PTO/AIA/15 (10-17)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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UTILITY PATENT APPLICATION TRANSMITTAL

(Only for new nonprovisional applications under 37 CFR 1.53(b))

Attorney Docket No.	128748-01502
First Named Inventor	Varsha Venkatachalam Iyer
Title	MITAPIVAT THERAPY AND MODULATORS OF CYTOCHROME P450
Priority Mail Express® Label No.	

APPLICATION ELEMENTS

See MPEP chapter 600 concerning utility patent application contents.

1. [ ] **Fee Transmittal Form** (PTO/SB/17 or equivalent)
2. [ ] **Applicant asserts small entity status.** See 37 CFR 1.27
3. [ ] **Applicant certifies micro entity status.** See 37 CFR 1.29. Applicant must attach form PTO/SB/15A or B or equivalent.
4. [X] **Specification** [*Total Pages* 70]
   Both the claims and abstract must start on a new page.
   (See MPEP § 608.01(a) for information on the preferred arrangement)
5. [X] **Drawing(s)** (35 U.S.C. 113) [*Total Sheets* 4]
6. **Inventor's Oath or Declaration** [*Total Pages*]
   (including substitute statements under 37 CFR 1.64 and assignments serving as an oath or declaration under 37 CFR 1.63(e))
   a. [ ] Newly executed (original or copy)
   b. [ ] A copy from a prior application (37 CFR 1.63(d))
7. [X] **Application Data Sheet** ** See note below.*
   See 37 CFR 1.76 (PTO/AIA/14 or equivalent)
8. **CD-ROM or CD-R** in duplicate, large table, or Computer Program (*Appendix*)
   [ ] Landscape Table on CD
9. **Nucleotide and/or Amino Acid Sequence Submission** (*if applicable, items a. – c. are required*)
   a. [ ] Computer Readable Form (CRF)
   b. [ ] Specification Sequence Listing on:
      i. [ ] CD-ROM or CD-R (2 copies); or
      ii. [ ] Paper
   c. [ ] Statements verifying identity of above copies

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

ACCOMPANYING APPLICATION PAPERS

10. [ ] **Assignment Papers** (cover sheet & document(s))
    Name of Assignee
11. [ ] **37 CFR 3.73(c) Statement** (*when there is an assignee*) [ ] **Power of Attorney**
12. [ ] **English Translation Document** (*if applicable*)
13. [ ] **Information Disclosure Statement** (PTO/SB/08 or PTO-1449)
    [ ] Copies of citations attached
14. [ ] **Preliminary Amendment**
15. [ ] **Return Receipt Postcard** (*MPEP § 503*) (*Should be specifically itemized*)
16. [ ] **Certified Copy of Priority Document(s)** (*if foreign priority is claimed*)
17. [X] **Nonpublication Request** Under 35 U.S.C. 122(b)(2)(B)(i). Applicant must attach form PTO/SB/35 or equivalent.
18. [ ] **Other:**

***Note:** (1) Benefit claims under 37 CFR 1.78 and foreign priority claims under 1.55 **must** be included in an Application Data Sheet (ADS).
(2) For applications filed under 35 U.S.C. 111, the application must contain an ADS specifying the applicant if the applicant is an assignee, person to whom the inventor is under an obligation to assign, or person who otherwise shows sufficient proprietary interest in the matter. See 37 CFR 1.46(b).

19. CORRESPONDENCE ADDRESS

[X] The address associated with Customer Number: 86738 **OR** [ ] Correspondence address below

Name					
Address					
City		State		Zip Code	
Country		Telephone		Email	

Signature	/Steven G. Davis/	Date	June 12, 2020
Name (Print/Type)	Steven G. Davis, Ph.D.	Registration No. (Attorney/Agent)	39,652

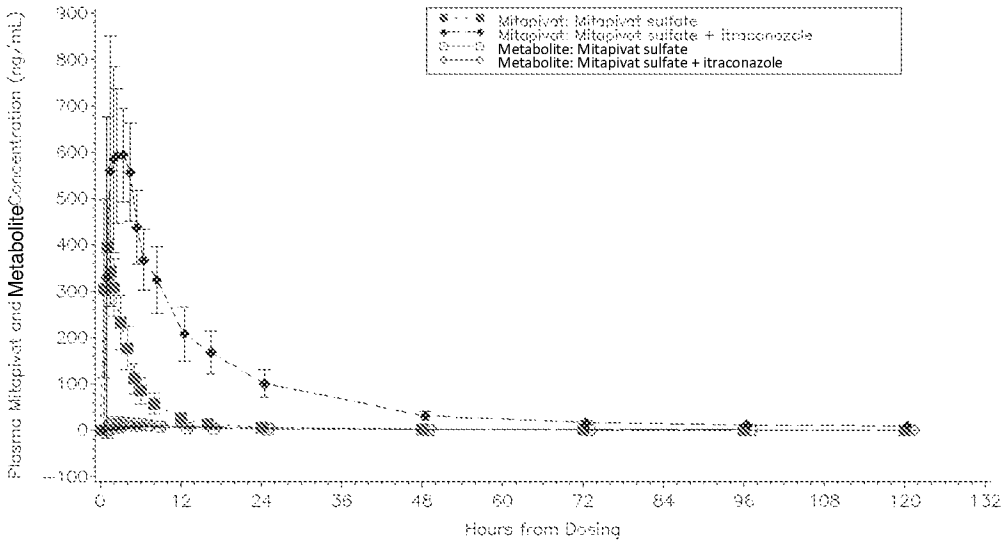

Mitapivat: Mitapivat sulfate
Mitapivat: Mitapivat sulfate + itraconazole
Metabolite: Mitapivat sulfate
Metabolite: Mitapivat sulfate + itraconazole
Plasma Mitapivat and Metabolite Concentration (ng/mL)
900
800
700
600
500
400
300
200
100
0
-100
0
12
24
36
48
60
72
84
96
108
120
132
Hours from Dosing

Figure 1

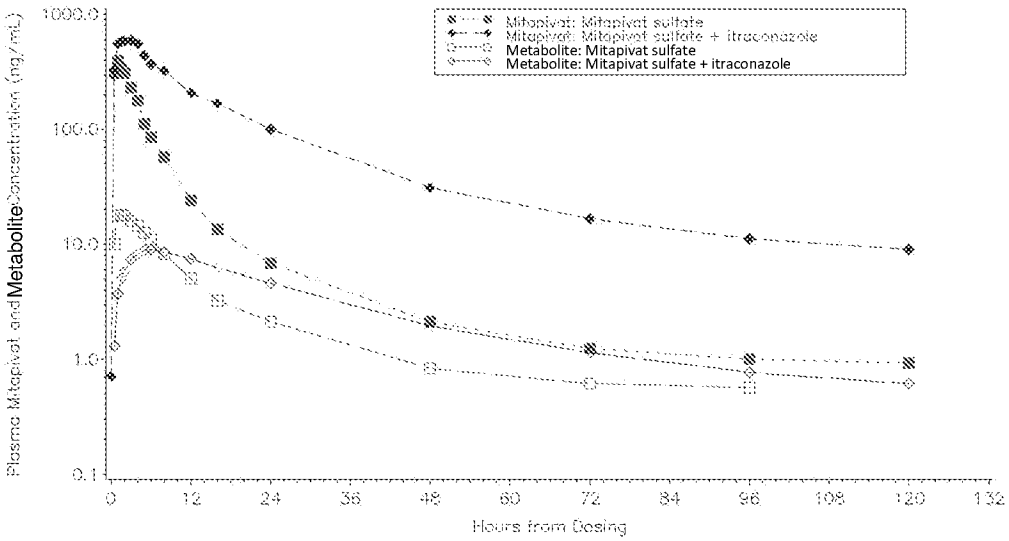

Mitapivat: Mitapivat sulfate
Mitapivat: Mitapivat sulfate + itraconazole
Metabolite: Mitapivat sulfate
Metabolite: Mitapivat sulfate + itraconazole
Plasma Mitapivat and Metabolite Concentration (ng/mL)
1000.0
100.0
10.0
1.0
0.1
0
12
24
36
48
60
72
84
96
108
120
132
Hours from Dosing

Figure 2

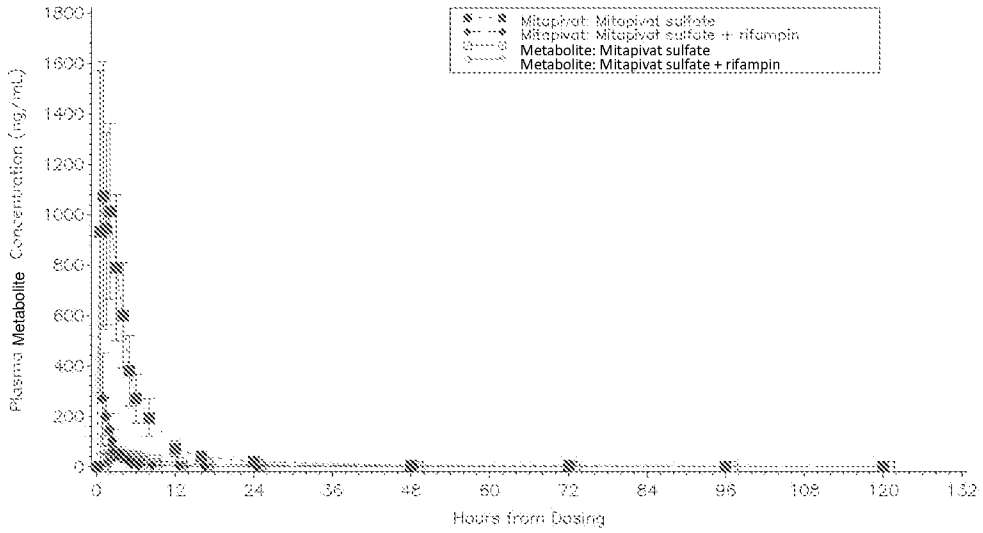

Mitapivat: Mitapivat sulfate
Mitapivat: Mitapivat sulfate + rifampin
Metabolite: Mitapivat sulfate
Metabolite: Mitapivat sulfate + rifampin
Plasma Metabolite Concentration (ng/mL)
1800
1600
1400
1200
1000
800
600
400
200
0
0
12
24
36
48
60
72
84
96
108
120
132
Hours from Dosing

Figure 3

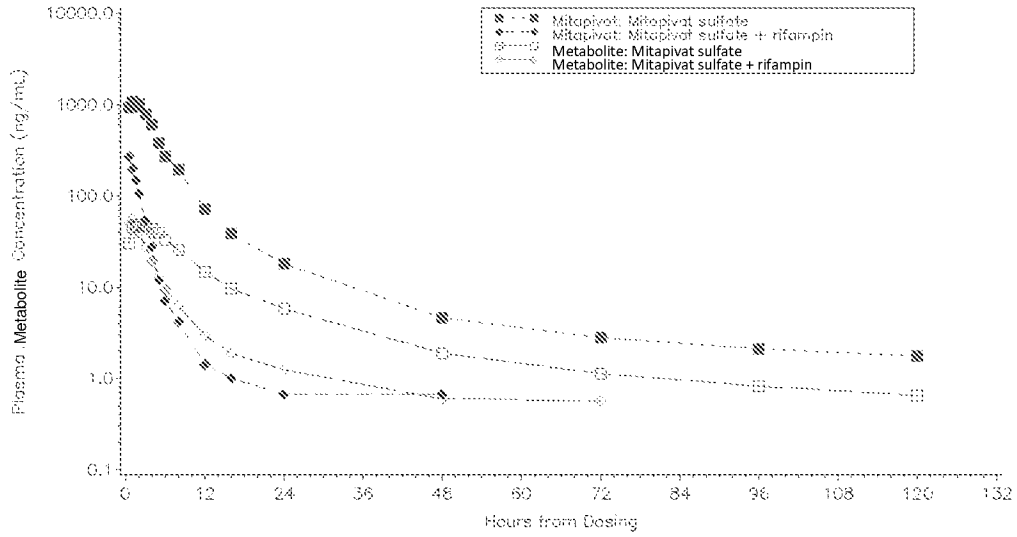

Mitapivat: Mitapivat sulfate
Mitapivat: Mitapivat sulfate + rifampin
Metabolite: Mitapivat sulfate
Metabolite: Mitapivat sulfate + rifampin
Plasma Metabolite Concentration (ng/mL)
10000.0
1000.0
100.0
10.0
1.0
0.1
0
12
24
36
48
60
72
84
96
108
120
132
Hours from Dosing

Figure 4

Electronic Patent Application Fee Transmittal

Application Number:	
Filing Date:	
Title of Invention:	MITAPIVAT THERAPY AND MODULATORS OF CYTOCHROME P450
First Named Inventor/Applicant Name:	Varsha Venkatachalam Iyer
Filer:	Steven G. Davis/Erin Shea
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:				
UTILITY APPLICATION FILING	1011	1	300	300
UTILITY SEARCH FEE	1111	1	660	660
UTILITY EXAMINATION FEE	1311	1	760	760
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				
Miscellaneous:				
		Total in USD ($)		**1720**

Electronic Acknowledgement Receipt

EFS ID:	39709998
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:	86738
Filer:	Steven G. Davis
Filer Authorized By:	
Attorney Docket Number:	128748-01502
Receipt Date:	12-JUN-2020
Filing Date:	
Time Stamp:	18:23:19
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	$1720
RAM confirmation Number	E20206BI23490036
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Application Data Sheet	128748-01502_-_Application_Data_Sheet_Fillable_PDF.pdf	1255683 50404fac34bcaf473e2152102a58aa24e989cadb	no	9
Warnings:					
Information:					
2	Nonpublication request from applicant	Nonpublication_Request.pdf	17330 639a8ba90447c12ffe8c2572751b550b7a5f8e33	no	1
Warnings:					
Information:					
3	Transmittal of New Application	Utility_Patent_Application_Transmittal.pdf	32100 91e7743ee623b3e3f56304c7a76a66338ae9bbe2	no	1
Warnings:					
Information:					
4	Specification	06122020_SPECIFICATION_.pdf	268736 3891c6d042fffd63732040701fd44ba810171573	no	70
Warnings:					
Information:					
5	Drawings-only black and white line drawings	06122020_FIGURES_FOR_FILING.pdf	745862 e8526be9c56c2aab29f775b0d5ae03c6490aea8c	no	4
Warnings:					
Information:					
6	Fee Worksheet (SB06)	fee-info.pdf	34922 b74e49cf202e9c61d17b902eebe0cd2ccf4d32a9	no	2
Warnings:					
Information:					

Total Files Size (in bytes):	2354633

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.

