CFR 1.313(c)(2), filed August 4, 2023 , to withdraw the above-identified application from issue after payment of the issue fee.

The petition is **GRANTED**.

The above-identified application is withdrawn from issue for consideration of a submission under 37 CFR 1.114 (request for continued examination). See 37 CFR 1.313(c)(2).

**Petitioner is advised that the issue fee paid in this application cannot be refunded. If, however, this application is again allowed, petitioner may request that it be applied towards the issue fee required by the new Notice of Allowance.**

Telephone inquiries concerning this decision should be directed to the Patent Electronic Business Center (EBC) at 866-217-9197.

This application file is being referred to Technology Center AU 1621 for processing of the request for continuing examination under 37 CFR 1.114 .

Office of Petitions

|                        |                               |                             |                          |
|------------------------|-------------------------------|-----------------------------|--------------------------|
| Notice of Allowability | Application No.<br>16/900,610 | Applicant(s)<br>Iyer et al. |                          |
|                        | Examiner<br>GEORGE W KOSTURKO | Art Unit<br>1621            | AIA (FITF) Status<br>Yes |

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address--

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to 08/04/2023.  
☐ A declaration(s)/affidavit(s) under 37 CFR 1.130(b) was/were filed on \_\_\_\_\_.

2. ☐ An election was made by the applicant in response to a restriction requirement set forth during the interview on \_\_\_\_\_; the restriction requirement and election have been incorporated into this action.

3. ☒ The allowed claim(s) is/are 59-77 . As a result of the allowed claim(s), you may be eligible to benefit from the **Patent Prosecution Highway** program at a participating intellectual property office for the corresponding application. For more information, please see [http://www.uspto.gov/patents/init\\_events/pph/index.jsp](http://www.uspto.gov/patents/init_events/pph/index.jsp) or send an inquiry to [PPHfeedback@uspto.gov](mailto:PPHfeedback@uspto.gov).

4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

**Certified copies:**

a) ☐ All      b) ☐ Some\*      c) ☐ None of the:

1. ☐ Certified copies of the priority documents have been received.

2. ☐ Certified copies of the priority documents have been received in Application No. \_\_\_\_\_ .

3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

\* Certified copies not received: \_\_\_\_\_ .

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.  
**THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.**

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.  
☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date \_\_\_\_\_ .

**Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).**

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

**Attachment(s)**

|                                                                                                                   |                                                                                      |
|-------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|
| 1. <input type="checkbox"/> Notice of References Cited (PTO-892)                                                  | 5. <input type="checkbox"/> Examiner's Amendment/Comment                             |
| 2. <input type="checkbox"/> Information Disclosure Statements (PTO/SB/08),<br>Paper No./Mail Date _____.          | 6. <input checked="" type="checkbox"/> Examiner's Statement of Reasons for Allowance |
| 3. <input type="checkbox"/> Examiner's Comment Regarding Requirement for Deposit<br>of Biological Material _____. | 7. <input type="checkbox"/> Other _____.                                             |
| 4. <input type="checkbox"/> Interview Summary (PTO-413),<br>Paper No./Mail Date. _____.                           |                                                                                      |

/THEODORE R. HOWELL/  
Primary Examiner, Art Unit 1628

## **DETAILED ACTION**

### ***Notice of Pre-AIA or AIA Status***

1. The present application, filed on or after March 16, 2013, is being examined under the first inventor to file provisions of the AIA. Claims 59-77 filed August 04, 2023 are currently pending.

### ***Continued Examination Under 37 CFR 1.114***

2. A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after allowance or after an Office action under *Ex Parte Quayle*, 25 USPQ 74, 453 O.G. 213 (Comm'r Pat. 1935). Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, prosecution in this application has been reopened pursuant to 37 CFR 1.114. Applicant's submission filed on 08/04/2023 has been entered.

3.

### ***4. Reasons for Allowance***

5. The following is an examiner's statement of reasons for allowance: Applicant's amendments are directed to incorporating the phrase "or a pharmaceutically acceptable salt thereof" within the scope of claims 59-77. Support is found in paragraphs [0021]-[0022] and [0031] of the specification and no new matter has been added. As indicated in the previous notice of allowance of 05/03/2023, Applicant's arguments filed 04/07/2023 were sufficient to overcome the rejection of record. Furthermore, there are no teaching or suggestions within the prior art to combine a moderate inducer of cytochrome P450 enzyme 3A4/5 (CYP 3A4/5) with the claimed doses of mitapivat or a

pharmaceutically acceptable salt thereof in order to treat hemolytic anemia in a subject in need and the therefore, claims 59-77 are allowed.

6. Any comments considered necessary by applicant must be submitted no later than the payment of the issue fee and, to avoid processing delays, should preferably accompany the issue fee. Such submissions should be clearly labeled "Comments on Statement of Reasons for Allowance."

7. Any inquiry concerning this communication or earlier communications from the examiner should be directed to GEORGE W KOSTURKO whose telephone number is (571)270-5903. The examiner can normally be reached M-F 9:00-5:30.

Examiner interviews are available via telephone, in-person, and video conferencing using a USPTO supplied web-based collaboration tool. To schedule an interview, applicant is encouraged to use the USPTO Automated Interview Request (AIR) at <http://www.uspto.gov/interviewpractice>.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, CLINTON A BROOKS can be reached on 571-270-7682. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of published or unpublished applications may be obtained from Patent Center. Unpublished application information in Patent Center is available to registered users. To file and manage patent submissions in Patent Center, visit: <https://patentcenter.uspto.gov>. Visit <https://www.uspto.gov/patents/apply/patent-center> for more information about Patent Center and <https://www.uspto.gov/patents/docx> for information about filing in DOCX format. For

Application/Control Number: 16/900,610  
Art Unit: 1621

Page 4

additional questions, contact the Electronic Business Center (EBC) at 866-217-9197  
(toll-free). If you would like assistance from a USPTO Customer Service  
Representative, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

**/G.W.K/  
Examiner, Art Unit 1621**

**/THEODORE R. HOWELL/  
Primary Examiner, Art Unit 1628**



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE  
United States Patent and Trademark Office  
Address: COMMISSIONER FOR PATENTS  
P.O. Box 1450  
Alexandria, Virginia 22313-1450  
www.uspto.gov

NOTICE OF ALLOWANCE AND FEE(S) DUE

190109 7590 08/30/2023  
McCarter & English / Agios Pharmaceuticals  
265 Franklin Street  
Boston, MA 02110

EXAMINER

KOSTURKO, GEORGE W

ART UNIT PAPER NUMBER

1621

DATE MAILED: 08/30/2023

| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR      | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|-----------------|-------------|---------------------------|---------------------|------------------|
| 16/900.610      | 06/12/2020  | Varsha Venkatachalam Iyer | 128748-01502        | 1054             |

TITLE OF INVENTION: MITAPIVAT THERAPY AND MODULATORS OF CYTOCHROME P450

| APPLN. TYPE    | ENTITY STATUS | ISSUE FEE DUE | PUBLICATION FEE DUE | PREV. PAID ISSUE FEE | TOTAL FEE(S) DUE | DATE DUE   |
|----------------|---------------|---------------|---------------------|----------------------|------------------|------------|
| nonprovisional | UNDISCOUNTED  | \$1200        | \$0.00              | \$1200.00            | \$0              | 11/30/2023 |

THE APPLICATION IDENTIFIED ABOVE HAS BEEN EXAMINED AND IS ALLOWED FOR ISSUANCE AS A PATENT. PROSECUTION ON THE MERITS IS CLOSED. THIS NOTICE OF ALLOWANCE IS NOT A GRANT OF PATENT RIGHTS. THIS APPLICATION IS SUBJECT TO WITHDRAWAL FROM ISSUE AT THE INITIATIVE OF THE OFFICE OR UPON PETITION BY THE APPLICANT. SEE 37 CFR 1.313 AND MPEP 1308.

THE ISSUE FEE AND PUBLICATION FEE (IF REQUIRED) MUST BE PAID WITHIN THREE MONTHS FROM THE MAILING DATE OF THIS NOTICE OR THIS APPLICATION SHALL BE REGARDED AS ABANDONED. THIS STATUTORY PERIOD CANNOT BE EXTENDED. SEE 35 U.S.C. 151. THE ISSUE FEE DUE INDICATED ABOVE DOES NOT REFLECT A CREDIT FOR ANY PREVIOUSLY PAID ISSUE FEE IN THIS APPLICATION. IF AN ISSUE FEE HAS PREVIOUSLY BEEN PAID IN THIS APPLICATION (AS SHOWN ABOVE), THE RETURN OF PART B OF THIS FORM WILL BE CONSIDERED A REQUEST TO REAPPLY THE PREVIOUSLY PAID ISSUE FEE TOWARD THE ISSUE FEE NOW DUE.

HOW TO REPLY TO THIS NOTICE:

I. Review the ENTITY STATUS shown above. If the ENTITY STATUS is shown as SMALL or MICRO, verify whether entitlement to that entity status still applies.

If the ENTITY STATUS is the same as shown above, pay the TOTAL FEE(S) DUE shown above.

If the ENTITY STATUS is changed from that shown above, on PART B - FEE(S) TRANSMITTAL, complete section number 5 titled "Change in Entity Status (from status indicated above)".

For purposes of this notice, small entity fees are 40% the amount of undiscounted fees, and micro entity fees are 20% the amount of undiscounted fees.

II. PART B - FEE(S) TRANSMITTAL, or its equivalent, must be completed and returned to the United States Patent and Trademark Office (USPTO) with your ISSUE FEE and PUBLICATION FEE (if required). If you are charging the fee(s) to your deposit account, section "4b" of Part B - Fee(s) Transmittal should be completed. If an equivalent of Part B is filed, a request to reapply a previously paid issue fee must be clearly made, and delays in processing may occur due to the difficulty in recognizing the paper as an equivalent of Part B.

III. All communications regarding this application must give the application number. Please direct all communications prior to issuance to Mail Stop ISSUE FEE unless advised to the contrary.

IMPORTANT REMINDER: Maintenance fees are due in utility patents issuing on applications filed on or after Dec. 12, 1980. It is patentee's responsibility to ensure timely payment of maintenance fees when due. More information is available at [www.uspto.gov/PatentMaintenanceFees](http://www.uspto.gov/PatentMaintenanceFees).

PART B - FEE(S) TRANSMITTAL

Complete and send this form, together with applicable fee(s), by mail or fax, or via EFS-Web.

By mail, send to: Mail Stop ISSUE FEE  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, Virginia 22313-1450

By fax, send to: (571)-273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications. **Because electronic patent issuance may occur shortly after issue fee payment, any desired continuing application should preferably be filed prior to payment of this issue fee in order not to jeopardize copendency.**

CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

190109 7590 08/30/2023  
McCarter & English / Agios Pharmaceuticals  
265 Franklin Street  
Boston, MA 02110

Note: A certificate of mailing can only be used for domestic mailings of the Fee(s) Transmittal. This certificate cannot be used for any other accompanying papers. Each additional paper, such as an assignment or formal drawing, must have its own certificate of mailing or transmission.