New Applications Under 35 U.S.C. 111

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.

National Stage of an International Application under 35 U.S.C. 371

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.

New International Application Filed with the USPTO as a Receiving Office

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.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	128748-01502
	Application Number	
Title of Invention	MITAPIVAT THERAPY AND MODULATORS OF CYTOCHROME P450	

The application data sheet is part of the provisional or nonprovisional application for which it is being submitted. The following form contains the bibliographic data arranged in a format specified by the United States Patent and Trademark Office as outlined in 37 CFR 1.76.
This document may be completed electronically and submitted to the Office in electronic format using the Electronic Filing System (EFS) or the document may be printed and included in a paper filed application.

Secrecy Order 37 CFR 5.2:

☐ Portions or all of the application associated with this Application Data Sheet may fall under a Secrecy Order pursuant to 37 CFR 5.2 (Paper filers only. Applications that fall under Secrecy Order may not be filed electronically.)

Inventor Information:

Inventor 1 — Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Varsha	Venkatachalam	Iyer	

Residence Information (Select One) ● US Residency Non US Residency Active US Military Service

City	State/Province	Country of Residence
Cambridge	MA	US

Mailing Address of Inventor:

Address 1	88 Sidney Street
Address 2	

City	State/Province	Postal Code	Country
Cambridge	MA	02139	US

Inventor 2 — Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Chandra	Agarwal	Prakash	

Residence Information (Select One) ◉ US Residency Non US Residency Active US Military Service

City	State/Province	Country of Residence
North Andover	MA	US

Mailing Address of Inventor:

Address 1	88 Sidney Street
Address 2	

City	State/Province	Postal Code	Country
Cambridge	MA	02139	US

Inventor 3 — Remove

Legal Name

Prefix	Given Name	Middle Name	Family Name	Suffix
	Hua		Yang	

Residence Information (Select One) ◉ US Residency Non US Residency Active US Military Service

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	128748-01502
	Application Number	
Title of Invention	MITAPIVAT THERAPY AND MODULATORS OF CYTOCHROME P450	

City	Acton	State/Province	MA	Country of Residence	US

Mailing Address of Inventor:

Address 1	88 Sidney Street		
Address 2			
City	Cambridge	**State/Province**	MA
Postal Code	02139	**Country**	US

All Inventors Must Be Listed - Additional Inventor Information blocks may be generated within this form by selecting the **Add** button. Add

Correspondence Information:

Enter either Customer Number or complete the Correspondence Information section below.
For further information see 37 CFR 1.33(a).

☐ **An Address is being provided for the correspondence Information of this application.**

Customer Number	86738		
Email Address	docket@mccarter.com	Add Email	Remove Email

Application Information:

Title of the Invention	MITAPIVAT THERAPY AND MODULATORS OF CYTOCHROME P450		
Attorney Docket Number	128748-01502	**Small Entity Status Claimed**	☐
Application Type	Nonprovisional		
Subject Matter	Utility		
Total Number of Drawing Sheets (if any)	4	**Suggested Figure for Publication (if any)**	

Filing By Reference:

Only complete this section when filing an application by reference under 35 U.S.C. 111(c) and 37 CFR 1.57(a). Do not complete this section if application papers including a specification and any drawings are being filed. Any domestic benefit or foreign priority information must be provided in the appropriate section(s) below (i.e., "Domestic Benefit/National Stage Information" and "Foreign Priority Information").

For the purposes of a filing date under 37 CFR 1.53(b), the description and any drawings of the present application are replaced by this reference to the previously filed application, subject to conditions and requirements of 37 CFR 1.57(a).

Application number of the previously filed application	Filing date (YYYY-MM-DD)	Intellectual Property Authority or Country

Publication Information:

☐ Request Early Publication (Fee required at time of Request 37 CFR 1.219)

☒ **Request Not to Publish.** I hereby request that the attached application not be published under 35 U.S.C. 122(b) and certify that the invention disclosed in the attached application **has not and will not** be the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	128748-01502
	Application Number	
Title of Invention	MITAPIVAT THERAPY AND MODULATORS OF CYTOCHROME P450	

Representative Information:

Representative information should be provided for all practitioners having a power of attorney in the application. Providing this information in the Application Data Sheet does not constitute a power of attorney in the application (see 37 CFR 1.32).
Either enter Customer Number or complete the Representative Name section below. If both sections are completed the customer Number will be used for the Representative Information during processing.

Please Select One:	● Customer Number	US Patent Practitioner	○ Limited Recognition (37 CFR 11.9)
Customer Number	86738		

Domestic Benefit/National Stage Information:

This section allows for the applicant to either claim benefit under 35 U.S.C. 119(e), 120, 121, 365(c), or 386(c) or indicate National Stage entry from a PCT application. Providing benefit claim information in the Application Data Sheet constitutes the specific reference required by 35 U.S.C. 119(e) or 120, and 37 CFR 1.78.
When referring to the current application, please leave the "Application Number" field blank.

Prior Application Status	Pending		Remove
Application Number	Continuity Type	Prior Application Number	Filing or 371(c) Date (YYYY-MM-DD)
	Claims benefit of provisional	62/861600	2019-06-14

Additional Domestic Benefit/National Stage Data may be generated within this form by selecting the **Add** button. Add

Foreign Priority Information:

This section allows for the applicant to claim priority to a foreign application. Providing this information in the application data sheet constitutes the claim for priority as required by 35 U.S.C. 119(b) and 37 CFR 1.55. When priority is claimed to a foreign application that is eligible for retrieval under the priority document exchange program (PDX)[i] the information will be used by the Office to automatically attempt retrieval pursuant to 37 CFR 1.55(i)(1) and (2). Under the PDX program, applicant bears the ultimate responsibility for ensuring that a copy of the foreign application is received by the Office from the participating foreign intellectual property office, or a certified copy of the foreign priority application is filed, within the time period specified in 37 CFR 1.55(g)(1).

Remove

Application Number	Country[i]	Filing Date (YYYY-MM-DD)	Access Code[i] (if applicable)

Additional Foreign Priority Data may be generated within this form by selecting the **Add** button. Add

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	128748-01502
	Application Number	
Title of Invention	MITAPIVAT THERAPY AND MODULATORS OF CYTOCHROME P450	

Statement under 37 CFR 1.55 or 1.78 for AIA (First Inventor to File) Transition Applications

☐ This application (1) claims priority to or the benefit of an application filed before March 16, 2013 and (2) also contains, or contained at any time, a claim to a claimed invention that has an effective filing date on or after March 16, 2013.
NOTE: By providing this statement under 37 CFR 1.55 or 1.78, this application, with a filing date on or after March 16, 2013, will be examined under the first inventor to file provisions of the AIA.

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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	128748-01502
	Application Number	
Title of Invention	MITAPIVAT THERAPY AND MODULATORS OF CYTOCHROME P450	

Authorization or Opt-Out of Authorization to Permit Access:

When this Application Data Sheet is properly signed and filed with the application, applicant has provided written authority to permit a participating foreign intellectual property (IP) office access to the instant application-as-filed (see paragraph A in subsection 1 below) and the European Patent Office (EPO) access to any search results from the instant application (see paragraph B in subsection 1 below).

Should applicant choose not to provide an authorization identified in subsection 1 below, applicant **must opt-out** of the authorization by checking the corresponding box A or B or both in subsection 2 below.

NOTE: This section of the Application Data Sheet is **ONLY** reviewed and processed with the **INITIAL** filing of an application. After the initial filing of an application, an Application Data Sheet cannot be used to provide or rescind authorization for access by a foreign IP office(s). Instead, Form PTO/SB/39 or PTO/SB/69 must be used as appropriate.

1. Authorization to Permit Access by a Foreign Intellectual Property Office(s)

A. Priority Document Exchange (PDX) - Unless box A in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the European Patent Office (EPO), the Japan Patent Office (JPO), the Korean Intellectual Property Office (KIPO), the State Intellectual Property Office of the People's Republic of China (SIPO), the World Intellectual Property Organization (WIPO), and any other foreign intellectual property office participating with the USPTO in a bilateral or multilateral priority document exchange agreement in which a foreign application claiming priority to the instant patent application is filed, access to: (1) the instant patent application-as-filed and its related bibliographic data, (2) any foreign or domestic application to which priority or benefit is claimed by the instant application and its related bibliographic data, and (3) the date of filing of this Authorization. See 37 CFR 1.14(h)(1).

B. Search Results from U.S. Application to EPO - Unless box B in subsection 2 (opt-out of authorization) is checked, the undersigned hereby **grants the USPTO authority** to provide the EPO access to the bibliographic data and search results from the instant patent application when a European patent application claiming priority to the instant patent application is filed. See 37 CFR 1.14(h)(2).

The applicant is reminded that the EPO's Rule 141(1) EPC (European Patent Convention) requires applicants to submit a copy of search results from the instant application without delay in a European patent application that claims priority to the instant application.

2. Opt-Out of Authorizations to Permit Access by a Foreign Intellectual Property Office(s)

☐ A. Applicant **DOES NOT** authorize the USPTO to permit a participating foreign IP office access to the instant application-as-filed. If this box is checked, the USPTO will not be providing a participating foreign IP office with any documents and information identified in subsection 1A above.

☐ B. Applicant **DOES NOT** authorize the USPTO to transmit to the EPO any search results from the instant patent application. If this box is checked, the USPTO will not be providing the EPO with search results from the instant application.

NOTE: Once the application has published or is otherwise publicly available, the USPTO may provide access to the application in accordance with 37 CFR 1.14.

PTO/AIA/14 (02-18)
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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	128748-01502
	Application Number	
Title of Invention	MITAPIVAT THERAPY AND MODULATORS OF CYTOCHROME P450	

Applicant Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

Applicant 1 — Remove

If the applicant is the inventor (or the remaining joint inventor or inventors under 37 CFR 1.45), this section should not be completed. The information to be provided in this section is the name and address of the legal representative who is the applicant under 37 CFR 1.43; or the name and address of the assignee, person to whom the inventor is under an obligation to assign the invention, or person who otherwise shows sufficient proprietary interest in the matter who is the applicant under 37 CFR 1.46. If the applicant is an applicant under 37 CFR 1.46 (assignee, person to whom the inventor is obligated to assign, or person who otherwise shows sufficient proprietary interest) together with one or more joint inventors, then the joint inventor or inventors who are also the applicant should be identified in this section.

Clear

● Assignee	Legal Representative under 35 U.S.C. 117	Joint Inventor
Person to whom the inventor is obligated to assign.	Person who shows sufficient proprietary interest	

If applicant is the legal representative, indicate the authority to file the patent application, the inventor is:

Name of the Deceased or Legally Incapacitated Inventor:

If the Applicant is an Organization check here. ☒

Organization Name	Agios Pharmaceuticals, Inc.		
Mailing Address Information For Applicant:			
Address 1	88 Sidney Street		
Address 2			
City	Cambridge	**State/Province**	MA
Country	US	Postal Code	02139
Phone Number		Fax Number	
Email Address			

Additional Applicant Data may be generated within this form by selecting the Add button. Add

Assignee Information including Non-Applicant Assignee Information:

Providing assignment information in this section does not substitute for compliance with any requirement of part 3 of Title 37 of CFR to have an assignment recorded by the Office.

PTO/AIA/14 (02-18)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	128748-01502
	Application Number	
Title of Invention	MITAPIVAT THERAPY AND MODULATORS OF CYTOCHROME P450	

Assignee 1

Complete this section if assignee information, including non-applicant assignee information, is desired to be included on the patent application publication. An assignee-applicant identified in the "Applicant Information" section will appear on the patent application publication as an applicant. For an assignee-applicant, complete this section only if identification as an assignee is also desired on the patent application publication.

Remove

If the Assignee or Non-Applicant Assignee is an Organization check here. ☐

Prefix	**Given Name**	Middle Name	**Family Name**	Suffix

Mailing Address Information For Assignee including Non-Applicant Assignee:

Address 1			
Address 2			
City		**State/Province**	
Country i		Postal Code	
Phone Number		Fax Number	
Email Address			

Additional Assignee or Non-Applicant Assignee Data may be generated within this form by selecting the Add button. Add

Signature:

Remove

NOTE: This Application Data Sheet must be signed in accordance with 37 CFR 1.33(b). **However, if this Application Data Sheet is submitted with the INITIAL filing of the application and either box A or B is not checked in subsection 2 of the "Authorization or Opt-Out of Authorization to Permit Access" section, then this form must also be signed in accordance with 37 CFR 1.14(c).**

This Application Data Sheet **must** be signed by a patent practitioner if one or more of the applicants is a **juristic entity** (e.g., corporation or association). If the applicant is two or more joint inventors, this form must be signed by a patent practitioner, **all** joint inventors who are the applicant, or one or more joint inventor-applicants who have been given power of attorney (e.g., see USPTO Form PTO/AIA/81) on behalf of **all** joint inventor-applicants.

See 37 CFR 1.4(d) for the manner of making signatures and certifications.

Signature	/Steven G. Davis/			Date (YYYY-MM-DD)	2020-06-12
First Name	Steven	Last Name	Davis	Registration Number	39,652

Additional Signature may be generated within this form by selecting the Add button. Add

PTO/AIA/14 (02-18)
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Application Data Sheet 37 CFR 1.76	Attorney Docket Number	128748-01502
	Application Number	
Title of Invention	MITAPIVAT THERAPY AND MODULATORS OF CYTOCHROME P450	

This collection of information is required by 37 CFR 1.76. 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 23 minutes to complete, including gathering, preparing, and submitting the completed application data sheet 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.**

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PTO/SB/35 (07-09)
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NONPUBLICATION REQUEST UNDER 35 U.S.C. 122(b)(2)(B)(i)	First Named Inventor	Varsha Venkatachalam Iyer
	Title	MITAPIVAT THERAPY AND MODULATORS OF CYTOCHROME P450
	Attorney Docket Number	128748-01502

I hereby certify that the invention disclosed in the attached application **has not and will not be** the subject of an application filed in another country, or under a multilateral international agreement, that requires publication at eighteen months after filing.

I hereby request that the attached application not be published under 35 U.S.C. 122(b).

/Steven G. Davis/	June 12, 2020
Signature	Date
Steven G. Davis, Ph.D.	39,652
Typed or printed name	Registration Number, if applicable
(617) 449-6580	
Telephone Number	

This request must be signed in compliance with 37 CFR 1.33(b) and submitted with the application **upon filing.**

Applicant may rescind this nonpublication request at any time. If applicant rescinds a request that an application not be published under 35 U.S.C. 122(b), the application will be scheduled for publication at eighteen months from the earliest claimed filing date for which a benefit is claimed.

If applicant subsequently files an application directed to the invention disclosed in the attached application in another country, or under a multilateral international agreement, that requires publication of applications eighteen months after filing, the applicant **must** notify the United States Patent and Trademark Office of such filing within forty-five (45) days after the date of the filing of such foreign or international application. **Failure to do so will result in abandonment of this application (35 U.S.C. 122(b)(2)(B)(iii)).**

MITAPIVAT THERAPY AND MODULATORS OF CYTOCHROME P450

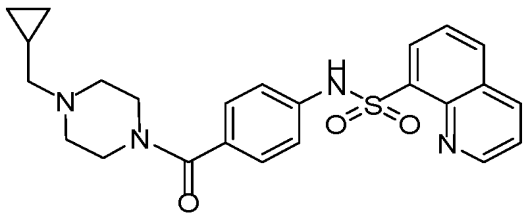
RELATED APPLICATIONS

This 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

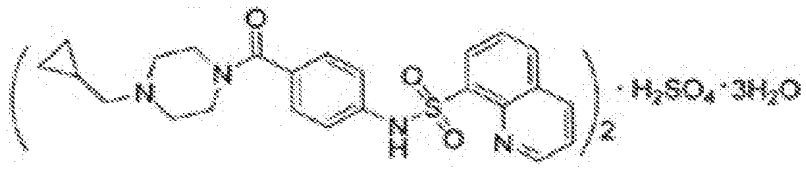
[0001] Pyruvate kinase deficiency (PKD) is a disease of the red blood cells caused by a deficiency of the pyruvate kinase R (PKR) enzyme due to recessive mutations of PKLR gene. PKR activators can be beneficial to treat PKD, thalassemia (*e.g.*, beta-thalassemia), abetalipoproteinemia or Bassen-Kornzweig 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. 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 hemylotic 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 hemylotic 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 thereof, a CYP3A4/5 inducer, or a CYP3A4/5 inhibitor.

[0022] An "effective amount" of mitavipat or a pharmaceutically acceptable 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 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 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 thereof means an amount of mitavipat or a pharmaceutically acceptable 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 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 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 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_2 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 ≥33% reduction in the number of RBC units transfused within at least 5 weeks of treatment. In certain embodiments, reduction of transfusion burden is ≥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 ±10%. In one embodiment, "about" means ±5%. When "about" is present before a series of numbers of number ranges, it is understood that the term can 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 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 equivalent to about 5 mg to about 200 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 5 mg to about 100 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 5 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 10 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 15 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 20 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 25 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 30 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 35 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 40 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 45 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 50 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 55 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 60 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 65 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 70 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 75 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 80 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 85 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 90 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 95 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 100 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt

thereof is formulated equivalent to about 110 mg of mitapivat. In certain embodiments, 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 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 thereofis 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 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 $AUC_{0\text{-}24}$, $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, $AUC_{\%estrap}$) and C_{max} 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 administrated 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.*, 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 here 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 embodiments, for the method of any one of the embodiments, the CYP3A4/5 inducer is a mild CYP3A4/5 inducer. In another embodiments, for the method of any one of the embodiments, the CYP3A4/5 inhibitor is a mild CYP3A4/5 inhibitor. In another embodiments, 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 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, armodafninil, 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) ≤12.0 g/dL (if male) or ≤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) ≤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 α- to β-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 β-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 α-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 β-thalassemia intermedia, Hb E β-thalassemia, α-thalassemia (Hb H disease), or β-thalassemia with mutations of 1 or more α 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 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.* 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-liproteinemia (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 bisphosphate (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 ^{13}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 6β-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.

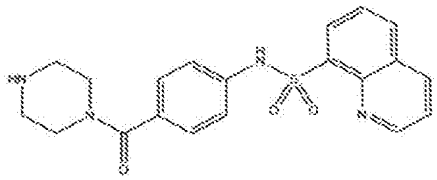
Example 2: Drug-drug Interaction (DDI) Studies of Mitapivat Sulfate and a CYP3A4 Inducer or a CYP3A4 Inhibitor

[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: $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, $AUC_{0\text{-}24}$, $AUC_{\%extrap}$, Cmax, CL/F (mitapivat only), Tlast, Tmax, t½, and Vz/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 t½) 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, $AUC_{0\text{-}t}$, and $AUC_{0\text{-}inf}$) 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_{0-t} (ng*hr/mL)	8860	14	1885	14	4.6998	4.2519 - 5.1949	15.05
AUC_{0-inf} (ng*hr/mL)	9475	14	1942	14	4.8780	4.4154 - 5.3890	14.97
C_{max} (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)
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)
Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the linear mixed effect model.
Geometric Mean Ratio (GMR) = test / reference
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_{0-t} and AUC_{0-inf} 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_{0-t} (ng*hr/mL)	300.0	14	197.1	14	1.5218	1.4268 - 1.6231	9.65
AUC_{0-inf} (ng*hr/mL)	338.6	14	216.2	14	1.5661	1.4784 - 1.6590	8.63
C_{max} (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)
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)
Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the linear mixed effect model.
Geometric Mean Ratio (GMR) = test / reference
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_{0-t} and AUC_{0-inf} 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_{0-t} (ng*hr/mL)	486.4	14	5565	14	0.0874	0.0719 - 0.1062	29.73
AUC_{0-inf} (ng*hr/mL)	496.4	14	5681	14	0.0874	0.0719 - 0.1062	29.84
C_{max} (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) 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) Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the linear mixed effect model. Geometric Mean Ratio (GMR) = test / reference 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_{0-t} and AUC_{0-inf} 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_{0-t} (ng*hr/mL)	230.5	14	623.0	14	0.3700	0.3316 - 0.4129	16.50
AUC_{0-inf} (ng*hr/mL)	244.9	14	662.6	14	0.3696	0.3321 - 0.4113	16.08
C_{max} (ng/mL)	64.47	14	53.80	14	1.1983	1.0642 - 1.3492	17.86

	Mitapivat sulfate + rifampin (Test)		Mitapivat sulfate alone (Reference)				
Parameter (unit)	Geometric LSM	n	Geometric LSM	n	GMR	90% Confidence Interval	Intra-subject CV%
Mitapivat sulfate alone: 50 mg mitapivat sulfate (1 x 50 mg tablet) at Hour 0 on Day 1 (Period 1) 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) Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the linear mixed effect model. Geometric Mean Ratio (GMR) = test / reference 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 (Emax and EC50) 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.

CLAIMS

WHAT IS CLAIMED IS:

1. 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).

2. 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).

3. 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 3A4/5 (CYP3A4/5).

4. The method of any one of claims 1-3, further comprising:

(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.

5. The method of claim 4, further comprising:

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

6. 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).

7. 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).

8. 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 3A4/5 (CYP3A4/5).

9. The method of any one of claims 6-8, further comprising:

(a) monitoring the subject for an effect of a drug-drug interaction between mitapivat or a pharmaceutically acceptable salt thereof and the CYP3A4/5 inhibitor; 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 inhibitor being administered to the subject to reduce the effect.

10. The method of claim 9, further comprising:

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

11. The method of any one of claims 1-10, comprising 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.

12. The method of any one of claims 1-10, comprising 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.

13. The method of any one of claims 1-10, comprising 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.

14. 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 3A4/5 (CYP3A4/5).

15. 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 3A4/5 (CYP3A4/5).

16. 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 3A4/5 (CYP3A4/5).

17. The method of any one of claims 14-16, wherein 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.

18. A method of treating a condition associated with 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 3A4/5 (CYP3A4/5).

19. 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 3A4/5 (CYP3A4/5).

20. 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 3A4/5 (CYP3A4/5).

21. The method of any one of claims 18-20, wherein 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.

22. The method of any one of claims 1-21, comprising 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.

23. The method of any one of claims 1-22, comprising administering to the subject about 1 mg to about 2500 mg of mitapivat or a pharmaceutically acceptable salt thereof.

24. The method of any one of claims 1-23, comprising administering to the subject about about 5 mg to 2500 mg of mitapivat or a pharmaceutically acceptable salt thereof.

25. The method of any one of claims 1-24, comprising administering to the subject about 5 mg to about 360 mg of mitapivat or a pharmaceutically acceptable salt thereof.

26. The method of any one of claims 1-25, comprising administering to the subject about 5 mg to about 300 mg of mitapivat or a pharmaceutically acceptable salt thereof.

27. The method of any one of claims 1-26, comprising administering to the subject about 50 mg to about 300 mg of mitapivat or a pharmaceutically acceptable salt thereof.

28. The method of any one of claims 1-26, comprising administering to the subject about 5 mg of mitapivat or a pharmaceutically acceptable salt thereof.

29. The method of any one of claims 1-27, comprising administering to the subject about 20 mg of mitapivat or a pharmaceutically acceptable salt thereof.

30. The method of any one of claims 1-27, comprising administering to the subject about 50 mg of mitapivat or a pharmaceutically acceptable salt thereof.

31. The method of any one of claims 1-27, comprising administering to the subject about 100 mg of mitapivat or a pharmaceutically acceptable salt thereof.

32. The method of any one of claims 1-31, comprising administering to the subject the effective amount of mitapivat or a pharmaceutically acceptable salt thereof once daily.

33. The method of any one of claims 1-31, comprising administering to the subject the effective amount of mitapivat or a pharmaceutically acceptable salt thereof twice daily.