Certificate of Mailing or Transmission

I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via EFS-Web or by facsimile to (571) 273-2885, on the date below.

|  |                         |
|--|-------------------------|
|  | (Typed or printed name) |
|  | (Signature)             |
|  | (Date)                  |

| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|-----------------|-------------|----------------------|---------------------|------------------|
|-----------------|-------------|----------------------|---------------------|------------------|

16/900,610 06/12/2020 Varsha Venkatachalam Iyer 128748-01502 1054

TITLE OF INVENTION: MITAPIVAT THERAPY AND MODULATORS OF CYTOCHROME P450

| APPLN. TYPE    | ENTITY STATUS | ISSUE FEE DUE | PUBLICATION FEE DUE | PREV. PAID ISSUE FEE | TOTAL FEE(S) DUE | DATE DUE   |
|----------------|---------------|---------------|---------------------|----------------------|------------------|------------|
| nonprovisional | UNDISCOUNTED  | \$1200        | \$0.00              | \$1200.00            | \$0              | 11/30/2023 |

| EXAMINER           | ART UNIT | CLASS-SUBCLASS |
|--------------------|----------|----------------|
| KOSTURKO, GEORGE W | 1621     | 424-094500     |

|                                                                                                                                                                                                                                                                                                                                                                                                                                         |                                                                                                                                                                                                                                                                                                                                                                    |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| 1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363).<br><br><input type="checkbox"/> Change of correspondence address (or Change of Correspondence Address form PTO/AIA/122 or PTO/SB/122) attached.<br><br><input type="checkbox"/> "Fee Address" indication (or "Fee Address" Indication form PTO/AIA/47 or PTO/SB/47; Rev 03-02 or more recent) attached. <b>Use of a Customer Number is required.</b> | 2. For printing on the patent front page, list<br>(1) The names of up to 3 registered patent attorneys or agents OR, alternatively, 1 _____<br>(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed. 2 _____<br>3 _____ |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ☐ Individual ☐ Corporation or other private group entity ☐ Government

4a. Fees submitted: ☐ Issue Fee ☐ Publication Fee (if required)

4b. Method of Payment: (Please first reapply any previously paid fee shown above)

☐ Electronic Payment via Patent Center or EFS-Web ☐ Enclosed check ☐ Non-electronic payment by credit card (Attach form PTO-2038)  
☐ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. \_\_\_\_\_

5. Change in Entity Status (from status indicated above)

☐ Applicant certifying micro entity status. See 37 CFR 1.29  
☐ Applicant asserting small entity status. See 37 CFR 1.27  
☐ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature \_\_\_\_\_ Date \_\_\_\_\_  
Typed or printed name \_\_\_\_\_ Registration No. \_\_\_\_\_



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|                                            |             |                           |                     |                  |
|--------------------------------------------|-------------|---------------------------|---------------------|------------------|
| APPLICATION NO.                            | FILING DATE | FIRST NAMED INVENTOR      | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
| 16/900,610                                 | 06/12/2020  | Varsha Venkatachalam Iyer | 128748-01502        | 1054             |
| 190109                                     | 7590        | 08/30/2023                | EXAMINER            |                  |
| McCarter & English / Agios Pharmaceuticals |             |                           | KOSTURKO, GEORGE W  |                  |
| 265 Franklin Street                        |             |                           | ART UNIT            |                  |
| Boston, MA 02110                           |             |                           | PAPER NUMBER        |                  |
|                                            |             |                           | 1621                |                  |
| DATE MAILED: 08/30/2023                    |             |                           |                     |                  |

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)  
(Applications filed on or after May 29, 2000)

The Office has discontinued providing a Patent Term Adjustment (PTA) calculation with the Notice of Allowance.

Section 1(h)(2) of the AIA Technical Corrections Act amended 35 U.S.C. 154(b)(3)(B)(i) to eliminate the requirement that the Office provide a patent term adjustment determination with the notice of allowance. See Revisions to Patent Term Adjustment, 78 Fed. Reg. 19416, 19417 (Apr. 1, 2013). Therefore, the Office is no longer providing an initial patent term adjustment determination with the notice of allowance. The Office will continue to provide a patent term adjustment determination with the Issue Notification Letter that is mailed to applicant approximately three weeks prior to the issue date of the patent, and will include the patent term adjustment on the patent. Any request for reconsideration of the patent term adjustment determination (or reinstatement of patent term adjustment) should follow the process outlined in 37 CFR 1.705.

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Customer Service Center of the Office of Patent Publication at 1-(888)-786-0101 or (571)-272-4200.

## OMB Clearance and PRA Burden Statement for PTOL-85 Part B

The Paperwork Reduction Act (PRA) of 1995 requires Federal agencies to obtain Office of Management and Budget approval before requesting most types of information from the public. When OMB approves an agency request to collect information from the public, OMB (i) provides a valid OMB Control Number and expiration date for the agency to display on the instrument that will be used to collect the information and (ii) requires the agency to inform the public about the OMB Control Number's legal significance in accordance with 5 CFR 1320.5(b).

The information collected by PTOL-85 Part B is required by 37 CFR 1.311. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 30 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, Virginia 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, Virginia 22313-1450. Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

### Privacy Act Statement

**The Privacy Act of 1974 (P.L. 93-579)** requires that you be given certain information in connection with your submission of the attached form related to a patent application or patent. Accordingly, pursuant to the requirements of the Act, please be advised that: (1) the general authority for the collection of this information is 35 U.S.C. 2(b) (2); (2) furnishing of the information solicited is voluntary; and (3) the principal purpose for which the information is used by the U.S. Patent and Trademark Office is to process and/or examine your submission related to a patent application or patent. If you do not furnish the requested information, the U.S. Patent and Trademark Office may not be able to process and/or examine your submission, which may result in termination of proceedings or abandonment of the application or expiration of the patent.

The information provided by you in this form will be subject to the following routine uses:

1. The information on this form will be treated confidentially to the extent allowed under the Freedom of Information Act (5 U.S.C. 552) and the Privacy Act (5 U.S.C. 552a). Records from this system of records may be disclosed to the Department of Justice to determine whether disclosure of these records is required by the Freedom of Information Act.
2. A record from this system of records may be disclosed, as a routine use, in the course of presenting evidence to a court, magistrate, or administrative tribunal, including disclosures to opposing counsel in the course of settlement negotiations.
3. A record in this system of records may be disclosed, as a routine use, to a Member of Congress submitting a request involving an individual, to whom the record pertains, when the individual has requested assistance from the Member with respect to the subject matter of the record.
4. A record in this system of records may be disclosed, as a routine use, to a contractor of the Agency having need for the information in order to perform a contract. Recipients of information shall be required to comply with the requirements of the Privacy Act of 1974, as amended, pursuant to 5 U.S.C. 552a(m).
5. A record related to an International Application filed under the Patent Cooperation Treaty in this system of records may be disclosed, as a routine use, to the International Bureau of the World Intellectual Property Organization, pursuant to the Patent Cooperation Treaty.
6. A record in this system of records may be disclosed, as a routine use, to another federal agency for purposes of National Security review (35 U.S.C. 181) and for review pursuant to the Atomic Energy Act (42 U.S.C. 218(c)).
7. A record from this system of records may be disclosed, as a routine use, to the Administrator, General Services, or his/her designee, during an inspection of records conducted by GSA as part of that agency's responsibility to recommend improvements in records management practices and programs, under authority of 44 U.S.C. 2904 and 2906. Such disclosure shall be made in accordance with the GSA regulations governing inspection of records for this purpose, and any other relevant (i.e., GSA or Commerce) directive. Such disclosure shall not be used to make determinations about individuals.
8. A record from this system of records may be disclosed, as a routine use, to the public after either publication of the application pursuant to 35 U.S.C. 122(b) or issuance of a patent pursuant to 35 U.S.C. 151. Further, a record may be disclosed, subject to the limitations of 37 CFR 1.14, as a routine use, to the public if the record was filed in an application which became abandoned or in which the proceedings were terminated and which application is referenced by either a published application, an application open to public inspection or an issued patent.
9. A record from this system of records may be disclosed, as a routine use, to a Federal, State, or local law enforcement agency, if the USPTO becomes aware of a violation or potential violation of law or regulation.



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APPLICATION #  
16/900,610

RECEIPT DATE / TIME  
11/29/2023 02:55:19 PM Z ET

ATTORNEY DOCKET #  
128748-01502

Title of Invention

MITAPIVAT THERAPY AND MODULATORS OF CYTOCHROME P450

Application Information

|                        |                                                          |                      |                           |
|------------------------|----------------------------------------------------------|----------------------|---------------------------|
| APPLICATION TYPE       | Utility - Nonprovisional Application under 35 USC 111(a) | PATENT #             | -                         |
| CONFIRMATION #         | 1054                                                     | FILED BY             | Lauren Margolis           |
| PATENT CENTER #        | 63419118                                                 | FILING DATE          | 06/12/2020                |
| CUSTOMER #             | 190109                                                   | FIRST NAMED INVENTOR | Varsha Venkatachalam Iyer |
| CORRESPONDENCE ADDRESS | -                                                        | AUTHORIZED BY        | Andrew Ehle               |

Documents

TOTAL DOCUMENTS: 1

| DOCUMENT                                             | PAGES | DESCRIPTION                 | SIZE (KB) |
|------------------------------------------------------|-------|-----------------------------|-----------|
| 128748_01502_PTOL85_Issue_Fee_Prev_Paid_Nov_2023.pdf | 1     | Issue Fee Payment (PTO-85B) | 37 KB     |

Digest

| DOCUMENT                                             | MESSAGE DIGEST(SHA-512)                                                                                                          |
|------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------|
| 128748_01502_PTOL85_Issue_Fee_Prev_Paid_Nov_2023.pdf | 3E99E732AC8F08D0410FD63BDEB60E2E3B62EC4D5F2E62E84A5988C44C0FF178B62DB41BDB39B40A37343E13DECD9DE6E55E16B8E4960883783FBBD7BE4AE96C |

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**New Applications Under 35 U.S.C. 111**

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**New International Application Filed with the USPTO as a Receiving Office**

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Complete and send this form, together with the applicable fee(s), by mail or fax, or via EFS-Web.

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Alexandria, VA 22313-1450

By fax, send to:(571) 273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications. **Because electronic patent issuance may occur shortly after issue fee payment, any desired continuing application should preferably be filed prior to payment of this issue fee in order not to jeopardize copendency.**

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265 Franklin Street  
Boston, Massachusetts 02110

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**Certificate of Mailing or Transmission**  
I hereby certify that this Fee(s) Transmittal is being deposited with the United States Postal Service with sufficient postage for first class mail in an envelope addressed to the Mail Stop ISSUE FEE address above, or being transmitted to the USPTO via EFS-Web or by facsimile to (571) 273-2885, on the date below.

(Typed or printed name)

(Signature)

(Date)

|                |             |                           |                    |                 |
|----------------|-------------|---------------------------|--------------------|-----------------|
| APPLICATION NO | FILING DATE | FIRST NAMED INVENTOR      | ATTORNEY DOCKET NO | CONFIRMATION NO |
| 16/900,610     | 06/12/2020  | Varsha Venkatachala. Iyer | 128748-01502       | 1054            |

TITLE OF INVENTION: MITAPIVAT THERAPY AND MODULATORS OF CYTOCHROME P450

|                |               |               |                     |                                 |                  |            |
|----------------|---------------|---------------|---------------------|---------------------------------|------------------|------------|
| APPLN: TYPE    | ENTITY STATUS | ISSUE FEE DUE | PUBLICATION FEE DUE | PREV. PAID ISSUE FEE            | TOTAL FEE(S) DUE | DATE DUE   |
| nonprovisional | UNDISCOUNTED  | \$1,200.00    |                     | \$1,200.00 (paid on 2023-08-02) | \$0.00           | 11/30/2023 |

|                                |                  |                               |
|--------------------------------|------------------|-------------------------------|
| EXAMINER<br>George W. Kosturko | ART UNIT<br>1621 | CLASS-SUBCLASS<br>424-094.500 |
|--------------------------------|------------------|-------------------------------|

1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363)  

☐ Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.  
☐ "Fee Address" indication (or "Fee Address" Indication form PTO/AIA/47 or PTO/SB/47; Rev 03-09 or more recent) attached. **Use of a Customer Number is required.**

2. For printing on the patent front page, list  

(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,  
(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed.

1. McCarter & English, LLP

2. Michael J. DeGrazia, Ph.D.

3.

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)  

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE  
Agios Pharmaceuticals, Inc.

(B) RESIDENCE: (CITY and STATE or COUNTRY)  
Cambridge, Massachusetts

Please check the appropriate assignee category or categories (will not be printed on the patent):  

☐ Individual ☒ Corporation or other private group entity ☐ Government

4a. Fees Submitted: ☐ Issue Fee ☐ Publication Fee (if required)

4b. Method of Payment (Please first reapply any previously paid fee shown above):  

☐ Electronic Payment via Patent Center or EFS-Web ☐ Enclosed check ☐ Non-electronic payment by credit card (Attach form PTO-2038)

☒ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. 50-4876

5. Change of Entity Status (from status indicated above)  

☐ Applicant certifying micro entity status. See 37 CFR 1.29.  
☐ Applicant asserting small entity status. See 37 CFR 1.27.  
☐ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid Certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

NOTE: If the application was previously under micro entity status, checking this box will be taken as a notification of loss of entitlement to micro entity status.