34. The method of any one of claims 1-33, comprising administering to the subject a sulfate salt of mitapivat.

35. The method of any one of claims 1-5, 11-17 and 22-34, wherein the CYP3A4/5 inducer is a strong inducer of CYP3A4/5.

36. The method of any one of claims 1-5, 11-17 and 22-34, wherein the CYP3A4/5 inducer is a moderate inducer of CYP3A4/5.

37. The method of any one of claims 1-5, 11-17 and 22-34, wherein the CYP3A4/5 inducer is a mild inducer of CYP3A4/5.

38. The method of any one of claims 1-5, 11-17 and 22-34, wherein 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, armodafninil, glycerol phenylbutyrate, asunaprevir, rifunamide, oritavancin, clobazam, felbamate, eslicarbazepine acetate, and genistein.

.39. The method of any one of claims 1-5, 11-17, 22-34 and 38, wherein the CYP3A4/5 inducer is rifampin.

40. The method of any one of claims 1-5, 11-17, 22-34, 38 and 39, comprising administering about 600 mg of rifampin to the subject.

41. The method of any one of claims 6-13 and 18-34, wherein the inhibitor of CYP3A4/5 is a strong inhibitor of CYP3A4/5.

42. The method of any one of claims 6-13 and 18-34, wherein the inhibitor of CYP3A4/5 is a moderate inhibitor of CYP3A4/5.

43. The method of any one of claims 6-13 and 18-34, wherein the inhibitor of CYP3A4/5 is a mild inhibitor of CYP3A4/5.

44. The method of any one of claims 6-13 and 18-34, wherein 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.

45. The method of any one of claims 6-13, 18-34 and 44, wherein the inhibitor of CYP3A4/5 is itraconazole.

45. The method of any one of claims 6-13, 18-34, 44 and 45, comprising administering about 200 mg of itraconazole to the subject.

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.

SCORE Placeholder Sheet for IFW Content

Application Number: 16900610 Document Date: 06/12/2020

The presence of this form in the IFW record indicates that the following document type was received in electronic format on the date identified above. This content is stored in the SCORE database.

Since this was an electronic submission, there is no physical artifact folder, no artifact folder is recorded in PALM, and no paper documents or physical media exist. The TIFF images in the IFW record were created from the original documents that are stored in SCORE.

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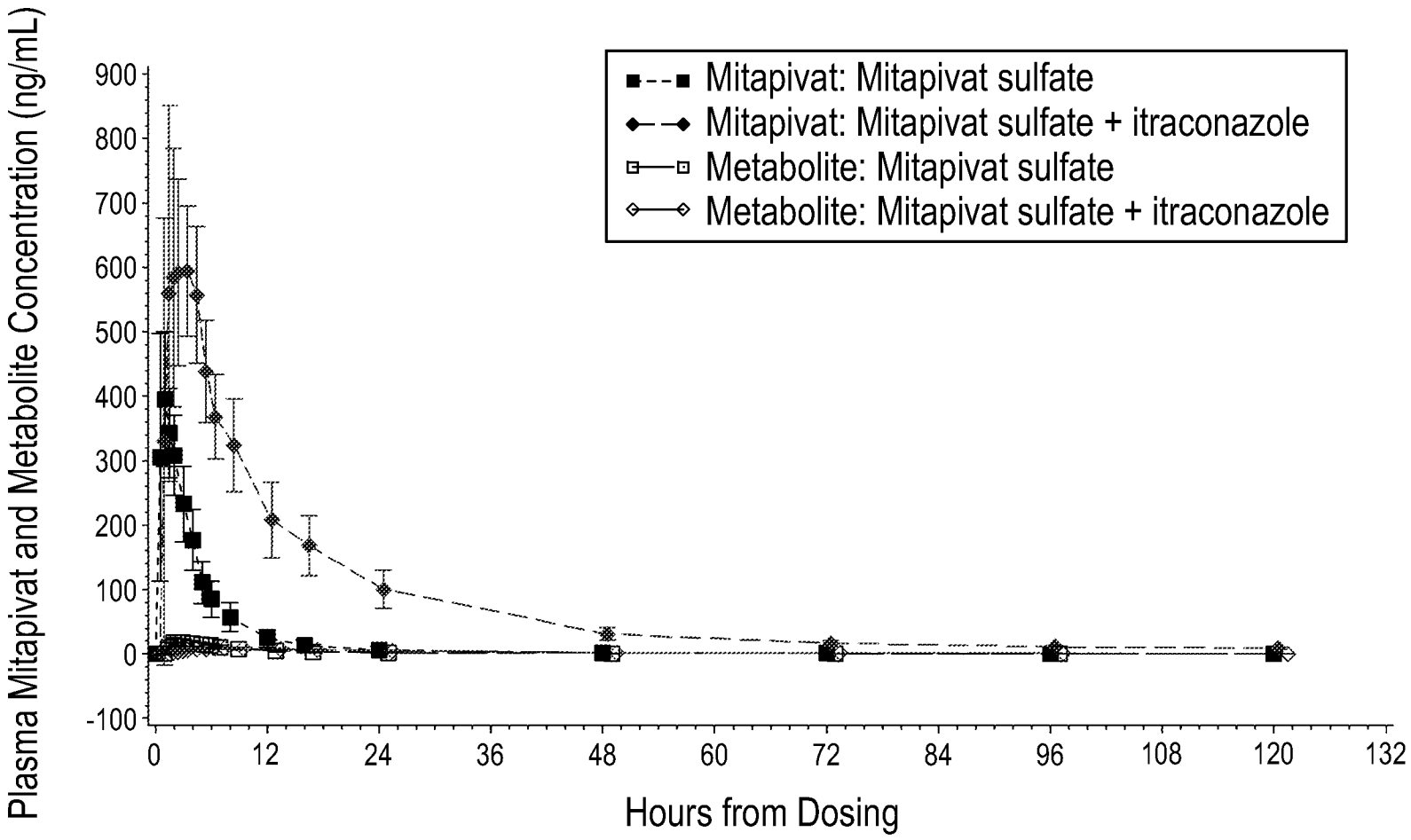

Plasma Mitapivat and Metabolite Concentration (ng/mL)
900
800
700
600
500
400
300
200
100
0
-100
0
12
24
36
48
60
72
84
96
108
120
132
Hours from Dosing
Mitapivat: Mitapivat sulfate
Mitapivat: Mitapivat sulfate + itraconazole
Metabolite: Mitapivat sulfate
Metabolite: Mitapivat sulfate + itraconazole

FIG. 1

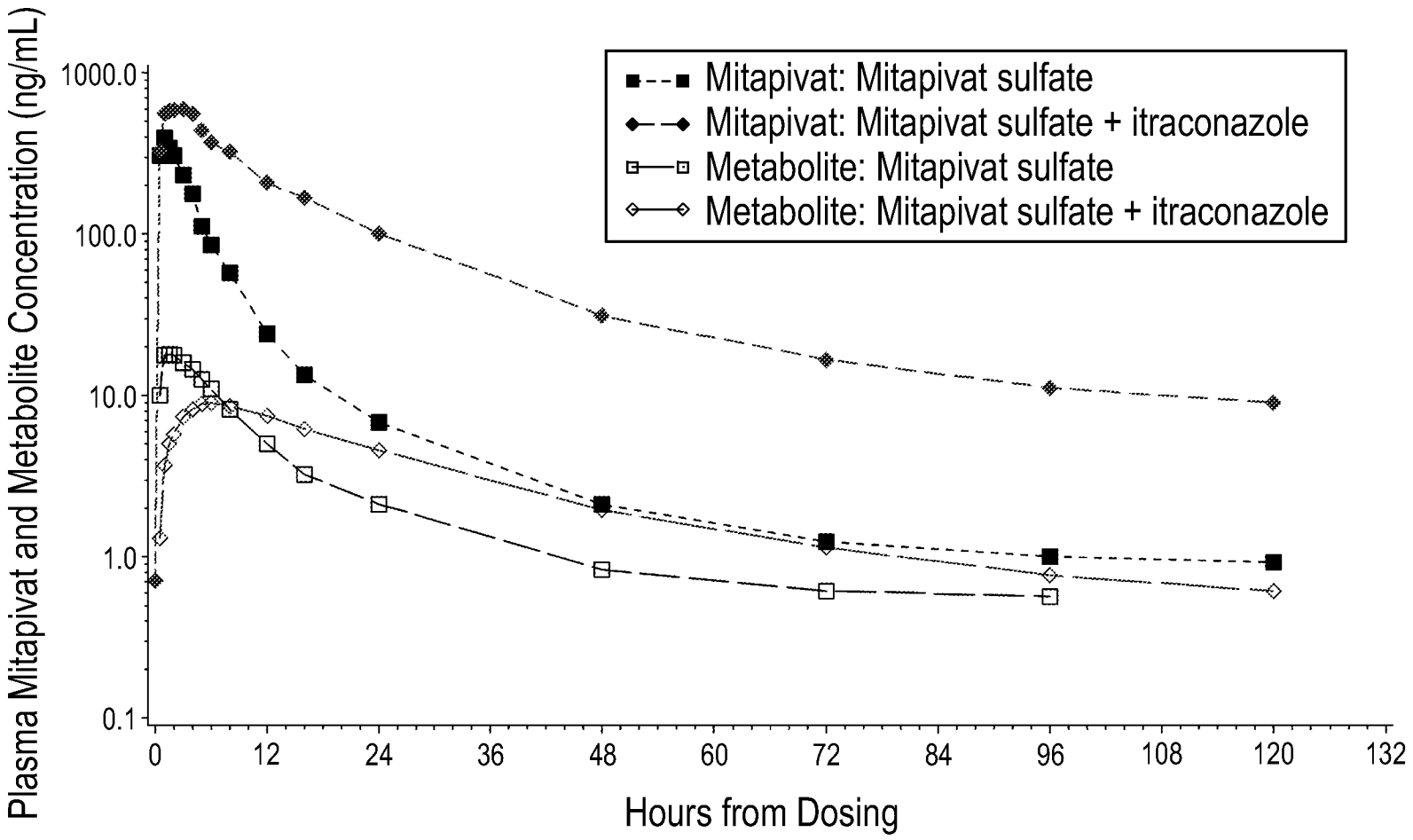

Mitapivat: Mitapivat sulfate
Mitapivat: Mitapivat sulfate + itraconazole
Metabolite: Mitapivat sulfate
Metabolite: Mitapivat sulfate + itraconazole
Plasma Mitapivat and Metabolite Concentration (ng/mL)
1000.0
100.0
10.0
1.0
0.1
0
12
24
36
48
60
72
84
96
108
120
132
Hours from Dosing

FIG. 2

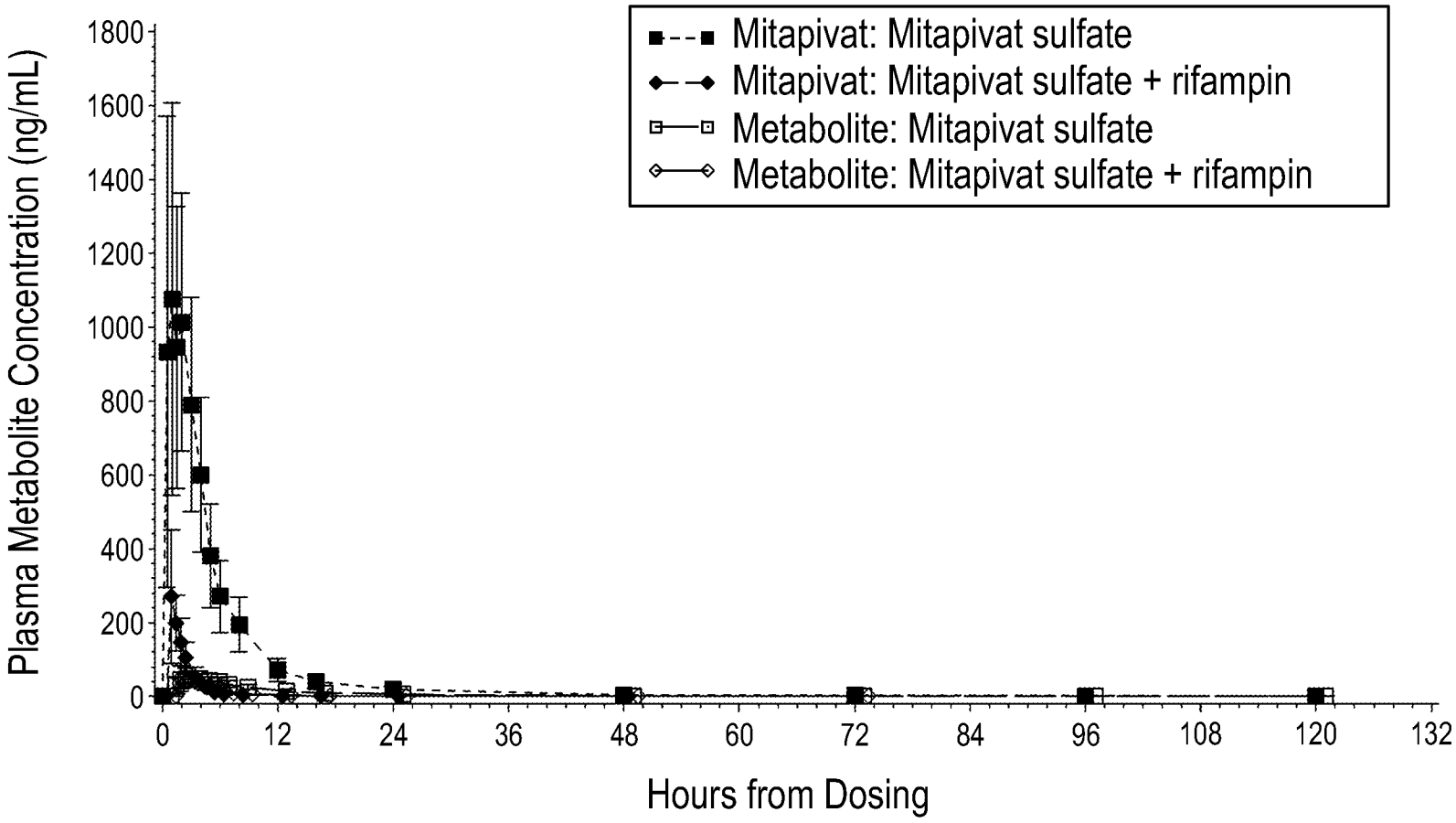

Mitapivat: Mitapivat sulfate
Mitapivat: Mitapivat sulfate + rifampin
Metabolite: Mitapivat sulfate
Metabolite: Mitapivat sulfate + rifampin
Plasma Metabolite Concentration (ng/mL)
1800
1600
1400
1200
1000
800
600
400
200
0
0
12
24
36
48
60
72
84
96
108
120
132
Hours from Dosing

FIG. 3

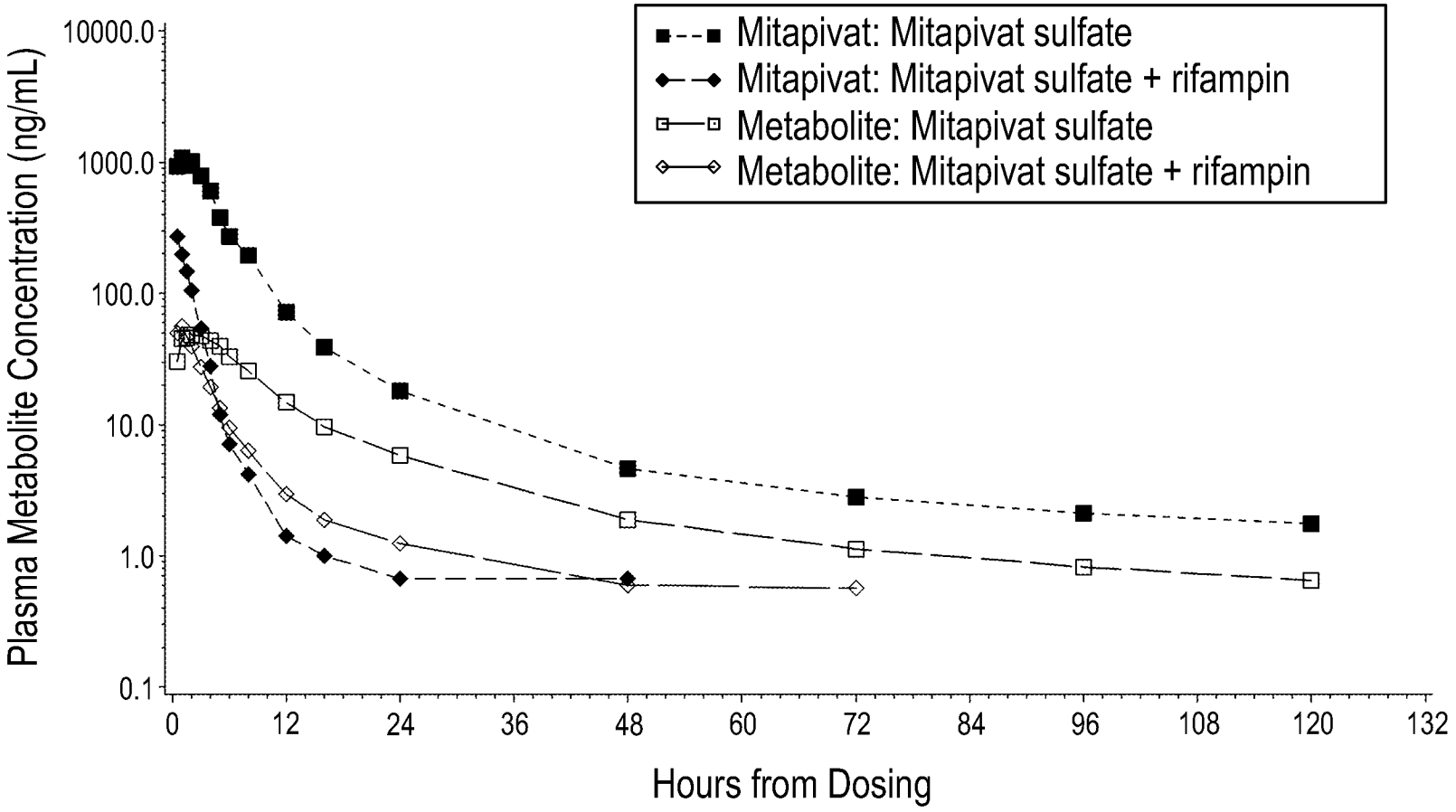

Mitapivat: Mitapivat sulfate
Mitapivat: Mitapivat sulfate + rifampin
Metabolite: Mitapivat sulfate
Metabolite: Mitapivat sulfate + rifampin
Plasma Metabolite Concentration (ng/mL)
10000.0
1000.0
100.0
10.0
1.0
0.1
0
12
24
36
48
60
72
84
96
108
120
132
Hours from Dosing

FIG. 4

Electronic Acknowledgement Receipt

EFS ID:	40326935
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:	86738
Filer:	Mei Bai
Filer Authorized By:	
Attorney Docket Number:	128748-01502
Receipt Date:	19-AUG-2020
Filing Date:	12-JUN-2020
Time Stamp:	16:41:35
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Preliminary Amendment	PAmend_Resp_to_NTFCAP.pdf	41541 4e425356cd8a0d098f0274105d712b75477e243a	no	8

Warnings:

Information:					
2	Drawings-only black and white line drawings	Replacement_drawings.PDF	143099 b5a707b67a89d7a1b4000e8120885ab3e7bdda72	no	4
Warnings:					
Information:					
			Total Files Size (in bytes):	184640	

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.

New Applications Under 35 U.S.C. 111

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.

National Stage of an International Application under 35 U.S.C. 371

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.

New International Application Filed with the USPTO as a Receiving Office

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.

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: 08/19/2020 Signature: /Mei Bai/
(Mei Bai, Ph.D.)

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: N/A

For: MITAPIVAT THERAPY AND MODULATORS OF CYTOCHROME P450 Examiner: Not Yet Assigned

PRELIMINARY AMENDMENT UNDER 37 C.F.R. § 1.115 AND RESPONSE TO NOTICE TO FILE CORRECTED APPLICATION PAPERS

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

Dear Sir:

INTRODUCTORY COMMENTS

In response to the Notice to File Corrected Application Papers mailed June 19, 2020, please amend the above-identified U.S. patent application as follows:

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Amendments to the Drawings begin on page 6 of this paper and include an attached replacement sheet.

Remarks/Arguments begin on page 7 of this paper.

AMENDMENTS TO THE CLAIMS

1. (Currently Amended) 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 or inhibitor of cytochrome P450 3A4/5 (CYP3A4/5).

2. (Currently Amended) 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 or inhibitor of cytochrome P450 3A4/5 (CYP3A4/5).

3. (Currently Amended) 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 or inhibitor of cytochrome P450 3A4/5 (CYP3A4/5).

4. (Currently Amended) The method of claim 1~~any one of claims 1-3~~, further comprising:

(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 or inhibitor; 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 or inhibitor being administered to the subject to reduce the effect.

5. (Original) The method of claim 4, further comprising:

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

6-10. (Cancelled)

11. (Currently Amended) The method of claim 1~~any one of claims 1-10~~, comprising 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.

12. (Currently Amended) The method of claim 1~~any one of claims 1-10~~, comprising 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.

13. (Cancelled)

14. (Currently Amended) 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 inhibitor of cytochrome P450 3A4/5 (CYP3A4/5).

15. (Currently Amended) 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 inhibitor of cytochrome P450 3A4/5 (CYP3A4/5).

16. (Currently Amended) 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 or inhibitor of cytochrome P450 3A4/5 (CYP3A4/5).

17. (Currently Amended) The method of claim 14~~any one of claims 14-16~~, wherein administration of a CYP3A4/5 inducer or 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 inducer or inhibitor.

18-21. (Cancelled)

22. (Currently Amended) The method of claim 1~~any one of claims 1-21~~, comprising 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.

23-31. (Cancelled)

32. (Currently Amended) The method of claim 1~~any one of claims 1-31~~, comprising administering to the subject the effective amount of mitapivat or a pharmaceutically acceptable salt thereof once daily or twice daily.

33. (Cancelled)

34. (Currently Amended)The method of claim 1~~any one of claims 1-33~~, comprising administering to the subject a sulfate salt of mitapivat.

35. (Cancelled)

36. (Currently Amended)The method of claim 1~~any one of claims 1-5, 11-17 and 22-34~~, wherein the CYP3A4/5 inducer or inhibitor is a moderate inducer or inhibitor of CYP3A4/5.

37. (Currently Amended)The method of claim 1~~any one of claims 1-5, 11-17 and 22-34~~, wherein the CYP3A4/5 inducer or inhibitor is a mild inducer or inhibitor of CYP3A4/5.

38. (Currently Amended)The method of claim 1~~any one of claims 1-5, 11-17 and 22-34~~, wherein 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, armodafninil, glycerol phenylbutyrate, asunaprevir, rifunamide, oritavancin, clobazam, felbamate, eslicarbazepine acetate, and genistein.

39-43. (Cancelled)

44. (Currently Amended) The method of claim 1~~any one of claims 6-13 and 18-34~~, wherein 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.

45-46. (Cancelled)

AMENDMENTS TO THE DRAWINGS

Please replace 4 sheets of drawings with 4 sheets of replacement drawings.

Attachment: Replacement sheets

REMARKS

Claim Status

Upon entry of this amendment, claims 1-5, 11, 12, 14-17, 22, 32, 34, 36-38 and 44 will be pending. Claims 6-10, 13, 18-21, 23-31, 33, 35, 39-43, 45 and 46 are currently canceled. Claims 1-4, 11, 12, 14-17, 22, 32, 34, 36-38 and 44 are currently amended. Support for the amendments to the claims can be found in the specification and claims as originally filed. For example, support for the amendments to claims 1-4 can be found in original claims 6-9, respectively. Support for the amendments to claims 14-17 can be found in original claims 18-21, respectively. Support for the amendments to claim 32 can be found in original claim 33. Support for the amendments to claims 36 and 37 can be found in original claims 42 and 43, respectively. No new matter has been added.

Response to File Corrected Application Papers

Applicant has submitted herewith replacement drawings, in which numbers, letters, and reference characters on the drawings measure at least 0.32 cm (1/8 inch) in height.

In view the amendments submitted herewith, the total number of claims is 18. In addition, the multiple claim dependencies have been removed throughout the claims. Accordingly, the surcharge for multiple dependent claim is no longer applicable. In response to the Notice of Insufficiency, Applicant submits herewith $1380 additional claim fees for 3 independent claims in excess of 3.

If the Examiner believes that a telephone conversation with the Applicant's Agent would be helpful in expediting prosecution of this application, the Examiner is invited to call the undersigned at (617) 449-6590.

Applicants believe no further fees are due with this response. 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: 08/19/2020

Respectfully submitted,

By_/Mei Bai/_____
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Attachments

UNITED STATES PATENT AND TRADEMARK OFFICE

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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

86738 7590 07/18/2022

MCCARTER & ENGLISH, LLP BOSTON
265 Franklin Street
Boston, MA 02110

EXAMINER	
KOSTURKO, GEORGE W	

ART UNIT	PAPER NUMBER
1628	

NOTIFICATION DATE	DELIVERY MODE
07/18/2022	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):

docket@mccarter.com

Office Action Summary	Application No. 16/900,610	Applicant(s) Iyer et al.	
	Examiner GEORGE W KOSTURKO	Art Unit 1628	AIA (FITF) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☑ Responsive to communication(s) filed on 08/19/2020.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☐ This action is **FINAL.** 2b) ☑ This action is non-final.