NOTE: Checking this box will be taken as a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature  
/Andrew R. Ehle/  
Typed or printed name  
Andrew R. Ehle

Date  
November 29, 2023  
Registration No.  
78,384

PTOL-85 Part B (03-23) Approved for use through 03/31/2023 OMB 0651-0033 U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

ME1 46855066v.1

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|                         |
|-------------------------|
| (Typed or printed name) |
| (Signature)             |
| (Date)                  |

| APPLICATION NO | FILING DATE | FIRST NAMED INVENTOR      | ATTORNEY DOCKET NO | CONFIRMATION NO |
|----------------|-------------|---------------------------|--------------------|-----------------|
| 16/900,610     | 06/12/2020  | Varsha Venkatachala. Iyer | 128748-01502       | 1054            |

TITLE OF INVENTION: MITAPIVAT THERAPY AND MODULATORS OF CYTOCHROME P450

| APPLN: TYPE    | ENTITY STATUS | ISSUE FEE DUE | PUBLICATION FEE DUE | PREV. PAID ISSUE FEE            | TOTAL FEE(S) DUE | DATE DUE   |
|----------------|---------------|---------------|---------------------|---------------------------------|------------------|------------|
| nonprovisional | UNDISCOUNTED  | \$1,200.00    |                     | \$1,200.00 (paid on 2023-08-02) | \$0.00           | 11/30/2023 |

| EXAMINER           | ART UNIT | CLASS-SUBCLASS |
|--------------------|----------|----------------|
| George W. Kosturko | 1621     | 424-094.500    |

|                                                                                                                                                                                                                                                                                                                                                                                                              |                                                                                                                                                                                                                                                                                                                                             |                                                                   |
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| 1. Change of correspondence address or indication of "Fee Address" (37 CFR 1.363)<br><br><input type="checkbox"/> Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.<br><input type="checkbox"/> "Fee Address" indication (or "Fee Address" Indication form PTO/AIA/47 or PTO/SB/47; Rev 03-09 or more recent) attached. Use of a Customer Number is required. | 2. For printing on the patent front page, list<br><br>(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,<br>(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed. | 1. McCarter & English, LLP<br>2. Michael J. DeGrazia, Ph.D.<br>3. |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------|

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(B) RESIDENCE: (CITY and STATE or COUNTRY) Cambridge, Massachusetts

Please check the appropriate assignee category or categories (will not be printed on the patent): ☐ Individual ☒ Corporation or other private group entity ☐ Government

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|                       |                  |                  |                   |
|-----------------------|------------------|------------------|-------------------|
| Authorized Signature  | /Andrew R. Ehle/ | Date             | November 29, 2023 |
| Typed or printed name | Andrew R. Ehle   | Registration No. | 78,384            |

PTOL-85 Part B (03-23) Approved for use through 03/31/2023 OMB 0651-0033 U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

ME1 46855066v.1



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| APPLICATION NO.                                                                       | ISSUE DATE | PATENT NO. | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---------------------------------------------------------------------------------------|------------|------------|---------------------|------------------|
| 16/900,610                                                                            | 01/23/2024 | 11878049   | 128748-01502        | 1054             |
| 190109                                                                                | 7590       | 01/03/2024 |                     |                  |
| McCarter & English / Agios Pharmaceuticals<br>265 Franklin Street<br>Boston, MA 02110 |            |            |                     |                  |

ISSUE NOTIFICATION

The projected patent number and issue date are specified above. The patent will issue electronically. The electronically issued patent is the official patent grant pursuant to 35 U.S.C. § 153. The patent may be accessed on or after the issue date through Patent Center at <https://patentcenter.uspto.gov/>. The patent will be available in both the public and the private sides of Patent Center. Further assistance in electronically accessing the patent, or about Patent Center, is available by calling the Patent Electronic Business Center at 1-888-217-9197.

The USPTO is implementing electronic patent issuance with a transition period, during which period the USPTO will mail a ceremonial paper copy of the electronic patent grant to the correspondence address of record. Additional copies of the patent (i.e., certified and presentation copies) may be ordered for a fee from the USPTO’s Certified Copy Center at <https://certifiedcopycenter.uspto.gov/index.html>. The Certified Copy Center may be reached at (800)972-6382.

Determination of Patent Term Adjustment under 35 U.S.C. 154 (b)  
(application filed on or after May 29, 2000)

The Patent Term Adjustment is 414 day(s). Any patent to issue from the above-identified application will include an indication of the adjustment on the front page.

If a Continued Prosecution Application (CPA) was filed in the above-identified application, the filing date that determines Patent Term Adjustment is the filing date of the most recent CPA.

Applicant will be able to obtain more detailed information by accessing the Patent Center (<https://patentcenter.uspto.gov>).

Any questions regarding the Patent Term Extension or Adjustment determination should be directed to the Office of Patent Legal Administration at (571)-272-7702. Questions relating to issue and publication fee payments should be directed to the Application Assistance Unit (AAU) of the Office of Patents Stakeholder Experience (OPSE), Stakeholder Support Division (SSD) at (571)-272-4200.

**INVENTOR(s)** (Please see PATENT CENTER site <https://patentcenter.uspto.gov> for additional inventors):

Varsha Venkatachalam Iyer, Cambridge, MA;  
Chandra Agarwal Prakash, North Andover, MA;  
Hua Yang, Acton, MA;

**APPLICANT(s)** (Please see PATENT CENTER site <https://patentcenter.uspto.gov> for additional applicants):

Agios Pharmaceuticals, Inc., Cambridge, MA;

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|                                            |             |                           |                     |                  |
|--------------------------------------------|-------------|---------------------------|---------------------|------------------|
| APPLICATION NO.                            | FILING DATE | FIRST NAMED INVENTOR      | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
| 16/900,610                                 | 06/12/2020  | Varsha Venkatachalam Iyer | 128748-01502        | 1054             |
| 190109                                     | 7590        | 01/23/2024                |                     |                  |
| McCarter & English / Agios Pharmaceuticals |             |                           | EXAMINER            |                  |
| 265 Franklin Street                        |             |                           | KOSTURKO, GEORGE W  |                  |
| Boston, MA 02110                           |             |                           | ART UNIT            | PAPER NUMBER     |
|                                            |             |                           | 1621                |                  |
|                                            |             |                           | NOTIFICATION DATE   | DELIVERY MODE    |
|                                            |             |                           | 01/23/2024          | ELECTRONIC       |

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The time period for reply, if any, is set in the attached communication.

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MDegrazia@McCarter.com  
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| APPLICATION NO. | ISSUE DATE  | PATENT NO. |
|-----------------|-------------|------------|
| 16/900,610      | 23-Jan-2024 | 11878049   |

McCarter & English / Agios Pharmaceuticals  
265 Franklin Street  
Boston, MA 02110

EGRANT NOTIFICATION

Your electronic patent grant (eGrant) is now available, which can be accessed via Patent Center at <https://patentcenter.uspto.gov>

The electronic patent grant is the official patent grant under 35 U.S.C. 153. For more information, please visit <https://www.uspto.gov/electronicgrants>

ELECTRONIC ACKNOWLEDGEMENT RECEIPT

|               |                             |                   |
|---------------|-----------------------------|-------------------|
| APPLICATION # | RECEIPT DATE / TIME         | ATTORNEY DOCKET # |
| 16/900,610    | 01/25/2024 05:46:36 PM Z ET | 128748-01502      |

Title of Invention

MITAPIVAT THERAPY AND MODULATORS OF CYTOCHROME P450

Application Information

|                        |                                                          |                      |                           |
|------------------------|----------------------------------------------------------|----------------------|---------------------------|
| APPLICATION TYPE       | Utility - Nonprovisional Application under 35 USC 111(a) | PATENT #             | 11878049                  |
| CONFIRMATION #         | 1054                                                     | FILED BY             | Kathleen Muzarol          |
| PATENT CENTER #        | 64092042                                                 | FILING DATE          | 06/12/2020                |
| CUSTOMER #             | 190109                                                   | FIRST NAMED INVENTOR | Varsha Venkatachalam Iyer |
| CORRESPONDENCE ADDRESS | -                                                        | AUTHORIZED BY        | Andrew Ehle               |

Documents

TOTAL DOCUMENTS: 2

| DOCUMENT                                             | PAGES | DESCRIPTION                           | SIZE (KB) |
|------------------------------------------------------|-------|---------------------------------------|-----------|
| 128748_01502_Req_for_Cert_of_Correction_Jan_2024.pdf | 2     | Request for Certificate of Correction | 23 KB     |
| 128748_01502_Certificate_of_CorrectionP_Jan_2024.pdf | 1     | Miscellaneous Incoming Letter         | 17 KB     |

Digest

| DOCUMENT                                             | MESSAGE DIGEST(SHA-512)                                                                                                          |
|------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------|
| 128748_01502_Req_for_Cert_of_Correction_Jan_2024.pdf | B5D120BCCF01CB3F46DC5A9F21729F478F77A85A49CDA9C67AB778AE0193B7BB27600D1A38E69961A56E6AF72BF2E85AC331F2BB48F97CFDA517EAE572C8201E |

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B0A1B9E583B278A0B54E8B84FF326F226182C92319C7414281B02CFFF2753F505E67ADE117334D0481788C85F65B5004743618A636DC39BCD1DEBD79E3444CEE

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**New Applications Under 35 U.S.C. 111**

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I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 C.F.R. § 1.6(a)(4).

Dated: January 25, 2024  
Electronic Signature for Andrew R. Ehle: /Andrew R. Ehle/

Docket No.: 128748-01502  
(PATENT)

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Letters Patent of:  
Varsha Venkatachalam Iyer et al.

Patent No.: 11,878,049

Issued: January 23, 2024

For: MITAPIVAT THERAPY AND MODULATORS  
OF CYTOCHROME P450

**REQUEST FOR CERTIFICATE OF CORRECTION  
PURSUANT TO 37 C.F.R. § 1.322**

Attention: Certificate of Correction Branch  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Dear Madam:

Upon reviewing the above-identified patent, Patentee noted a typographical error which should be corrected.

In the Claims:

In Column 46, Claim 9, line 10, please replace “The method of claim 6,” with -- The method of claim 8, --.

The error was incurred through the fault of the Office as evidenced by the record of this case. *See* the last set of the amended claims submitted on August 4, 2023, particularly Claim 67. Accordingly no fee is required for this Request under 37 C.F.R. 1.322(a)(1)(i).

Transmitted herewith is a proposed Certificate of Correction effecting such amendment. Patentee respectfully solicits the granting of the requested Certificate of Correction.

ME1 47461415v.1

Patent No.: 11,878,049

Docket No.: 128748-01502

Applicant believes no fee is due with this request. However, if a fee is due, please charge our Deposit Account No. 50-4876, under Order No. 128748-01502 from which the undersigned is authorized to draw.

Dated: January 25, 2024

Respectfully submitted,

Electronic signature: /Andrew R. Ehle/  
Andrew R. Ehle

Registration No.: 78,384  
MCCARTER & ENGLISH, LLP  
265 Franklin Street  
Boston, Massachusetts 02110  
(617) 449-6500  
(617) 607-9200 (Fax)  
Attorney/Agent For Applicant

UNITED STATES PATENT AND TRADEMARK OFFICE  
CERTIFICATE OF CORRECTION

Page 1 of 1

PATENT NO. : 11,878,049  
APPLICATION NO. : 16/900,610  
ISSUE DATE : January 23, 2024  
INVENTOR(S) : Varsha Venkatachalam Iyer et al.

It is certified that an error appears or errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:  
  
In Column 46, Claim 9, line 10, please replace "The method of claim 6," with -- The method of claim 8, --.

I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted via the Office electronic filing system in accordance with 37 C.F.R. § 1.6(a)(4).  
Dated: January 25, 2024 Electronic Signature for Andrew R. Ehle: /Andrew R. Ehle/

MAILING ADDRESS OF SENDER (Please do not use Customer Number below):  
Andrew R. Ehle  
MCCARTER & ENGLISH, LLP  
265 Franklin Street  
Boston, Massachusetts 02110  
  
ME1 47461424v.1

# File History Content Report

The following content is missing from the original file history record obtained from the United States Patent and Trademark Office. No additional information is available.

Document Date - 2024-02-05

Document Title - Certificate of Correction - Post Issue Communication



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE  
United States Patent and Trademark Office  
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P.O. Box 1450  
Alexandria, Virginia 22313-1450  
www.uspto.gov

|                                            |             |                           |                     |                  |
|--------------------------------------------|-------------|---------------------------|---------------------|------------------|
| APPLICATION NO.                            | FILING DATE | FIRST NAMED INVENTOR      | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
| 16/900,610                                 | 06/12/2020  | Varsha Venkatachalam Iyer | 128748-01502        | 1054             |
| 190109                                     | 7590        | 02/27/2024                |                     |                  |
| McCarter & English / Agios Pharmaceuticals |             |                           | EXAMINER            |                  |
| 265 Franklin Street                        |             |                           | KOSTURKO, GEORGE W  |                  |
| Boston, MA 02110                           |             |                           | ART UNIT            | PAPER NUMBER     |
|                                            |             |                           | 1621                |                  |
|                                            |             |                           | NOTIFICATION DATE   | DELIVERY MODE    |
|                                            |             |                           | 02/27/2024          | ELECTRONIC       |

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Notice of the Office communication was sent electronically on above-indicated "Notification Date" to the following e-mail address(es):

MDegrazia@McCarter.com  
docket@mccarter.com  
pair\_agios@firsttofile.com

| APPLICATION NO. | ISSUE DATE  | PATENT NO. |
|-----------------|-------------|------------|
| 16/900,610      | 27-Feb-2024 | 11878049   |

McCarter & English / Agios Pharmaceuticals  
265 Franklin Street  
Boston, MA 02110

**ELECTRONIC CERTIFICATE OF CORRECTION NOTIFICATION**

Your electronic certificate of correction is now available, and can be accessed via Patent Center at <https://patentcenter.uspto.gov>.