3)☐ 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.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 1-5,11-12,14-17,22,32,34,36-38 and 44 is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☑ Claim(s) 1-5,11-12,14-17,22,32,34,36-38 and 44 is/are rejected.

8) ☐ Claim(s) _____ is/are objected to.

9) ☐ Claim(s) _____ are subject to restriction and/or election requirement

* If any claims have been determined allowable, 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 or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☑ The drawing(s) filed on 08/19/2020 is/are: a)☑ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ 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)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☑ Notice of References Cited (PTO-892)

2) ☑ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.

4) ☐ Other: _____.

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 1-5, 11-12, 14-17, 22, 32, 34, 36-38 and 44, filed August 19th, 2020 are the subject matter of this Office Action.

Priority

2. Acknowledgement is made of Applicant's claim to priority to U.S. Provisional Application 62861600 filed 06/14/2009.

Information Disclosure Statement

3. The information disclosure statement (IDS) submitted on 04/12/2021 is in compliance with the provisions of 37 CFR 1.97. Accordingly, the information disclosure statement is being considered by the examiner.

Claim Rejections - 35 USC § 112-Paragraph B

4. The following is a quotation of 35 U.S.C. 112(b):

> (b) CONCLUSION.—The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the inventor or a joint inventor regards as the invention.

The following is a quotation of 35 U.S.C. 112 (pre-AIA), second paragraph:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

5. Claim 17 is rejected under 35 U.S.C. 112(b) or 35 U.S.C. 112 (pre-AIA), second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the inventor or a joint inventor (or for applications subject to pre-AIA 35 U.S.C. 112, the applicant), regards as the invention.

6. Claim 14 is directed to the method of treating pyruvate kinase deficiency 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 inhibitor of cytochrome P450 3A4/5. Claim 17 is directed to the method of claim 14, wherein the administering of a CYP3A4/5 inducer or inhibitor, if any, is terminated at a sufficient time interval prior to the administration of the effective amount of mitapivat or pharmaceutically acceptable salt thereof such that there is no detectable symptom of a drug-drug interaction between the mitapivat or a pharmaceutically acceptable salt thereof and the CYP3A4/5 inducer or inhibitor.

7. There is insufficient antecedent basis for the limitation of "an inducer or inhibitor of CYP3A4/5" in combination with mitapivat or a pharmaceutically acceptable salt thereof in claim 17, as the claim from which it depends (claim 14) *requires* that mitapivat or a pharmaceutically acceptable salt thereof is administered in the *absence* of an inducer or an inhibitor of CYP3A4/5. Accordingly, one of ordinary skill in the art prior to the time of the invention would not have been reasonably apprised of the metes and bounds of the subject matter for which Applicant was presently seeking protection.

8.

Claim Rejections - 35 USC § 102

9. In the event the determination of the status of the application as subject to AIA 35 U.S.C. 102 and 103 (or as subject to pre-AIA 35 U.S.C. 102 and 103) is incorrect, any correction of the statutory basis for the rejection will not be considered a new ground of rejection if the prior art relied upon, and the rationale supporting the rejection, would be the same under either status.

10. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –

(a)(1) the claimed invention was patented, described in a printed publication, or in public use, on sale, or otherwise available to the public before the effective filing date of the claimed invention.

11. Claim(s) 14-17 are rejected under 35 U.S.C. 102(a)(1) as being anticipated by Agresta (WO2016/201227 published 12/15/2016).

12. Agresta teaches the method of treating pyruvate kinase deficiency in a subject comprising administering a therapeutically effective amount of Compound 1 (claims 1-9). As shown in page 28 of the specification, compound 1 or N-(4-(4-(cyclopropylmethyl)piperazine-1-carbonyl)phenyl)quinoline-8-sulfonamide corresponds to the claimed mitapivat. See ([0002] of the instant specification). Administration of said compound 1 to treat sickle cell disease as well as beta-thalassemia is embraced in the methodology of Agresta (page 11). As Agresta does not teach that compound 1 is administered with an inhibitor or alternatively, an inducer of CYP3A4/5, the teachings of Agresta read on the limitation wherein mitapivat is administered in the absence of an inhibitor or inducer of CYP3A4/5, which reads on the instant claims.

13.

Claim Rejections - 35 USC § 103

14. In the event the determination of the status of the application as subject to AIA 35 U.S.C. 102 and 103 (or as subject to pre-AIA 35 U.S.C. 102 and 103) is incorrect, any correction of the statutory basis for the rejection will not be considered a new ground of rejection if the prior art relied upon, and the rationale supporting the rejection, would be the same under either status.

15. The factual inquiries for establishing a background for determining obviousness under 35 U.S.C. 103 are summarized as follows:

1. Determining the scope and contents of the prior art.

2. Ascertaining the differences between the prior art and the claims at issue.

3. Resolving the level of ordinary skill in the pertinent art.

4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

16. Claim(s) 1-5, 11-12, 22, 32 are rejected under 35 U.S.C. 103 as being unpatentable over Agresta (WO2016/201227 published 12/15/2016) in view of Ghosal (WO2012/015712 published 02/02/2012).

17. Agresta teaches the method of treating pyruvate kinase deficiency in a subject comprising administering a therapeutically effective amount of Compound 1 (claims 1-9). As shown in page 28 of the specification, compound 1 or N-(4-(4-(cyclopropylmethyl)piperazine-1-carbonyl)phenyl)quinoline-8-sulfonamide corresponds to the claimed mitapivat. See ([0002] of the instant specification). Administration of said compound 1 to treat sickle cell disease as well as beta-thalassemia is embraced in the methodology of Agresta (page 11). Agresta teaches administering mitapivat in a dose of 50-300 mg, once or twice per day, which reads on the limitation of claims 22 and 32.

18. Agresta further teaches that during administration of mitapivat, the pharmacokinetic parameters of mitapivat were lower on day 14 compared to that on day 1, suggesting that multiple doses of mitapivat may result in an increase in the rate of drug metabolism. The decrease in exposure on day 14 is consistent with pre-clinical data suggesting that mitapivat is a moderate inducer of cytochrome P450 3A4, which is also the major route of mitapivat's metabolic pathway (page 40).

19. However, Agresta does not specifically teach the incorporation of an inhibitor of cytochrome P450 3A4/5 to the mitapivat therapeutic regimen.

20. Ghosal teaches improving the pharmacokinetics of therapeutic compounds that are metabolized by the cytochrome P450 3A4/5 enzyme by the co-administration of said therapeutic compound in combination with boceprevir, a CYP3A4/5 inhibitor (abstract, page 2 lines 10-30, claims 1-2). Concomitant and sequential administration of the CYP3A4/5 inhibitor with the additional therapeutic is embraced in the methodology of Ghosal (page 5 lines 5-30). Improvement in increased half-life, AUC between doses and Cmax are embraced in the methodology of Ghosal (page 11 lines 25-35, page 56). Regarding the scope of claim 4-5, monitoring the interaction of the therapeutic agent and boceprevir and adjusting the dose of the CYP3A4/5 inhibitor when administered in combination with the therapeutic agent in order to arrive at the targeted pharmacokinetic parameter is embodied within the methodology of Ghosal (page 5 lines 10-20).

21. It would therefore have been prima facie obvious to one of ordinary skill in the art prior to the time of the invention to administer a CYP3A4/5 inhibitor, such as boceprevir with mitapivat in order to treat pyruvate kinase disorders, sickle cell anemia or beta thalassemia in a subject in view of Ghosal. Motivation to incorporate a CYP3A4/5 inhibitor to the mitapivat regimen logically flows from the fact that mitapivat is both metabolized by and induces cytochrome P450 3A4, resulting in a poor pharmacokinetic profile in administered subjects . Considering it was known in the art to incorporate the CYP3A4/5 inhibitor boceprevir to improve the pharmacokinetic profile of therapeutic agents metabolized by CYP3A4/5, said artisan would have applied the teachings of Ghosal to the mitapivat regimen of Agresta and readily predicted that incorporation of

boceprevir to the mitapivat regimen of Agresta would have improved the pharmacokinetic profile of mitapivat in the administered subject.

22.

23. Claim(s) 34 is rejected under 35 U.S.C. 103 as being unpatentable over the combination of Agresta (WO2016/201227 published 12/15/2016) in view of Ghosal (WO2012/015712 published 02/02/2012) as applied from claims 1-5, 11-12, 22 and 32 in view of Salituro (US2010/0331307 published 12/30/2010).

24.

25. As disclosed above, Agresta and Ghosal teaches the method of administering a CYP3A4/5 inhibitor, such as boceprevir with mitapivat in order to treat pyruvate kinase disorders, sickle cell anemia or beta thalassemia in a subject, as mitapivat is both metabolized by and induces cytochrome P450 3A4, resulting in a poor pharmacokinetic profile in administered subjects and it was known in the art to incorporate the CYP3A4/5 inhibitor boceprevir to improve the pharmacokinetic profile of therapeutic agents metabolized by CYP3A4/5.

26. However, the combination of Agresta and Ghosal does not specifically teach wherein mitapivat is present as the sulfate salt.

27. Salituro teaches compounds of Formula (I). Embraced within Formula (I) is example 78 shown above which corresponds to the claimed mitapivat ([0432]). Salituro additionally teaches preparation of said compounds of Formula (I) in pharmaceutical salt formulations. The sulfate counter anion is one of 11 suitable anionic salt formulation for compounds of Formula (I) ([0174]).

28. It would therefore have been prima facie obvious to one of ordinary skill in the art to administer mitapivat as taught by the combination of Agresta and Ghosal, as a sulfate salt in view of Salituro.

29. MPEP 2143 provides rationale for a conclusion of obviousness including (E): "Obvious to try": choosing from a finite number of identified predictable solutions with a reasonable expectation of success. Considering the sulfate counter anion is one of 11 predictable counter anion salts taught by Salituro to formulate with mitapivat, said artisan would have readily predicted that administering the sulfate salt formulation of mitapivat in the regimen of Agresta and Ghosal would have effectively treated the disclosed disorder.

30.

31. Claim(s) 36-37 and 44 are rejected under 35 U.S.C. 103 as being unpatentable over the combination of Agresta (WO2016/201227 published 12/15/2016) in view of Ghosal (WO2012/015712 published 02/02/2012) as applied from claims 1-5, 11-12, 22 and 32 in view of Synold (US2001/0041706 published 11/15/2001).

32.

33. As disclosed above, Agresta and Ghosal teaches the method of administering a CYP3A4/5 inhibitor, such as boceprevir with mitapivat in order to treat pyruvate kinase disorders, sickle cell anemia or beta thalassemia in a subject, as mitapivat is both metabolized by and induces cytochrome P450 3A4, resulting in a poor pharmacokinetic profile in administered subjects and it was known in the art to incorporate the CYP3A4/5

inhibitor boceprevir to improve the pharmacokinetic profile of therapeutic agents metabolized by CYP3A4/5.

34. However, the combination of Agresta and Ghosal does not specifically teach employing a mild or moderate CYP3A4/5 inhibitor to improve the pharmacokinetic profile of mitapivat.

35. Synold teaches the administration of CYP3A4 inhibitors in order to inhibit the metabolism of drugs that are actively metabolized by cytochrome P450 enzyme 3A4 (CYP3A4) ([0007]-[0009]). Clotrimazole and erythromycin are taught by Synold as suitable CYP3A4 inhibitors to employ to improve the metabolism (decrease clearance, increase AUC) of the therapeutic agent that is metabolized by CYP3A4, either concurrently or sequentially with said agent ([0016]-[0018], [0029]-[0030]). As evidenced by paragraphs [0108]-[0109] of the instant specification, erythromycin is taught as a moderate CYP3A4 inhibitor while clotrimazole is taught as a mild CYP3A4 inhibitor.

36. Accordingly, one of ordinary skill in the art prior to the time of the invention would have found it prima facie obvious to substitute the CYP3A4 inhibitor boceprevir in the regimen of Agresta and Ghosal above, for an alternative CYP3A4 inhibitor such as erythromycin or clotrimazole in order to arrive at the instantly claimed methodology in view of Synold. MPEP 2143 provides rationale for a conclusion of obviousness including (B): Simple substitution of one known element for another to obtain predictable results;

37. In the present case, clotrimazole and erythromycin are each taught by Synold as suitable CYP3A4 inhibitors to employ to improve the metabolism (decrease clearance, increase AUC) of the therapeutic agent that is metabolized by CYP3A4, either concurrently or sequentially with said agent ([0016]-[0018], [0029]-[0030]). Thus, said

artisan would have predicted with a reasonable expected that substitution of CYP3A4 inhibitor boceprevir in the regimen above for clotrimazole or erythromycin, the resulting combination would have effectively improved the pharmacokinetic profile of mitapivat in the administered subject.

38.

39. Claim(s) 1, 22, 32 and 38 are rejected under 35 U.S.C. 103 as being unpatentable over Agresta (WO2016/201227 published 12/15/2016) and Jannetta (US2002/0055495 published 05/09/2002).

40.

41. Agresta teaches the method of treating pyruvate kinase deficiency in a subject comprising administering a therapeutically effective amount of Compound 1 (claims 1-9). As shown in page 28 of the specification, compound 1 or N-(4-(4-(cyclopropylmethyl)piperazine-1-carbonyl)phenyl)quinoline-8-sulfonamide corresponds to the claimed mitapivat. See ([0002] of the instant specification). Administration of said compound 1 to treat sickle cell disease as well as beta-thalassemia is embraced in the methodology of Agresta (page 11). Agresta teaches a therapeutic range of 10 mg-3000 mg, administered once or twice a day (page 33). Agresta also teaches that 700 mg doses of mitapivat yields undesirable Grade 1-2 nausea and vomiting in patients (page 41).

42. However, Agresta does not specifically teach incorporating a CYP 3A4/5 inducer with the mitapivat regimen, such as dexamethasone.

43. Jannetta teaches the CYP3A4 inducer dexamethasone is art-recognized as relieving nausea, emesis and symptoms associated with it ([0005], [0013], claims 1, 14).

44. Therefore, one of ordinary skill in the art prior to the time of the invention would have found it prima facie obvious to incorporate the anti-emetic dexamethasone to patient being treated with 700 mg doses of mitapivat in view of Jannetta. Considering 700 mg doses of mitapivat yields undesirable Grade 1-2 nausea and vomiting in patients as taught by Agresta above, and that the CYP3A4 inducer dexamethasone is art-recognized as relieving nausea and emesis, said artisan would have readily predicted that the incorporation of the CYP3A4 inducer dexamethasone to the 700 mg mitapivat regimen of Agresta would have inhibited emesis in the administered patient.

45.

Double Patenting

46. The nonstatutory double patenting rejection is based on a judicially created doctrine grounded in public policy (a policy reflected in the statute) so as to prevent the unjustified or improper timewise extension of the "right to exclude" granted by a patent and to prevent possible harassment by multiple assignees. A nonstatutory double patenting rejection is appropriate where the conflicting claims are not identical, but at least one examined application claim is not patentably distinct from the reference claim(s) because the examined application claim is either anticipated by, or would have been obvious over, the reference claim(s). See, e.g., *In re Berg*, 140 F.3d 1428, 46 USPQ2d 1226 (Fed. Cir. 1998); *In re Goodman*, 11 F.3d 1046, 29 USPQ2d 2010 (Fed. Cir. 1993); *In re Longi*, 759 F.2d 887, 225 USPQ 645 (Fed. Cir. 1985); *In re Van Ornum*, 686 F.2d 937, 214 USPQ 761 (CCPA 1982); *In re Vogel*, 422 F.2d 438, 164 USPQ 619 (CCPA 1970); *In re Thorington*, 418 F.2d 528, 163 USPQ 644 (CCPA 1969).

A timely filed terminal disclaimer in compliance with 37 CFR 1.321(c) or 1.321(d) may be used to overcome an actual or provisional rejection based on nonstatutory double patenting provided the reference application or patent either is shown to be commonly owned with the examined application, or claims an invention made as a result of activities undertaken within the scope of a joint research agreement. See MPEP § 717.02 for applications subject to examination under the first inventor to file provisions of the AIA as explained in MPEP § 2159. See MPEP § 2146 *et seq.* for applications not subject to examination under the first inventor to file provisions of the AIA. A terminal disclaimer must be signed in compliance with 37 CFR 1.321(b).

The USPTO Internet website contains terminal disclaimer forms which may be used. Please visit www.uspto.gov/patent/patents-forms. The filing date of the application in which the form is filed determines what form (e.g., PTO/SB/25, PTO/SB/26, PTO/AIA/25, or PTO/AIA/26) should be used. A web-based eTerminal Disclaimer may be filled out completely online using web-screens. An eTerminal Disclaimer that meets all requirements is auto-processed and approved immediately upon submission. For more information about eTerminal Disclaimers, refer to www.uspto.gov/patents/process/file/efs/guidance/eTD-info-I.jsp.

47. Claim 1, 4-5, 11-12, 14, 17, 22, 32 are are rejected on the ground of nonstatutory double patenting as being unpatentable over claim 1-16 of U.S. Patent No. 11,234,976 in view of over Agresta (WO2016/201227 published 12/15/2016) in view of Ghosal (WO2012/015712 published 02/02/2012).

48.

49. .

50. Although the claims at issue are not identical, they are not patentably distinct from each other because each of the claims are directed to treating pyruvate kinase deficiency in a subject in need comprising administering mitapivat in the absence of a CYP 3A4 inducer or inhibitor. The amount of mitapivat and the dosing frequency in the instant claims overlaps with the amounts embraced in claims 1-16 of U.S. Patent 11,234,976.

51. However, claims 1-16 of U.S. Patent 11,234,976 do not specifically teach combining compound 1/mitapivat with an inhibitor of CYP 3A4/5.

52. It is also noted that Agresta further teaches that during administration of mitapivat/compound 1, the pharmacokinetic parameters of mitapivat were lower on day 14 compared to that on day 1, suggesting that multiple doses of mitapivat may result in an increase in the rate of drug metabolism. The decrease in exposure on day 14 is consistent with pre-clinical data suggesting that mitapivat is a moderate inducer of cytochrome P450 3A4, which is also the major route of mitapivat's metabolic pathway (page 40).

53. And while Agresta does not specifically teach the incorporation of an inhibitor of cytochrome P450 3A4/5 to the mitapivat therapeutic regimen, Ghosal teaches improving the pharmacokinetics of therapeutic compounds that are metabolized by the cytochrome P450 3A4/5 enzyme by the co-administration of said therapeutic compound in combination with boceprevir, a CYP3A4/5 inhibitor (abstract, page 2 lines 10-30, claims 1-2). Concomitant and sequential administration of the CYP3A4/5 inhibitor with the additional therapeutic is embraced in the methodology of Ghosal (page 5 lines 5-30). Improvement in increased half-life, AUC between doses and Cmax are embraced

in the methodology of Ghosal (page 11 lines 25-35, page 56). Regarding the scope of claim 4-5, monitoring the interaction of the therapeutic agent and boceprevir and adjusting the dose of the CYP3A4/5 inhibitor when administered in combination with the therapeutic agent in order to arrive at the targeted pharmacokinetic parameter is embodied within the methodology of Ghosal (page 5 lines 10-20).

54. It would therefore have been prima facie obvious to one of ordinary skill in the art prior to the time of the invention to administer a CYP3A4/5 inhibitor, such as boceprevir with mitapivat into the pyruvate kinase disorder treating regimen of claims 1-16 of U.S. Patent 11,234,976 in view of Agresta and Ghosal. Motivation to incorporate a CYP3A4/5 inhibitor to the mitapivat regimen of U.S. Patent 11,234,976 flows logically from the fact that mitapivat is both metabolized by and induces cytochrome P450 3A4, resulting in a poor pharmacokinetic profile in administered subjects and it was known in the art to incorporate the CYP3A4/5 inhibitor boceprevir to improve the pharmacokinetic profile of therapeutic agents metabolized by CYP3A4/5. As such, said artisan would have applied the teachings of Ghosal and Agresta to the mitapivat regimen of U.S. Patent 11,234,976 and readily predicted that incorporation of boceprevir to the mitapivat regimen would have improved the pharmacokinetic profile of mitapivat in the administered subject.

55.

Conclusion

56. In view of the rejections set forth above, no claim is allowed.

57. 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, Winston Shen can be reached on 571-272-3157. 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 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 1628

58. /THEODORE R. HOWELL/
Primary Examiner, Art Unit 1628

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/Michelle Lombardo
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:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
Extension-of-Time:				

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension - 1 month with $0 paid	1251	1	220	220
Miscellaneous:				
			Total in USD ($)	**220**

Electronic Acknowledgement Receipt

EFS ID:	47056396
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
Filer:	Andrew Ehle
Filer Authorized By:	
Attorney Docket Number:	128748-01502
Receipt Date:	18-NOV-2022
Filing Date:	12-JUN-2020
Time Stamp:	16:26:51
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	$220
RAM confirmation Number	E2022AHG27301791
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Amendment/Request for Reconsideration-After Non-Final Rejection	Response_to_Non-Final_Office_Action.pdf	52262 d5c5def4c5f56ef49fb689aa6c8822907efd1b7b	no	9
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	38395 7408b044a3c580e44061ffbaa63bc5b8057d2941	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	90657		

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.

New Applications Under 35 U.S.C. 111

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.

National Stage of an International Application under 35 U.S.C. 371

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.

New International Application Filed with the USPTO as a Receiving Office

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.

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: 1628

For: MITAPIVAT THERAPY AND MODULATORS OF CYTOCHROME P450 | Examiner: George W. Kosturko

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

AMENDMENT IN RESPONSE TO NON-FINAL OFFICE ACTION UNDER 37 C.F.R. § 1.111

This response, along with a request for one-month extension of time, is filed in reply to the Office Action mailed July 18, 2022. Please amend the above-identified U.S. patent application as follows:

Amendments to the Claims are reflected in the listing of claims which begins on page 2 of this paper.

Remarks begin on page 4 of this paper.

AMENDMENTS TO THE CLAIMS

11-46. (**Cancelled**)

47. (**New**) A method of treating hemolytic anemia in a subject, comprising administering to the subject about 10 mg/day, about 40 mg/day, or about 50 mg/day of mitapivat and a moderate inducer of cytochrome P450 3A4/5 (CYP3A4/5).

48. (**New**) The method of claim 47, wherein the subject is administered about 10 mg/day of mitapivat.

49. (**New**) The method of claim 48, wherein the subject is administered about 5 mg of mitapivat twice per day.

50. (**New**) The method of claim 47, wherein the subject is administered about 40 mg/day of mitapivat.

51. (**New**) The method of claim 50, wherein the subject is administered about 20 mg of mitapivat twice per day.

52. (**New**) The method of claim 47, wherein the subject is administered about 50 mg/day of mitapivat.

53. **(New)** The method of claim 52, wherein the subject is administered about 50 mg of mitapivat once per day.

51. (**New**) The method of claim 47, wherein the mitapivat is orally administered as part of a tablet composition.

52. (**New**) The method of claim 49, wherein the mitapivat is orally administered as part of a tablet composition.

53. (**New**) The method of claim 51, wherein the mitapivat is orally administered as part of a tablet composition.

54. (**New**) The method of claim 53, wherein the mitapivat is orally administered as part of a tablet composition.

55. (**New**) The method of claim 51, wherein the tablet composition further comprises microcrystalline cellulose, mannitol, croscarmellose sodium, and sodium stearyl fumarate.

56. (**New**) The method of claim 52, wherein the tablet composition further comprises microcrystalline cellulose, mannitol, croscarmellose sodium, and sodium stearyl fumarate.

57. (**New**) The method of claim 53, wherein the tablet composition further comprises microcrystalline cellulose, mannitol, croscarmellose sodium, and sodium stearyl fumarate.

58. (**New**) The method of claim 54, wherein the tablet composition further comprises microcrystalline cellulose, mannitol, croscarmellose sodium, and sodium stearyl fumarate.

REMARKS

I. Status of Claims

Prior to the entry of the instant amendments, claims 1-5, 11-12, 14-17, 22, 32, 34, 36-38, and 44 were pending in this application. Claims 1-5, 11-12, 14-17, 22, 32, 34, 36-38, and 44 have been cancelled without prejudice. Claims 47-58 are new. Support for new claims 47, 48, 50, and 52 can be found, at least, for example, on page 3, lines 6-9, page 17, lines 9-1, 13-14, and 24-26, page 32, line 26, original claim 36, and throughout the specification as filed. Support for new claims 49, 51, and 53 can be found, at least, for example, on page 19, lines 13-15; page 19, lines 18-20; and page 17, lines 24-26, respectively. Support for new claims 51-58, can be found, at least, for example, on page 13, lines 4-9. Thus, upon entry of these amendments, claims 47-55 will be pending in this application.

The amendments to the claims were made solely in the interest of expediting prosecution of the instant application. *No new matter has been added.* Applicants reserve the right to pursue the claims as originally filed in this or a separate application(s).

II. 35 U.S.C. §112 – Indefiniteness

Claim 17 has been rejected under 35 U.S.C. 112(b), for allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter. *See Office Action, pages 2-3.* Claim 17, however, has been cancelled without prejudice. Reconsideration and withdrawal of this rejection is respectfully requested.