The electronic certificate of correction is the official certificate of correction under 35 U.S.C. 254 or 255. For more information, please visit <https://www.uspto.gov/electronicgrants>.

UNITED STATES PATENT AND TRADEMARK OFFICE  
**CERTIFICATE OF CORRECTION**

PATENT NO. : 11,878,049 B1  
APPLICATION NO. : 16/900610  
DATED : January 23, 2024  
INVENTOR(S) : Varsha Venkatachalam Iyer et al.

Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

In the Claims

In Column 46, Claim 9, Line 10, replace “The method of claim 6,” with -- The method of claim 8, --.

Signed and Sealed this  
Twenty-seventh Day of February, 2024



Katherine Kelly Vidal  
*Director of the United States Patent and Trademark Office*

# File History Content Report

The following content is missing from the original file history record obtained from the United States Patent and Trademark Office. No additional information is available.

Document Date - 2026-05-18

Document Title - USPTO Communication Re: Petition Requesting Trial

|                                                                                                                                                                                                                                                                                                                                                                                                            |                                                                                                                                                                                                                               |                                  |       |                                    |               |                                            |                         |         |                         |                    |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------|-------|------------------------------------|---------------|--------------------------------------------|-------------------------|---------|-------------------------|--------------------|
| PATENT APPLICATION FEE DETERMINATION RECORD<br>Substitute for Form PTO-875                                                                                                                                                                                                                                                                                                                                 |                                                                                                                                                                                                                               |                                  |       |                                    |               | Application or Docket Number<br>16/900,610 |                         |         |                         |                    |
| APPLICATION AS FILED - PART I                                                                                                                                                                                                                                                                                                                                                                              |                                                                                                                                                                                                                               |                                  |       |                                    |               |                                            |                         |         |                         |                    |
| (Column 1)                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                                                                                                                                                                               | (Column 2)                       |       | SMALL ENTITY                       |               | OR                                         | OTHER THAN SMALL ENTITY |         |                         |                    |
| FOR                                                                                                                                                                                                                                                                                                                                                                                                        | NUMBER FILED                                                                                                                                                                                                                  | NUMBER EXTRA                     |       | RATE(\$)                           | FEE(\$)       |                                            | RATE(\$)                | FEE(\$) |                         |                    |
| BASIC FEE<br>(37 CFR 1.16(a), (b), or (c))                                                                                                                                                                                                                                                                                                                                                                 | N/A                                                                                                                                                                                                                           | N/A                              |       | N/A                                |               |                                            | N/A                     | 300     |                         |                    |
| SEARCH FEE<br>(37 CFR 1.16(k), (l), or (m))                                                                                                                                                                                                                                                                                                                                                                | N/A                                                                                                                                                                                                                           | N/A                              |       | N/A                                |               |                                            | N/A                     | 660     |                         |                    |
| EXAMINATION FEE<br>(37 CFR 1.16(o), (p), or (q))                                                                                                                                                                                                                                                                                                                                                           | N/A                                                                                                                                                                                                                           | N/A                              |       | N/A                                |               |                                            | N/A                     | 760     |                         |                    |
| TOTAL CLAIMS<br>(37 CFR 1.16(j))                                                                                                                                                                                                                                                                                                                                                                           | 57                                                                                                                                                                                                                            | minus 20 =                       | *     | 37                                 |               | OR                                         | x 100 =                 | 3700    |                         |                    |
| INDEPENDENT CLAIMS<br>(37 CFR 1.16(h))                                                                                                                                                                                                                                                                                                                                                                     | 12                                                                                                                                                                                                                            | minus 3 =                        | *     | 9                                  |               |                                            | x 460 =                 | 4140    |                         |                    |
| APPLICATION SIZE FEE<br>(37 CFR 1.16(s))                                                                                                                                                                                                                                                                                                                                                                   | If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$310 (\$155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s). |                                  |       |                                    |               |                                            |                         |         |                         |                    |
| MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))                                                                                                                                                                                                                                                                                                                                                          |                                                                                                                                                                                                                               |                                  |       |                                    |               |                                            |                         | 820     |                         |                    |
| * If the difference in column 1 is less than zero, enter "0" in column 2.                                                                                                                                                                                                                                                                                                                                  |                                                                                                                                                                                                                               |                                  |       |                                    |               | TOTAL                                      |                         | TOTAL   | 10380                   |                    |
| APPLICATION AS AMENDED - PART II                                                                                                                                                                                                                                                                                                                                                                           |                                                                                                                                                                                                                               |                                  |       |                                    |               |                                            |                         |         |                         |                    |
| (Column 1)                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                                                                                                                                                                               | (Column 2)                       |       | (Column 3)                         |               | SMALL ENTITY                               |                         | OR      | OTHER THAN SMALL ENTITY |                    |
| AMENDMENT A                                                                                                                                                                                                                                                                                                                                                                                                |                                                                                                                                                                                                                               | CLAIMS REMAINING AFTER AMENDMENT |       | HIGHEST NUMBER PREVIOUSLY PAID FOR | PRESENT EXTRA | RATE(\$)                                   | ADDITIONAL FEE(\$)      |         | RATE(\$)                | ADDITIONAL FEE(\$) |
|                                                                                                                                                                                                                                                                                                                                                                                                            | Total<br>(37 CFR 1.16(i))                                                                                                                                                                                                     | *                                | Minus | **                                 | =             | x                                          | =                       | OR      | x                       | =                  |
|                                                                                                                                                                                                                                                                                                                                                                                                            | Independent<br>(37 CFR 1.16(h))                                                                                                                                                                                               | *                                | Minus | ***                                | =             | x                                          | =                       | OR      | x                       | =                  |
|                                                                                                                                                                                                                                                                                                                                                                                                            | Application Size Fee (37 CFR 1.16(s))                                                                                                                                                                                         |                                  |       |                                    |               |                                            |                         | OR      |                         |                    |
|                                                                                                                                                                                                                                                                                                                                                                                                            | FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))                                                                                                                                                               |                                  |       |                                    |               |                                            |                         | OR      |                         |                    |
|                                                                                                                                                                                                                                                                                                                                                                                                            |                                                                                                                                                                                                                               |                                  |       |                                    |               | TOTAL<br>ADD'L FEE                         |                         | OR      | TOTAL<br>ADD'L FEE      |                    |
| (Column 1)                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                                                                                                                                                                               | (Column 2)                       |       | (Column 3)                         |               | SMALL ENTITY                               |                         | OR      | OTHER THAN SMALL ENTITY |                    |
| AMENDMENT B                                                                                                                                                                                                                                                                                                                                                                                                |                                                                                                                                                                                                                               | CLAIMS REMAINING AFTER AMENDMENT |       | HIGHEST NUMBER PREVIOUSLY PAID FOR | PRESENT EXTRA | RATE(\$)                                   | ADDITIONAL FEE(\$)      |         | RATE(\$)                | ADDITIONAL FEE(\$) |
|                                                                                                                                                                                                                                                                                                                                                                                                            | Total<br>(37 CFR 1.16(i))                                                                                                                                                                                                     | *                                | Minus | **                                 | =             | x                                          | =                       | OR      | x                       | =                  |
|                                                                                                                                                                                                                                                                                                                                                                                                            | Independent<br>(37 CFR 1.16(h))                                                                                                                                                                                               | *                                | Minus | ***                                | =             | x                                          | =                       | OR      | x                       | =                  |
|                                                                                                                                                                                                                                                                                                                                                                                                            | Application Size Fee (37 CFR 1.16(s))                                                                                                                                                                                         |                                  |       |                                    |               |                                            |                         | OR      |                         |                    |
|                                                                                                                                                                                                                                                                                                                                                                                                            | FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))                                                                                                                                                               |                                  |       |                                    |               |                                            |                         | OR      |                         |                    |
|                                                                                                                                                                                                                                                                                                                                                                                                            |                                                                                                                                                                                                                               |                                  |       |                                    |               | TOTAL<br>ADD'L FEE                         |                         | OR      | TOTAL<br>ADD'L FEE      |                    |
| * If the entry in column 1 is less than the entry in column 2, write "0" in column 3.<br>** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".<br>*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".<br>The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1. |                                                                                                                                                                                                                               |                                  |       |                                    |               |                                            |                         |         |                         |                    |

|                                                                                                                                                                                                                                                                                                                                                                                                            |                                                                                                                                                                                                                               |                                  |       |                                    |               |                                            |                         |         |                         |                    |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------|-------|------------------------------------|---------------|--------------------------------------------|-------------------------|---------|-------------------------|--------------------|
| <div>PATENT APPLICATION FEE DETERMINATION RECORD</div> <div>Substitute for Form PTO-875</div>                                                                                                                                                                                                                                                                                                              |                                                                                                                                                                                                                               |                                  |       |                                    |               | Application or Docket Number<br>16/900,610 |                         |         |                         |                    |
| APPLICATION AS FILED - PART I                                                                                                                                                                                                                                                                                                                                                                              |                                                                                                                                                                                                                               |                                  |       |                                    |               |                                            |                         |         |                         |                    |
| (Column 1)                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                                                                                                                                                                               | (Column 2)                       |       | SMALL ENTITY                       |               | OR                                         | OTHER THAN SMALL ENTITY |         |                         |                    |
| FOR                                                                                                                                                                                                                                                                                                                                                                                                        | NUMBER FILED                                                                                                                                                                                                                  | NUMBER EXTRA                     |       | RATE(\$)                           | FEE(\$)       |                                            | RATE(\$)                | FEE(\$) |                         |                    |
| BASIC FEE<br>(37 CFR 1.16(a), (b), or (c))                                                                                                                                                                                                                                                                                                                                                                 | N/A                                                                                                                                                                                                                           | N/A                              |       | N/A                                |               |                                            | N/A                     | 300     |                         |                    |
| SEARCH FEE<br>(37 CFR 1.16(k), (l), or (m))                                                                                                                                                                                                                                                                                                                                                                | N/A                                                                                                                                                                                                                           | N/A                              |       | N/A                                |               |                                            | N/A                     | 660     |                         |                    |
| EXAMINATION FEE<br>(37 CFR 1.16(o), (p), or (q))                                                                                                                                                                                                                                                                                                                                                           | N/A                                                                                                                                                                                                                           | N/A                              |       | N/A                                |               |                                            | N/A                     | 760     |                         |                    |
| TOTAL CLAIMS<br>(37 CFR 1.16(j))                                                                                                                                                                                                                                                                                                                                                                           | 18                                                                                                                                                                                                                            | minus 20 =                       | *     |                                    |               | OR                                         | x 100 =                 | 0.00    |                         |                    |
| INDEPENDENT CLAIMS<br>(37 CFR 1.16(h))                                                                                                                                                                                                                                                                                                                                                                     | 6                                                                                                                                                                                                                             | minus 3 =                        | *     | 3                                  |               |                                            | x 460 =                 | 1380    |                         |                    |
| APPLICATION SIZE FEE<br>(37 CFR 1.16(s))                                                                                                                                                                                                                                                                                                                                                                   | If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$310 (\$155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s). |                                  |       |                                    |               |                                            |                         | 0.00    |                         |                    |
| MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))                                                                                                                                                                                                                                                                                                                                                          |                                                                                                                                                                                                                               |                                  |       |                                    |               |                                            |                         | 0.00    |                         |                    |
| * If the difference in column 1 is less than zero, enter "0" in column 2.                                                                                                                                                                                                                                                                                                                                  |                                                                                                                                                                                                                               |                                  |       | TOTAL                              |               |                                            | TOTAL                   | 3100    |                         |                    |
| APPLICATION AS AMENDED - PART II                                                                                                                                                                                                                                                                                                                                                                           |                                                                                                                                                                                                                               |                                  |       |                                    |               |                                            |                         |         |                         |                    |
| (Column 1)                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                                                                                                                                                                               | (Column 2)                       |       | (Column 3)                         |               | SMALL ENTITY                               |                         | OR      | OTHER THAN SMALL ENTITY |                    |
| AMENDMENT A                                                                                                                                                                                                                                                                                                                                                                                                |                                                                                                                                                                                                                               | CLAIMS REMAINING AFTER AMENDMENT |       | HIGHEST NUMBER PREVIOUSLY PAID FOR | PRESENT EXTRA | RATE(\$)                                   | ADDITIONAL FEE(\$)      |         | RATE(\$)                | ADDITIONAL FEE(\$) |
|                                                                                                                                                                                                                                                                                                                                                                                                            | Total<br>(37 CFR 1.16(i))                                                                                                                                                                                                     | *                                | Minus | **                                 | =             | x =                                        |                         | OR      | x =                     |                    |
|                                                                                                                                                                                                                                                                                                                                                                                                            | Independent<br>(37 CFR 1.16(h))                                                                                                                                                                                               | *                                | Minus | ***                                | =             | x =                                        |                         | OR      | x =                     |                    |
|                                                                                                                                                                                                                                                                                                                                                                                                            | Application Size Fee (37 CFR 1.16(s))                                                                                                                                                                                         |                                  |       |                                    |               |                                            |                         | OR      |                         |                    |
|                                                                                                                                                                                                                                                                                                                                                                                                            | FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))                                                                                                                                                               |                                  |       |                                    |               |                                            |                         | OR      |                         |                    |
|                                                                                                                                                                                                                                                                                                                                                                                                            |                                                                                                                                                                                                                               |                                  |       |                                    |               | TOTAL<br>ADD'L FEE                         |                         | OR      | TOTAL<br>ADD'L FEE      |                    |
| AMENDMENT B                                                                                                                                                                                                                                                                                                                                                                                                |                                                                                                                                                                                                                               | CLAIMS REMAINING AFTER AMENDMENT |       | HIGHEST NUMBER PREVIOUSLY PAID FOR | PRESENT EXTRA | RATE(\$)                                   | ADDITIONAL FEE(\$)      |         | RATE(\$)                | ADDITIONAL FEE(\$) |
|                                                                                                                                                                                                                                                                                                                                                                                                            | Total<br>(37 CFR 1.16(i))                                                                                                                                                                                                     | *                                | Minus | **                                 | =             | x =                                        |                         | OR      | x =                     |                    |
|                                                                                                                                                                                                                                                                                                                                                                                                            | Independent<br>(37 CFR 1.16(h))                                                                                                                                                                                               | *                                | Minus | ***                                | =             | x =                                        |                         | OR      | x =                     |                    |
|                                                                                                                                                                                                                                                                                                                                                                                                            | Application Size Fee (37 CFR 1.16(s))                                                                                                                                                                                         |                                  |       |                                    |               |                                            |                         | OR      |                         |                    |
|                                                                                                                                                                                                                                                                                                                                                                                                            | FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))                                                                                                                                                               |                                  |       |                                    |               |                                            |                         | OR      |                         |                    |
|                                                                                                                                                                                                                                                                                                                                                                                                            |                                                                                                                                                                                                                               |                                  |       |                                    |               | TOTAL<br>ADD'L FEE                         |                         | OR      | TOTAL<br>ADD'L FEE      |                    |
| * If the entry in column 1 is less than the entry in column 2, write "0" in column 3.<br>** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".<br>*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".<br>The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1. |                                                                                                                                                                                                                               |                                  |       |                                    |               |                                            |                         |         |                         |                    |

|                                                                                                                                                                                                                                                                                                                                                                                                            |                                                                                                                                                                                                                               |                                  |       |                                    |               |                                            |                         |         |                         |                    |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------|-------|------------------------------------|---------------|--------------------------------------------|-------------------------|---------|-------------------------|--------------------|
| PATENT APPLICATION FEE DETERMINATION RECORD<br>Substitute for Form PTO-875                                                                                                                                                                                                                                                                                                                                 |                                                                                                                                                                                                                               |                                  |       |                                    |               | Application or Docket Number<br>16/900,610 |                         |         |                         |                    |
| APPLICATION AS FILED - PART I                                                                                                                                                                                                                                                                                                                                                                              |                                                                                                                                                                                                                               |                                  |       |                                    |               |                                            |                         |         |                         |                    |
| (Column 1)                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                                                                                                                                                                               | (Column 2)                       |       | SMALL ENTITY                       |               | OR                                         | OTHER THAN SMALL ENTITY |         |                         |                    |
| FOR                                                                                                                                                                                                                                                                                                                                                                                                        | NUMBER FILED                                                                                                                                                                                                                  | NUMBER EXTRA                     |       | RATE(\$)                           | FEE(\$)       |                                            | RATE(\$)                | FEE(\$) |                         |                    |
| BASIC FEE<br>(37 CFR 1.16(a), (b), or (c))                                                                                                                                                                                                                                                                                                                                                                 | N/A                                                                                                                                                                                                                           | N/A                              |       | N/A                                |               |                                            | N/A                     | 300     |                         |                    |
| SEARCH FEE<br>(37 CFR 1.16(k), (l), or (m))                                                                                                                                                                                                                                                                                                                                                                | N/A                                                                                                                                                                                                                           | N/A                              |       | N/A                                |               |                                            | N/A                     | 660     |                         |                    |
| EXAMINATION FEE<br>(37 CFR 1.16(o), (p), or (q))                                                                                                                                                                                                                                                                                                                                                           | N/A                                                                                                                                                                                                                           | N/A                              |       | N/A                                |               |                                            | N/A                     | 760     |                         |                    |
| TOTAL CLAIMS<br>(37 CFR 1.16(j))                                                                                                                                                                                                                                                                                                                                                                           | 18                                                                                                                                                                                                                            | minus 20 =                       | *     |                                    |               | OR                                         | x 100 =                 | 0.00    |                         |                    |
| INDEPENDENT CLAIMS<br>(37 CFR 1.16(h))                                                                                                                                                                                                                                                                                                                                                                     | 6                                                                                                                                                                                                                             | minus 3 =                        | *     | 3                                  |               |                                            | x 460 =                 | 1380    |                         |                    |
| APPLICATION SIZE FEE<br>(37 CFR 1.16(s))                                                                                                                                                                                                                                                                                                                                                                   | If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$310 (\$155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s). |                                  |       |                                    |               |                                            |                         | 0.00    |                         |                    |
| MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))                                                                                                                                                                                                                                                                                                                                                          |                                                                                                                                                                                                                               |                                  |       |                                    |               |                                            |                         | 0.00    |                         |                    |
| * If the difference in column 1 is less than zero, enter "0" in column 2.                                                                                                                                                                                                                                                                                                                                  |                                                                                                                                                                                                                               |                                  |       | TOTAL                              |               |                                            | TOTAL                   | 3100    |                         |                    |
| APPLICATION AS AMENDED - PART II                                                                                                                                                                                                                                                                                                                                                                           |                                                                                                                                                                                                                               |                                  |       |                                    |               |                                            |                         |         |                         |                    |
| (Column 1)                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                                                                                                                                                                               | (Column 2)                       |       | (Column 3)                         |               | SMALL ENTITY                               |                         | OR      | OTHER THAN SMALL ENTITY |                    |
| AMENDMENT A                                                                                                                                                                                                                                                                                                                                                                                                |                                                                                                                                                                                                                               | CLAIMS REMAINING AFTER AMENDMENT |       | HIGHEST NUMBER PREVIOUSLY PAID FOR | PRESENT EXTRA | RATE(\$)                                   | ADDITIONAL FEE(\$)      |         | RATE(\$)                | ADDITIONAL FEE(\$) |
|                                                                                                                                                                                                                                                                                                                                                                                                            | Total<br>(37 CFR 1.16(i))                                                                                                                                                                                                     | *                                | Minus | **                                 | =             | x                                          | =                       | OR      | x                       | =                  |
|                                                                                                                                                                                                                                                                                                                                                                                                            | Independent<br>(37 CFR 1.16(h))                                                                                                                                                                                               | *                                | Minus | ***                                | =             | x                                          | =                       | OR      | x                       | =                  |
|                                                                                                                                                                                                                                                                                                                                                                                                            | Application Size Fee (37 CFR 1.16(s))                                                                                                                                                                                         |                                  |       |                                    |               |                                            |                         | OR      |                         |                    |
|                                                                                                                                                                                                                                                                                                                                                                                                            | FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))                                                                                                                                                               |                                  |       |                                    |               |                                            |                         | OR      |                         |                    |
|                                                                                                                                                                                                                                                                                                                                                                                                            |                                                                                                                                                                                                                               |                                  |       |                                    |               | TOTAL<br>ADD'L FEE                         |                         | OR      | TOTAL<br>ADD'L FEE      |                    |
| AMENDMENT B                                                                                                                                                                                                                                                                                                                                                                                                |                                                                                                                                                                                                                               | CLAIMS REMAINING AFTER AMENDMENT |       | HIGHEST NUMBER PREVIOUSLY PAID FOR | PRESENT EXTRA | RATE(\$)                                   | ADDITIONAL FEE(\$)      |         | RATE(\$)                | ADDITIONAL FEE(\$) |
|                                                                                                                                                                                                                                                                                                                                                                                                            | Total<br>(37 CFR 1.16(i))                                                                                                                                                                                                     | *                                | Minus | **                                 | =             | x                                          | =                       | OR      | x                       | =                  |
|                                                                                                                                                                                                                                                                                                                                                                                                            | Independent<br>(37 CFR 1.16(h))                                                                                                                                                                                               | *                                | Minus | ***                                | =             | x                                          | =                       | OR      | x                       | =                  |
|                                                                                                                                                                                                                                                                                                                                                                                                            | Application Size Fee (37 CFR 1.16(s))                                                                                                                                                                                         |                                  |       |                                    |               |                                            |                         | OR      |                         |                    |
|                                                                                                                                                                                                                                                                                                                                                                                                            | FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j))                                                                                                                                                               |                                  |       |                                    |               |                                            |                         | OR      |                         |                    |
|                                                                                                                                                                                                                                                                                                                                                                                                            |                                                                                                                                                                                                                               |                                  |       |                                    |               | TOTAL<br>ADD'L FEE                         |                         | OR      | TOTAL<br>ADD'L FEE      |                    |
| * If the entry in column 1 is less than the entry in column 2, write "0" in column 3.<br>** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".<br>*** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".<br>The "Highest Number Previously Paid For" (Total or Independent) is the highest found in the appropriate box in column 1. |                                                                                                                                                                                                                               |                                  |       |                                    |               |                                            |                         |         |                         |                    |

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|                                                                                                                                 |                                                                                          |                                                                                                                                                                                                                               |       |                                            |               |                           |  |                                       |  |
|---------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|--------------------------------------------|---------------|---------------------------|--|---------------------------------------|--|
| <b>PATENT APPLICATION FEE DETERMINATION RECORD</b><br>Substitute for Form PTO-875                                               |                                                                                          |                                                                                                                                                                                                                               |       | Application or Docket Number<br>16/900,610 |               | Filing Date<br>06/12/2020 |  | <input type="checkbox"/> To be Mailed |  |
| ENTITY: <input checked="" type="checkbox"/> LARGE <input type="checkbox"/> SMALL <input type="checkbox"/> MICRO                 |                                                                                          |                                                                                                                                                                                                                               |       |                                            |               |                           |  |                                       |  |
| APPLICATION AS FILED - PART I                                                                                                   |                                                                                          |                                                                                                                                                                                                                               |       |                                            |               |                           |  |                                       |  |
|                                                                                                                                 |                                                                                          | (Column 1)                                                                                                                                                                                                                    |       | (Column 2)                                 |               |                           |  |                                       |  |
| FOR                                                                                                                             |                                                                                          | NUMBER FILED                                                                                                                                                                                                                  |       | NUMBER EXTRA                               |               | RATE (\$)                 |  | FEE (\$)                              |  |
| <input type="checkbox"/> BASIC FEE<br>(37 CFR 1.16(a), (b), or (c))                                                             |                                                                                          | N/A                                                                                                                                                                                                                           |       | N/A                                        |               | N/A                       |  |                                       |  |
| <input type="checkbox"/> SEARCH FEE<br>(37 CFR 1.16(k), (i), or (m))                                                            |                                                                                          | N/A                                                                                                                                                                                                                           |       | N/A                                        |               | N/A                       |  |                                       |  |
| <input type="checkbox"/> EXAMINATION FEE<br>(37 CFR 1.16(o), (p), or (q))                                                       |                                                                                          | N/A                                                                                                                                                                                                                           |       | N/A                                        |               | N/A                       |  |                                       |  |
| TOTAL CLAIMS<br>(37 CFR 1.16(i))                                                                                                |                                                                                          | minus 20 =                                                                                                                                                                                                                    |       | *                                          |               | x \$100 =                 |  |                                       |  |
| INDEPENDENT CLAIMS<br>(37 CFR 1.16(h))                                                                                          |                                                                                          | minus 3 =                                                                                                                                                                                                                     |       | *                                          |               | x \$460 =                 |  |                                       |  |
| <input type="checkbox"/> APPLICATION SIZE FEE (37 CFR 1.16(s))                                                                  |                                                                                          | If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$310 (\$155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s). |       |                                            |               |                           |  |                                       |  |
| <input type="checkbox"/> MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))                                                      |                                                                                          |                                                                                                                                                                                                                               |       |                                            |               |                           |  |                                       |  |
| * If the difference in column 1 is less than zero, enter "0" in column 2.                                                       |                                                                                          |                                                                                                                                                                                                                               |       |                                            |               | TOTAL                     |  |                                       |  |
| APPLICATION AS AMENDED - PART II                                                                                                |                                                                                          |                                                                                                                                                                                                                               |       |                                            |               |                           |  |                                       |  |
|                                                                                                                                 |                                                                                          | (Column 1)                                                                                                                                                                                                                    |       | (Column 2)                                 |               | (Column 3)                |  |                                       |  |
| AMENDMENT                                                                                                                       | 11/18/2022                                                                               | CLAIMS REMAINING AFTER AMENDMENT                                                                                                                                                                                              |       | HIGHEST NUMBER PREVIOUSLY PAID FOR         | PRESENT EXTRA | RATE (\$)                 |  | ADDITIONAL FEE (\$)                   |  |
|                                                                                                                                 | Total<br>(37 CFR 1.16(i))                                                                | * 15                                                                                                                                                                                                                          | Minus | ** 20                                      | = 0           | x \$ 100 =                |  | 0                                     |  |
|                                                                                                                                 | Independent<br>(37 CFR 1.16(h))                                                          | * 1                                                                                                                                                                                                                           | Minus | *** 3                                      | = 0           | x \$ 480 =                |  | 0                                     |  |
|                                                                                                                                 | <input type="checkbox"/> Application Size Fee (37 CFR 1.16(s))                           |                                                                                                                                                                                                                               |       |                                            |               |                           |  |                                       |  |
|                                                                                                                                 | <input type="checkbox"/> FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j)) |                                                                                                                                                                                                                               |       |                                            |               |                           |  |                                       |  |
|                                                                                                                                 |                                                                                          |                                                                                                                                                                                                                               |       |                                            |               | TOTAL ADD'L FEE           |  | 0                                     |  |
|                                                                                                                                 |                                                                                          | (Column 1)                                                                                                                                                                                                                    |       | (Column 2)                                 |               | (Column 3)                |  |                                       |  |
| AMENDMENT                                                                                                                       |                                                                                          | CLAIMS REMAINING AFTER AMENDMENT                                                                                                                                                                                              |       | HIGHEST NUMBER PREVIOUSLY PAID FOR         | PRESENT EXTRA | RATE (\$)                 |  | ADDITIONAL FEE (\$)                   |  |
|                                                                                                                                 | Total<br>(37 CFR 1.16(i))                                                                | *                                                                                                                                                                                                                             | Minus | **                                         | =             | x \$ 0 =                  |  |                                       |  |
|                                                                                                                                 | Independent<br>(37 CFR 1.16(h))                                                          | *                                                                                                                                                                                                                             | Minus | ***                                        | =             | x \$ 0 =                  |  |                                       |  |
|                                                                                                                                 | <input type="checkbox"/> Application Size Fee (37 CFR 1.16(s))                           |                                                                                                                                                                                                                               |       |                                            |               |                           |  |                                       |  |
|                                                                                                                                 | <input type="checkbox"/> FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j)) |                                                                                                                                                                                                                               |       |                                            |               |                           |  |                                       |  |
|                                                                                                                                 |                                                                                          |                                                                                                                                                                                                                               |       |                                            |               | TOTAL ADD'L FEE           |  |                                       |  |
| * If the entry in column 1 is less than the entry in column 2, write "0" in column 3.                                           |                                                                                          |                                                                                                                                                                                                                               |       |                                            |               | SLIE                      |  |                                       |  |
| ** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".                                       |                                                                                          |                                                                                                                                                                                                                               |       |                                            |               | /TONYA Q MCBRIDE/         |  |                                       |  |
| *** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".                                        |                                                                                          |                                                                                                                                                                                                                               |       |                                            |               |                           |  |                                       |  |
| The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1. |                                                                                          |                                                                                                                                                                                                                               |       |                                            |               |                           |  |                                       |  |

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**  
*If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.*

PTO/SB/06 (09-11)

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|                                                                                                                                 |                                                                                          |                                                                                                                                                                                                                               |       |                                            |               |                           |  |                                       |  |
|---------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|--------------------------------------------|---------------|---------------------------|--|---------------------------------------|--|
| <b>PATENT APPLICATION FEE DETERMINATION RECORD</b><br>Substitute for Form PTO-875                                               |                                                                                          |                                                                                                                                                                                                                               |       | Application or Docket Number<br>16/900,610 |               | Filing Date<br>06/12/2020 |  | <input type="checkbox"/> To be Mailed |  |
| ENTITY: <input checked="" type="checkbox"/> LARGE <input type="checkbox"/> SMALL <input type="checkbox"/> MICRO                 |                                                                                          |                                                                                                                                                                                                                               |       |                                            |               |                           |  |                                       |  |
| APPLICATION AS FILED - PART I                                                                                                   |                                                                                          |                                                                                                                                                                                                                               |       |                                            |               |                           |  |                                       |  |
|                                                                                                                                 |                                                                                          | (Column 1)                                                                                                                                                                                                                    |       | (Column 2)                                 |               |                           |  |                                       |  |
| FOR                                                                                                                             |                                                                                          | NUMBER FILED                                                                                                                                                                                                                  |       | NUMBER EXTRA                               |               | RATE (\$)                 |  | FEE (\$)                              |  |
| <input type="checkbox"/> BASIC FEE<br>(37 CFR 1.16(a), (b), or (c))                                                             |                                                                                          | N/A                                                                                                                                                                                                                           |       | N/A                                        |               | N/A                       |  |                                       |  |
| <input type="checkbox"/> SEARCH FEE<br>(37 CFR 1.16(k), (i), or (m))                                                            |                                                                                          | N/A                                                                                                                                                                                                                           |       | N/A                                        |               | N/A                       |  |                                       |  |
| <input type="checkbox"/> EXAMINATION FEE<br>(37 CFR 1.16(o), (p), or (q))                                                       |                                                                                          | N/A                                                                                                                                                                                                                           |       | N/A                                        |               | N/A                       |  |                                       |  |
| TOTAL CLAIMS<br>(37 CFR 1.16(i))                                                                                                |                                                                                          | minus 20 =                                                                                                                                                                                                                    |       | *                                          |               | x \$100 =                 |  |                                       |  |
| INDEPENDENT CLAIMS<br>(37 CFR 1.16(h))                                                                                          |                                                                                          | minus 3 =                                                                                                                                                                                                                     |       | *                                          |               | x \$460 =                 |  |                                       |  |
| <input type="checkbox"/> APPLICATION SIZE FEE (37 CFR 1.16(s))                                                                  |                                                                                          | If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$310 (\$155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s). |       |                                            |               |                           |  |                                       |  |
| <input type="checkbox"/> MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))                                                      |                                                                                          |                                                                                                                                                                                                                               |       |                                            |               |                           |  |                                       |  |
| * If the difference in column 1 is less than zero, enter "0" in column 2.                                                       |                                                                                          |                                                                                                                                                                                                                               |       |                                            |               | TOTAL                     |  |                                       |  |
| APPLICATION AS AMENDED - PART II                                                                                                |                                                                                          |                                                                                                                                                                                                                               |       |                                            |               |                           |  |                                       |  |
|                                                                                                                                 |                                                                                          | (Column 1)                                                                                                                                                                                                                    |       | (Column 2)                                 |               | (Column 3)                |  |                                       |  |
| AMENDMENT                                                                                                                       | 04/07/2023                                                                               | CLAIMS REMAINING AFTER AMENDMENT                                                                                                                                                                                              |       | HIGHEST NUMBER PREVIOUSLY PAID FOR         | PRESENT EXTRA | RATE (\$)                 |  | ADDITIONAL FEE (\$)                   |  |
|                                                                                                                                 | Total<br>(37 CFR 1.16(i))                                                                | * 19                                                                                                                                                                                                                          | Minus | ** 20                                      | = 0           | x \$ 100 =                |  | 0                                     |  |
|                                                                                                                                 | Independent<br>(37 CFR 1.16(h))                                                          | * 1                                                                                                                                                                                                                           | Minus | *** 6                                      | = 0           | x \$ 480 =                |  | 0                                     |  |
|                                                                                                                                 | <input type="checkbox"/> Application Size Fee (37 CFR 1.16(s))                           |                                                                                                                                                                                                                               |       |                                            |               |                           |  |                                       |  |
|                                                                                                                                 | <input type="checkbox"/> FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j)) |                                                                                                                                                                                                                               |       |                                            |               |                           |  |                                       |  |
|                                                                                                                                 |                                                                                          |                                                                                                                                                                                                                               |       |                                            |               | TOTAL ADD'L FEE           |  | 0                                     |  |
|                                                                                                                                 |                                                                                          | (Column 1)                                                                                                                                                                                                                    |       | (Column 2)                                 |               | (Column 3)                |  |                                       |  |
| AMENDMENT                                                                                                                       |                                                                                          | CLAIMS REMAINING AFTER AMENDMENT                                                                                                                                                                                              |       | HIGHEST NUMBER PREVIOUSLY PAID FOR         | PRESENT EXTRA | RATE (\$)                 |  | ADDITIONAL FEE (\$)                   |  |
|                                                                                                                                 | Total<br>(37 CFR 1.16(i))                                                                | *                                                                                                                                                                                                                             | Minus | **                                         | =             | x \$ 0 =                  |  |                                       |  |
|                                                                                                                                 | Independent<br>(37 CFR 1.16(h))                                                          | *                                                                                                                                                                                                                             | Minus | ***                                        | =             | x \$ 0 =                  |  |                                       |  |
|                                                                                                                                 | <input type="checkbox"/> Application Size Fee (37 CFR 1.16(s))                           |                                                                                                                                                                                                                               |       |                                            |               |                           |  |                                       |  |
|                                                                                                                                 | <input type="checkbox"/> FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j)) |                                                                                                                                                                                                                               |       |                                            |               |                           |  |                                       |  |
|                                                                                                                                 |                                                                                          |                                                                                                                                                                                                                               |       |                                            |               | TOTAL ADD'L FEE           |  |                                       |  |
| * If the entry in column 1 is less than the entry in column 2, write "0" in column 3.                                           |                                                                                          |                                                                                                                                                                                                                               |       |                                            |               | LIE                       |  |                                       |  |
| ** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".                                       |                                                                                          |                                                                                                                                                                                                                               |       |                                            |               | /LINDA HUMES/             |  |                                       |  |
| *** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".                                        |                                                                                          |                                                                                                                                                                                                                               |       |                                            |               |                           |  |                                       |  |
| The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1. |                                                                                          |                                                                                                                                                                                                                               |       |                                            |               |                           |  |                                       |  |

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

*If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.*

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|---------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------|--------------------------------------------|---------------|---------------------------|--|---------------------------------------|--|
| <b>PATENT APPLICATION FEE DETERMINATION RECORD</b><br>Substitute for Form PTO-875                                               |                                                                                          |                                                                                                                                                                                                                               |       | Application or Docket Number<br>16/900,610 |               | Filing Date<br>06/12/2020 |  | <input type="checkbox"/> To be Mailed |  |
| ENTITY: <input checked="" type="checkbox"/> LARGE <input type="checkbox"/> SMALL <input type="checkbox"/> MICRO                 |                                                                                          |                                                                                                                                                                                                                               |       |                                            |               |                           |  |                                       |  |
| APPLICATION AS FILED - PART I                                                                                                   |                                                                                          |                                                                                                                                                                                                                               |       |                                            |               |                           |  |                                       |  |
|                                                                                                                                 |                                                                                          | (Column 1)                                                                                                                                                                                                                    |       | (Column 2)                                 |               |                           |  |                                       |  |
| FOR                                                                                                                             |                                                                                          | NUMBER FILED                                                                                                                                                                                                                  |       | NUMBER EXTRA                               |               | RATE (\$)                 |  | FEE (\$)                              |  |
| <input type="checkbox"/> BASIC FEE<br>(37 CFR 1.16(a), (b), or (c))                                                             |                                                                                          | N/A                                                                                                                                                                                                                           |       | N/A                                        |               | N/A                       |  |                                       |  |
| <input type="checkbox"/> SEARCH FEE<br>(37 CFR 1.16(k), (i), or (m))                                                            |                                                                                          | N/A                                                                                                                                                                                                                           |       | N/A                                        |               | N/A                       |  |                                       |  |
| <input type="checkbox"/> EXAMINATION FEE<br>(37 CFR 1.16(o), (p), or (q))                                                       |                                                                                          | N/A                                                                                                                                                                                                                           |       | N/A                                        |               | N/A                       |  |                                       |  |
| TOTAL CLAIMS<br>(37 CFR 1.16(i))                                                                                                |                                                                                          | minus 20 =                                                                                                                                                                                                                    |       | *                                          |               | x \$100 =                 |  |                                       |  |
| INDEPENDENT CLAIMS<br>(37 CFR 1.16(h))                                                                                          |                                                                                          | minus 3 =                                                                                                                                                                                                                     |       | *                                          |               | x \$460 =                 |  |                                       |  |
| <input type="checkbox"/> APPLICATION SIZE FEE (37 CFR 1.16(s))                                                                  |                                                                                          | If the specification and drawings exceed 100 sheets of paper, the application size fee due is \$310 (\$155 for small entity) for each additional 50 sheets or fraction thereof. See 35 U.S.C. 41(a)(1)(G) and 37 CFR 1.16(s). |       |                                            |               |                           |  |                                       |  |
| <input type="checkbox"/> MULTIPLE DEPENDENT CLAIM PRESENT (37 CFR 1.16(j))                                                      |                                                                                          |                                                                                                                                                                                                                               |       |                                            |               |                           |  |                                       |  |
| * If the difference in column 1 is less than zero, enter "0" in column 2.                                                       |                                                                                          |                                                                                                                                                                                                                               |       |                                            |               | TOTAL                     |  |                                       |  |
| APPLICATION AS AMENDED - PART II                                                                                                |                                                                                          |                                                                                                                                                                                                                               |       |                                            |               |                           |  |                                       |  |
|                                                                                                                                 |                                                                                          | (Column 1)                                                                                                                                                                                                                    |       | (Column 2)                                 |               | (Column 3)                |  |                                       |  |
| AMENDMENT                                                                                                                       | 08/04/2023                                                                               | CLAIMS REMAINING AFTER AMENDMENT                                                                                                                                                                                              |       | HIGHEST NUMBER PREVIOUSLY PAID FOR         | PRESENT EXTRA | RATE (\$)                 |  | ADDITIONAL FEE (\$)                   |  |
|                                                                                                                                 | Total<br>(37 CFR 1.16(i))                                                                | * 19                                                                                                                                                                                                                          | Minus | ** 20                                      | = 0           | x \$ 100 =                |  | 0                                     |  |
|                                                                                                                                 | Independent<br>(37 CFR 1.16(h))                                                          | * 1                                                                                                                                                                                                                           | Minus | *** 6                                      | = 0           | x \$ 480 =                |  | 0                                     |  |
|                                                                                                                                 | <input type="checkbox"/> Application Size Fee (37 CFR 1.16(s))                           |                                                                                                                                                                                                                               |       |                                            |               |                           |  |                                       |  |
|                                                                                                                                 | <input type="checkbox"/> FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j)) |                                                                                                                                                                                                                               |       |                                            |               |                           |  |                                       |  |
|                                                                                                                                 |                                                                                          |                                                                                                                                                                                                                               |       |                                            |               | TOTAL ADD'L FEE           |  | 0                                     |  |
|                                                                                                                                 |                                                                                          | (Column 1)                                                                                                                                                                                                                    |       | (Column 2)                                 |               | (Column 3)                |  |                                       |  |
| AMENDMENT                                                                                                                       |                                                                                          | CLAIMS REMAINING AFTER AMENDMENT                                                                                                                                                                                              |       | HIGHEST NUMBER PREVIOUSLY PAID FOR         | PRESENT EXTRA | RATE (\$)                 |  | ADDITIONAL FEE (\$)                   |  |
|                                                                                                                                 | Total<br>(37 CFR 1.16(i))                                                                | *                                                                                                                                                                                                                             | Minus | **                                         | =             | x \$ 0 =                  |  |                                       |  |
|                                                                                                                                 | Independent<br>(37 CFR 1.16(h))                                                          | *                                                                                                                                                                                                                             | Minus | ***                                        | =             | x \$ 0 =                  |  |                                       |  |
|                                                                                                                                 | <input type="checkbox"/> Application Size Fee (37 CFR 1.16(s))                           |                                                                                                                                                                                                                               |       |                                            |               |                           |  |                                       |  |
|                                                                                                                                 | <input type="checkbox"/> FIRST PRESENTATION OF MULTIPLE DEPENDENT CLAIM (37 CFR 1.16(j)) |                                                                                                                                                                                                                               |       |                                            |               |                           |  |                                       |  |
|                                                                                                                                 |                                                                                          |                                                                                                                                                                                                                               |       |                                            |               | TOTAL ADD'L FEE           |  |                                       |  |
| * If the entry in column 1 is less than the entry in column 2, write "0" in column 3.                                           |                                                                                          |                                                                                                                                                                                                                               |       |                                            |               | SLIE                      |  |                                       |  |
| ** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 20, enter "20".                                       |                                                                                          |                                                                                                                                                                                                                               |       |                                            |               | /ANISSA D DEVLIN/         |  |                                       |  |
| *** If the "Highest Number Previously Paid For" IN THIS SPACE is less than 3, enter "3".                                        |                                                                                          |                                                                                                                                                                                                                               |       |                                            |               |                           |  |                                       |  |
| The "Highest Number Previously Paid For" (Total or Independent) is the highest number found in the appropriate box in column 1. |                                                                                          |                                                                                                                                                                                                                               |       |                                            |               |                           |  |                                       |  |

This collection of information is required by 37 CFR 1.16. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.**

*If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.*

|                                                                                                                                         |          |        |                       |        |                        |        |                                                   |       |             |       |        |       |        |
|-----------------------------------------------------------------------------------------------------------------------------------------|----------|--------|-----------------------|--------|------------------------|--------|---------------------------------------------------|-------|-------------|-------|--------|-------|--------|
| <div>MULTIPLE DEPENDENT CLAIM<br/>FEE CALCULATION SHEET</div> <div>Substitute for Form PTO-1360<br/>(For use with Form PTO/SB/06)</div> |          |        |                       |        |                        |        | Application Number                                |       | Filing Date |       |        |       |        |
|                                                                                                                                         |          |        |                       |        |                        |        | 16900610                                          |       |             |       |        |       |        |
|                                                                                                                                         |          |        |                       |        |                        |        | Applicant(s) Varsha Iyer                          |       |             |       |        |       |        |
|                                                                                                                                         |          |        |                       |        |                        |        | * May be used for additional claims or amendments |       |             |       |        |       |        |
| CLAIMS                                                                                                                                  | AS FILED |        | AFTER FIRST AMENDMENT |        | AFTER SECOND AMENDMENT |        |                                                   | *     |             | *     |        | *     |        |
|                                                                                                                                         | Indep    | Depend | Indep                 | Depend | Indep                  | Depend |                                                   | Indep | Depend      | Indep | Depend | Indep | Depend |
| 1                                                                                                                                       | 1        |        |                       |        |                        |        |                                                   | 51    |             |       |        |       |        |
| 2                                                                                                                                       | 1        |        |                       |        |                        |        |                                                   | 52    |             |       |        |       |        |
| 3                                                                                                                                       | 1        |        |                       |        |                        |        |                                                   | 53    |             |       |        |       |        |
| 4                                                                                                                                       |          | 3      |                       |        |                        |        |                                                   | 54    |             |       |        |       |        |
| 5                                                                                                                                       |          | 3      |                       |        |                        |        |                                                   | 55    |             |       |        |       |        |
| 6                                                                                                                                       | 1        |        |                       |        |                        |        |                                                   | 56    |             |       |        |       |        |
| 7                                                                                                                                       | 1        |        |                       |        |                        |        |                                                   | 57    |             |       |        |       |        |
| 8                                                                                                                                       | 1        |        |                       |        |                        |        |                                                   | 58    |             |       |        |       |        |
| 9                                                                                                                                       |          | 3      |                       |        |                        |        |                                                   | 59    |             |       |        |       |        |
| 10                                                                                                                                      |          | 3      |                       |        |                        |        |                                                   | 60    |             |       |        |       |        |
| 11                                                                                                                                      |          | ( 1 )  |                       |        |                        |        |                                                   | 61    |             |       |        |       |        |
| 12                                                                                                                                      |          | ( 1 )  |                       |        |                        |        |                                                   | 62    |             |       |        |       |        |
| 13                                                                                                                                      |          | ( 1 )  |                       |        |                        |        |                                                   | 63    |             |       |        |       |        |
| 14                                                                                                                                      | 1        |        |                       |        |                        |        |                                                   | 64    |             |       |        |       |        |
| 15                                                                                                                                      | 1        |        |                       |        |                        |        |                                                   | 65    |             |       |        |       |        |
| 16                                                                                                                                      | 1        |        |                       |        |                        |        |                                                   | 66    |             |       |        |       |        |
| 17                                                                                                                                      |          | 3      |                       |        |                        |        |                                                   | 67    |             |       |        |       |        |
| 18                                                                                                                                      | 1        |        |                       |        |                        |        |                                                   | 68    |             |       |        |       |        |
| 19                                                                                                                                      | 1        |        |                       |        |                        |        |                                                   | 69    |             |       |        |       |        |
| 20                                                                                                                                      | 1        |        |                       |        |                        |        |                                                   | 70    |             |       |        |       |        |
| 21                                                                                                                                      |          | 3      |                       |        |                        |        |                                                   | 71    |             |       |        |       |        |
| 22                                                                                                                                      |          | ( 1 )  |                       |        |                        |        |                                                   | 72    |             |       |        |       |        |
| 23                                                                                                                                      |          | ( 1 )  |                       |        |                        |        |                                                   | 73    |             |       |        |       |        |
| 24                                                                                                                                      |          | ( 1 )  |                       |        |                        |        |                                                   | 74    |             |       |        |       |        |
| 25                                                                                                                                      |          | ( 1 )  |                       |        |                        |        |                                                   | 75    |             |       |        |       |        |
| 26                                                                                                                                      |          | ( 1 )  |                       |        |                        |        |                                                   | 76    |             |       |        |       |        |
| 27                                                                                                                                      |          | ( 1 )  |                       |        |                        |        |                                                   | 77    |             |       |        |       |        |
| 28                                                                                                                                      |          | ( 1 )  |                       |        |                        |        |                                                   | 78    |             |       |        |       |        |
| 29                                                                                                                                      |          | ( 1 )  |                       |        |                        |        |                                                   | 79    |             |       |        |       |        |
| 30                                                                                                                                      |          | ( 1 )  |                       |        |                        |        |                                                   | 80    |             |       |        |       |        |
| 31                                                                                                                                      |          | ( 1 )  |                       |        |                        |        |                                                   | 81    |             |       |        |       |        |
| 32                                                                                                                                      |          | ( 1 )  |                       |        |                        |        |                                                   | 82    |             |       |        |       |        |
| 33                                                                                                                                      |          | ( 1 )  |                       |        |                        |        |                                                   | 83    |             |       |        |       |        |
| 34                                                                                                                                      |          | ( 1 )  |                       |        |                        |        |                                                   | 84    |             |       |        |       |        |
| 35                                                                                                                                      |          | ( 1 )  |                       |        |                        |        |                                                   | 85    |             |       |        |       |        |
| 36                                                                                                                                      |          | ( 1 )  |                       |        |                        |        |                                                   | 86    |             |       |        |       |        |
| 37                                                                                                                                      |          | ( 1 )  |                       |        |                        |        |                                                   | 87    |             |       |        |       |        |
| 38                                                                                                                                      |          | ( 1 )  |                       |        |                        |        |                                                   | 88    |             |       |        |       |        |
| 39                                                                                                                                      |          | ( 1 )  |                       |        |                        |        |                                                   | 89    |             |       |        |       |        |
| 40                                                                                                                                      |          | ( 1 )  |                       |        |                        |        |                                                   | 90    |             |       |        |       |        |
| 41                                                                                                                                      |          | ( 1 )  |                       |        |                        |        |                                                   | 91    |             |       |        |       |        |
| 42                                                                                                                                      |          | ( 1 )  |                       |        |                        |        |                                                   | 92    |             |       |        |       |        |
| 43                                                                                                                                      |          | ( 1 )  |                       |        |                        |        |                                                   | 93    |             |       |        |       |        |
| 44                                                                                                                                      |          | ( 1 )  |                       |        |                        |        |                                                   | 94    |             |       |        |       |        |
| 45                                                                                                                                      |          | ( 1 )  |                       |        |                        |        |                                                   | 95    |             |       |        |       |        |
| 46                                                                                                                                      |          |        |                       |        |                        |        |                                                   | 96    |             |       |        |       |        |
| 47                                                                                                                                      |          |        |                       |        |                        |        |                                                   | 97    |             |       |        |       |        |
| 48                                                                                                                                      |          |        |                       |        |                        |        |                                                   | 98    |             |       |        |       |        |
| 49                                                                                                                                      |          |        |                       |        |                        |        |                                                   | 99    |             |       |        |       |        |
| 50                                                                                                                                      |          |        |                       |        |                        |        |                                                   | 100   |             |       |        |       |        |
| Total Indep                                                                                                                             | 12       |        | 0                     |        | 0                      |        |                                                   |       |             |       |        |       |        |
| Total Depend                                                                                                                            | 45       | ↙      | 0                     | ↙      | 0                      | ↙      |                                                   |       |             |       |        |       |        |
| Total Claims                                                                                                                            | 57       |        | 0                     |        | 0                      |        |                                                   |       |             |       |        |       |        |

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**Patent Export, 2026-05-27 03:46:46 +0000**

Search results for: PNS=(US11878049);

Collections searched: DWPI; ARIPO grant; Argentina UM; Argentina app; Armenia grant; Australia UM; Australia app; Australia grant; Austria UM; Austria app; Austria grant; Belarus UM; Belarus grant; Belgium app; Belgium grant; Brazil UM; Brazil app; Brazil grant; Bulgaria UM; Bulgaria app; Bulgaria grant; Canada app; Canada grant; China, Mainland UM; China, Mainland app; China, Mainland grant; Colombia app; Costa Rica UM; Costa Rica app; Croatia UM; Croatia app; Croatia grant; Cuba app; Cuba grant; Czech Republic UM; Czech Republic app; Czech Republic grant; Czechoslovakia grant; Denmark UM; Denmark app; Denmark grant; Estonia UM; Estonia app; Estonia grant; Eurasian (EAPO) grant; Europe (EP) app; Europe (EP) grant; Finland app; Finland grant; France app; France grant; Georgia grant; German Democratic Republic app; German Democratic Republic grant; Germany UM; Germany app; Germany grant; Greece app; Greece grant; Gulf Cooperation Council grant; Hong Kong UM; Hong Kong app; Hong Kong grant; Hungary grant; Iceland grant; India app; India grant; Indonesia UM; Indonesia app; Ireland UM; Ireland app; Ireland grant; Israel grant; Italy grant; Italy grant; Italy grant; Italy grant; Japan UM; Japan app; Japan grant; Korea UM; Korea app; Korea grant; Latvia grant; Lithuania grant; Luxembourg app; Luxembourg grant; Malaysia app; Malaysia grant; Mexico app; Mexico grant; Moldova UM; Moldova grant; Monaco grant; Mongolia UM; Mongolia grant; Morocco app; Morocco grant; Netherlands app; Netherlands grant; New Zealand grant; Norway app; Norway grant; OAPI grant; PCT (WO) app; Philippines UM; Philippines app; Philippines grant; Poland UM; Poland grant; Portugal UM; Portugal app; Portugal grant; Romania UM; Romania app; Romania granted; Russia UM; Russia app; Russia grant; Saudi Arabia app; Saudi Arabia grant; Serbia UM; Serbia app; Serbia grant; Singapore app; Singapore grant; Slovakia UM; Slovakia app; Slovakia grant; Slovenia UM; Slovenia grant; South Africa app; Spain UM; Spain app; Spain grant; Sweden app; Sweden grant; Switzerland app; Switzerland grant; Taiwan UM; Taiwan appl; Taiwan grant; Thailand UM; Thailand app; Thailand grant; Tunisia app; Turkey UM; Turkey app; UK app; UK grant; USSR grant; Ukraine UM; Ukraine grant; United States app; United States grant; Uruguay app; Vietnam UM; Vietnam app; Vietnam grant; Other

**Table of Contents**

- 
1. US11878049B1    Mitapivat therapy and modulators of cytochrome P450
-

Family 1/1  
1 record(s) per family

Record 1/1 US11878049B1 Mitapivat therapy and modulators of cytochrome P450

**Title:** Mitapivat therapy and modulators of cytochrome P450  
**Title - DWPI:** Treating hemolytic anemia, sickle cell disease, pyruvate kinase deficiency, or thalassemia in subject, involves administering specific amount of N-(4-(4-(cyclopropylmethyl)piperazine-1-carbonyl)phenyl) quinoline-8-sulfonamide or its salt and moderate inducer of cytochrome P450 3A4/5 to subject  
**Priority Number:** US62861600P  
**Priority Number - DWPI:** US2020900610A | US2019861600P  
**Priority Date:** 2019-06-14  
**Priority Date - DWPI:** 2020-06-12 | 2019-06-14  
**Application Number:** US16900610A  
**Application Date:** 2020-06-12  
**Publication Date:** 2024-01-23  
**IPC Class Table:**

| IPC        | Section | Class | Subclass | Class Group | Subgroup   |
|------------|---------|-------|----------|-------------|------------|
| A61K003845 | A       | A61   | A61K     | A61K0038    | A61K003845 |
| A61K004506 | A       | A61   | A61K     | A61K0045    | A61K004506 |

IPC Class Table - DWPI:

| IPC - DWPI | Section - DWPI | Class - DWPI | Subclass - DWPI | Class Group - DWPI | Subgroup - DWPI |
|------------|----------------|--------------|-----------------|--------------------|-----------------|
| A61K003845 | A              | A61          | A61K            | A61K0038           | A61K003845      |
| A61K004506 | A              | A61          | A61K            | A61K0045           | A61K004506      |

**Assignee/Applicant:** Agios Pharmaceuticals Inc.,Cambridge,MA,US  
**Assignee - Current US:** AGIOS PHARMACEUTICALS INC.  
**JP F Terms:**  
**JP FI Codes:**  
**Item Number:** 1

**Assignee - Original:** Agios Pharmaceuticals Inc.

**Any CPC Table:**

| Type                 | CPC - Current -<br>Invention | CPC - Current -<br>Additional | Version              | Office           |
|----------------------|------------------------------|-------------------------------|----------------------|------------------|
| Current              | A61K 38/45                   | -                             | 20130101             | US               |
| Current              | A61K 31/496                  | -                             | 20130101             | EP               |
| Current<br>Current   | -                            | A61K 45/06<br>C12Y 207/0104   | 20130101<br>20130101 | EP; US<br>EP; US |
| Original<br>Original | A61K 38/45                   | A61K 45/06<br>C12Y 207/0104   | 20130101<br>20130101 | US<br>US         |

**ECLA:**

**Abstract:**

Described herein are methods of treating pyruvate kinase deficiency (PKD), sickle cell disease or thalassemia with mitapivat or a pharmaceutically acceptable salt thereof, or use of the drug for the treatment of these conditions, in combination with or in the absence of with a secondary drug, such as an inducer or an inhibitor of cytochrome P450. Various doses and dosing regimens of mitapivat in monotherapy and in concomitant medications are described.

**Language of Publication:** EN

**INPADOC Legal Status Table:**

| Gazette Date                                                                                                                                         | Code | INPADOC Legal Status Impact |
|------------------------------------------------------------------------------------------------------------------------------------------------------|------|-----------------------------|
| 2024-02-27                                                                                                                                           | CC   | -                           |
| <b>Description:</b> CERTIFICATE OF CORRECTION                                                                                                        |      |                             |
| 2024-01-03                                                                                                                                           | STCF | -                           |
| <b>Description:</b> INFORMATION ON STATUS: PATENT GRANT PATENTED CASE                                                                                |      |                             |
| 2020-06-12                                                                                                                                           | FEPP | -                           |
| <b>Description:</b> FEE PAYMENT PROCEDURE ENTITY STATUS SET TO UNDISCOUNTED (ORIGINAL EVENT CODE: BIG.); ENTITY STATUS OF PATENT OWNER: LARGE ENTITY |      |                             |

**Post-Issuance (US):** CORR-CERT Certificate of Correction 2024-02-27 2024 2024-03-12 2024 A

Certificate of Correction was issued for this patent

Reassignment (US) Table:

| Assignee                                                                         | Assignor                      | Date Signed | Reel/Frame  | Date       |
|----------------------------------------------------------------------------------|-------------------------------|-------------|-------------|------------|
| AGIOS PHARMACEUTICALS INC.,CAMBRIDGE,MA,US                                       | IYER, VARSHA<br>VENKATACHALAM | 2022-10-05  | 061931/0718 | 2022-11-11 |
| Conveyance: ASSIGNMENT OF ASSIGNORS INTEREST (SEE DOCUMENT FOR DETAILS).         |                               |             |             |            |
| Corresponent: MCCARTER & ENGLISH LLP BOSTON 265 FRANKLIN STREET BOSTON, MA 02110 |                               |             |             |            |
| AGIOS PHARMACEUTICALS INC.,CAMBRIDGE,MA,US                                       | IYER, VARSHA<br>VENKATACHALAM | 2020-08-27  | 054079/0224 | 2020-10-16 |
|                                                                                  | PRAKASH, CHANDRA<br>AGARWAL   | 2020-09-11  | -           | -          |
|                                                                                  | YANG, HUA                     | 2020-07-31  | -           | -          |
| Conveyance: ASSIGNMENT OF ASSIGNORS INTEREST (SEE DOCUMENT FOR DETAILS).         |                               |             |             |            |
| Corresponent: MCCARTER & ENGLISH, LLP 265 FRANKLIN STREET BOSTON, MA 02110       |                               |             |             |            |

Maintenance Status (US): CC

Litigation (US):

Opposition (EP):

License (EP):

EPO Procedural Status:

Front Page Drawing:

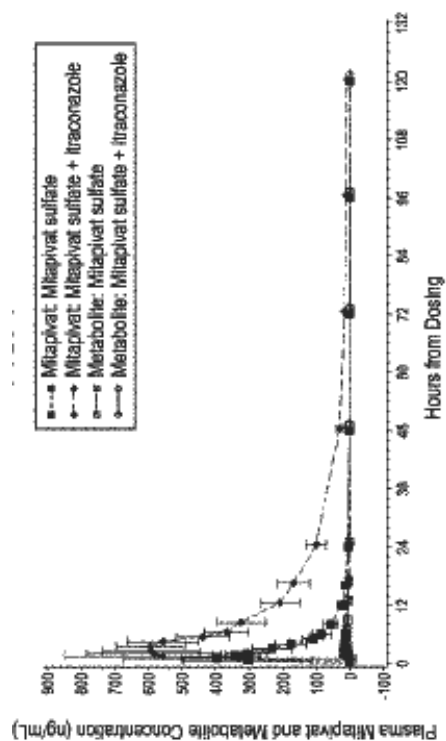


FIG. 1



United States Patent and Trademark Office

Office of the Commissioner for Patents

MITAPIVAT THERAPY AND MODULATORS OF CYTOCHROME P450

PATENT #

11878049

APPLICATION #

16900610

FILING DATE

06/12/2020

ISSUE DATE

01/23/2024

Payment Window Status

| WINDOW    |                  | STATUS           |                 | FEES     |         |
|-----------|------------------|------------------|-----------------|----------|---------|
| 3.5 Year  |                  | Not Open         |                 | Not Due  |         |
| Window    | First Day to Pay | Surcharge Starts | Last Day to Pay | Status   | Fees    |
| 3.5 Year  | 01/23/2027       | 07/24/2027       | 01/24/2028      | Not Open | Not Due |
| 7.5 Year  | 01/23/2031       | 07/24/2031       | 01/23/2032      | Not Open | Not Due |
| 11.5 Year | 01/23/2035       | 07/24/2035       | 01/23/2036      | Not Open | Not Due |

No maintenance fees are due at this time. 3.5 year window opens on 01/23/2027.

Patent Holder Information

Customer #

190109

Entity Status

UNDISCOUNTED

Phone Number

6174496500

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