III. 35 U.S.C. §102 – Novelty

Claims 14-17 have been rejected under 35 U.S.C. § 102(a)(1), as allegedly anticipated by International Patent Publication No. WO 2016201227 (hereafter referred to as "Agresta"). *See Office Action, pages 3-4.* Applicant respectfully disagrees. However, to facilitate prosecution, claims 14-17 have been cancelled and new claims 47-56 require administration of a moderate CYP3A4/5 inducer. As noted by the Examiner, Agresta does not disclose administration with inhibitors or inducers of CYP3A4/5. Thus, Agresta fails to teach or disclose all elements of the

instant claims. Accordingly, the pending claims are novel. Reconsideration and withdrawal of this rejection is respectfully requested.

IV. 35 U.S.C. § 103(a) – Obviousness

A. Standard of Review

Procedurally, the USPTO bears the initial burden of establishing a *prima facie* case of obviousness. *MPEP § 2142.* To establish a *prima facie* case of obviousness within the meaning of § 103(a), it is necessary to establish that "a person of ordinary skill at the time of the invention would have selected and combined those prior art elements [relied upon to support the rejection] in the normal course of research and development to yield the claimed invention." *Unigene Laboratories, Inc. v. Apotex, Inc.*, 655 F.3d 1352, 1360 (Fed. Cir. 2011). That is, an Examiner must step back in time and into the shoes of the hypothetical person of one of ordinary skill in the art when the invention was unknown and just before it was made. *MPEP § 2142.*

In doing so, it is important to "identify a reason that would have prompted [one of skill in the art] to combine the elements in the way the claimed new invention does." *See KSR Int'l Co. v. Teleflex Inc., 550 U.S. 398 (2007).* Extending that decision, the Federal Circuit has stated that it remains necessary in an obviousness rejection to (1) "articulate a reason why a PHOSITA would combine the prior art references"; (2) have an adequate evidentiary basis for that finding; and (3) provide a "satisfactory explanation" for the motivation finding that includes an express and "rational" connection with the evidence presented. *See, In re NuVasive, Inc., 842 F.3d 1376 (2016).*

Here, based on the prior art relied in the present Office Action, one of skill in the art would not have had the requisite motivation to arrive at the pending claims.

B. Agresta in view of Ghosal

Prior pending claims 1-5, 11-12, 22, and 32 were rejected under 35 U.S.C. § 103(a), for allegedly being obvious in view of Agresta in combination with International Patent Publication No. WO 2012015712 (hereafter referred to as "Ghosal"). *See Office Action, pages 5-7.* The Examiner relies on Agresta for allegedly teaching that mitapivit induces CYP3A4/5, which has been found to metabolize mitapivit and therefore lead to reduced exposure. *See id at 5.* Because, however, Agresta

fails to specifically describe the addition of a CYP3A4/5 to mitigate this effect, the Examiner relies on Ghosal, who allegedly shows that adding CYP3A4/5 inhibitors in combination with drugs that are metabolized by CYP3A4/5 improve the drugs pharmacokinetics. *See id at 6*. From this, the Examiner contends that one of skill in the art would have been motivated to include a CYP3A4/5 inhibitor as part of the treatment regimen with mitapivit. Applicant disagrees.

However, to facilitate prosecution, the pending claims are no longer directed to administering an inhibitor of CYP3A4/5 with mitapivit, but rather a CYP3A4/5 inducer. One of skill in the art armed with Agresta and Ghosal would *not* have been motivated to administer an inducer of CYP3A4/5 with mitapivit because, as the Examiner contends, doing so would have decreased exposure and led to an overall reduction in the pharmacokinetics of the drug. Indeed, this would be the opposite of what Ghosal is trying to prevent by adding an CYP3A4/5 inhibitor.

A *prima facie* case of obviousness does not exist over the combination of Agresta and Ghosal. Reconsideration and withdrawal of this rejection is respectfully requested.

C. *Agresta in view of Ghosal and Salituro*

Claim 34 has been rejected under 35 U.S.C. § 103(a), for allegedly being obvious in view of Agresta in combination with Ghosal in further combination with U.S. Patent Publication US 2010/0331307 (hereafter referred to as "Salituro"). *See Office Action, pages 7-8.* Applicant disagrees. Nonetheless, to expedite prosecution, the pending claims now require administering a moderate inducer of cytochrome P450 3A4/5 (CYP3A4/5). As discussed above, the combination of Agresta and Ghosal do not motivate one of skill in the art to administer an inducer of CYP3A4/5, much less with the specific amounts of mitapivit claimed, and Salituro fails to remedy this lack of motivation. Reconsideration and withdrawal of this rejection is respectfully requested.

D. Agresta in view of Ghosal and Synold

Claims 36-37 and 44 have been rejected under 35 U.S.C. § 103(a), for allegedly being obvious in view of Agresta in combination with Ghosal in further combination with U.S. Patent Publication US 2001/0041706 (hereafter referred to as "Synold"). *See Office Action, pages 8-10.* Applicant disagrees for the reasons, above, i.e., Synold, like Salituro, fails to motivate one of skill in

the art to administer an inducer of CYP3A4/5 when read in combination with Agresta and Ghosal. As such, a *prima facie* case of obviousness is not established based on this combination fo references. Reconsideration and withdrawal of this rejection is respectfully requested.

E. Agresta in view of Jannetta

Claims 1, 22, 32, and 38 have been rejected under 35 U.S.C. § 103(a), for allegedly being obvious in view of Agresta in combination with U.S. Patent Publication US 2002/0055495 (hereafter referred to as "Jannetta"). *See Office Action, pages 10-11.* Agresta is discussed above, and because Agresta does not teach the use of a CYP3A4/5 inducer, the Examiner relies on Jannetta to allegedly show that dexamethasone is an art recognized inducer of CYP3A4/5 which can be used to treat nausea and associate symptoms. *See id at 10.* From this, the Examiner contends that it would have been obvious to administer dexamethasone with mitaptivit at higher dosages of 700 mg to reduce nausea and emesis. *See id.* Applicant disagrees.

Reiterated form above, a prima facie case requires (1) an articulated reason why a one of skill in the art would combine the prior art references; (2) have an adequate evidentiary basis for that finding; and (3) provide a "satisfactory explanation" for the motivation finding that includes an express and "rational" connection with the evidence presented. *See, In re NuVasive, Inc., 842 F.3d 1376 (2016).* It was argued on page 5 of the Office Action that because Agresta allegedly teaches mitapivit is metabolized by CYP3A4/5, which therefore leads to reduced efficacy, that one of skill in the art would have found it obvious to administer a CYP3A4/5 inhibitor. This alleged teaching, however, has been ignored in this part of the rejection. Specifically, there is no evidence or explanation provided to support a reason why one of skill in the art would have considered Jannetta in the first place when Agresta states that mitapivit is metabolized by CYP3A4/5. The combination of mitapivit with a CYP3A4/5 does not flow logically from the references cited. For this reason alone, a *prima facie* case does not exist over Agresta and Jannetta.

Hypothetically, for arguments sake, even if one of skill in the art were to ignore the teachings of Agresta, the Examiner relies on Agresta's teaching that high dosages of mitapivit (e.g, 700 mg) leads to undesirable effects such as nausea and vomiting which could allegedly be mitigated by the CYP3A4/5 inducer, dexamethasone, of Janetta. First, Applicant claims are directed

to mitapivat dosages of about 10 mg/day, about 40 mg/day, or about 50 mg/day, not 700 mg. Agresta does not evidence that nausea and vomiting effects would occur at these lower dosages. Therefore, the allegations relating to dexamethasone effects does not apply to the pending claims. Next, dexamethasone is strong inducer of CYP3A4/5, whereas the pending claims required the use of a moderate CYP3A4/5 inducer. Similarly, there is no evidence of record to support that a moderate CYP3A4/5 inducer would have the same anti-nausea and anti-vomiting effects relied upon in the present rejection.

For at least these reasons, a *prima facie* case of obviousness does not exist. Reconsideration and withdrawal of this rejection is respectfully requested.

V. Double Patenting

Claims 1, 4-5, 11-12, 14, 17, 22, and 32 have been provisionally rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 1-16 of U.S. Patent No. 11,234,976 in view of Agresta and Ghosal. *See Office Action at 11-14*. Applicant disagrees.

An obvious-type double patenting analysis double patenting rejection is based on the claims, not the teachings in the prior art specifications or references. The Federal Circuit has made it clear that "[t]he focus of the obviousness-type double patenting doctrine … rests on preventing a patentee from claiming an obvious variant of what it has previously claimed, not what it has previously disclosed." See Eli Lilly & Co. v. Teva Parenteral Medicines, Inc., Nos. 11-1561, -1562 (Fed. Cir. Aug. 24, 2012). The "earlier patent's written description considered only to the extent necessary to construe its claims." See id. This is further stated in the examination guidelines in MPEP § 804(II)(B)(2)(a) where it states "when considering whether the invention defined in a claim of an application would have been an obvious variation of the invention defined in the claim of a patent or copending application, the disclosure of the patent [or reference] may not be used as prior art." Rather, the specification may be used a dictionary to construe terms or to assist in claim support.

Here, that is not the case. The present double patenting analysis is based on allegations stemming from the teaching of not one, but multiple prior art references. This is not proper and the rejection cannot stand.

Furthermore, the present double patenting rejection is predicated on the administration of a CYP3A4/5 inhibitor, not a CYP3A4/5 inducer to which the present claims are now drawn. Therefore, for the reason expressed above in the § 103 section, the pending claims are distinguished from Agresta and Ghosal. Reconsideration and withdrawal of this rejection is respectfully requested.

VI. Final Comments

Favorable reconsideration of the present application and timely allowance of the pending claims is respectfully requested. If a telephone conversation with Applicant's attorney would help expedite the prosecution of the above-identified application, the Examiner is respectfully invited to call the undersigned attorney at (617) 449-6585. Please charge any deficiency in the fees related to the filing of this paper to our Deposit Account No. 50-4876, under Order No. 128748-01502.

Dated: November 18, 2022

Respectfully submitted,

By /Andrew R. Ehle/

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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 16/900,610	Filing Date 06/12/2020	☐ To be Mailed

ENTITY: ☑ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA		RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A		N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A		N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*		x $100 =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*		x $460 =	
☐ 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).				
☐ 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 (37 CFR 1.16(i))	* 15	Minus	** 20	= 0		x $100 =	0
	Independent (37 CFR 1.16(h))	* 1	Minus	*** 3	= 0		x $480 =	0
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ 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 (37 CFR 1.16(i))	*	Minus	**	=		x $0 =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=		x $0 =	
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ 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.**

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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 02/07/2023

McCarter & English / Agios Pharmaceuticals
265 Franklin Street
Boston, MA 02110

EXAMINER	
KOSTURKO, GEORGE W	

ART UNIT	PAPER NUMBER
1628	

NOTIFICATION DATE	DELIVERY MODE
02/07/2023	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):

docket@mccarter.com

Office Action Summary	Application No. 16/900,610	Applicant(s) Iyer et al.	
	Examiner GEORGE W KOSTURKO	Art Unit 1628	AIA (FITF) Status Yes

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTHS FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☑ Responsive to communication(s) filed on 11/18/2022.

☐ A declaration(s)/affidavit(s) under **37 CFR 1.130(b)** was/were filed on _____.

2a)☑ This action is **FINAL.** 2b) ☐ This action is non-final.

3)☐ 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.

4)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims*

5) ☑ Claim(s) 47-58 is/are pending in the application.

5a) Of the above claim(s) _____ is/are withdrawn from consideration.

6) ☐ Claim(s) _____ is/are allowed.

7) ☑ Claim(s) 47-58 is/are rejected.

8) ☐ Claim(s) _____ is/are objected to.

9) ☐ Claim(s) _____ are subject to restriction and/or election requirement

* If any claims have been determined allowable, 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 or send an inquiry to **PPHfeedback@uspto.gov.**

Application Papers

10)☐ The specification is objected to by the Examiner.

11)☑ The drawing(s) filed on 08/19/2020 is/are: a)☑ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

Priority under 35 U.S.C. § 119

12)☐ 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)).

** See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☑ Notice of References Cited (PTO-892)

2) ☐ Information Disclosure Statement(s) (PTO/SB/08a and/or PTO/SB/08b) Paper No(s)/Mail Date _____.

3) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date _____.

4) ☐ Other: _____.

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 47-58 filed November 18th 2022 are currently pending.

2.

Response to Amendment

3. Applicant's amendments, file 11/18/2022 are acknowledged. Claims 11-46 have been canceled in their entirety. New claim 47 is directed to the treatment of hemolytic anemia in a subject comprising administering to the subject about 10 mg/day, about 40 mg/day or about 50 mg/day mitapivat and a moderate inducer of cytochrome P450 3A4/5.

4. Applicant's arguments, filed 11/18/2022 have been fully considered. Rejections and/or objections not reiterated from the previous Office Action are hereby withdrawn. The following rejections and/or objections are either reiterated or newly applied. They constitute the complete set of rejections and objections presently being applied to the instant application.

5.

Claim Rejections - 35 USC § 112-Paragraph D

6. The following is a quotation of 35 U.S.C. 112(d):

> (d) REFERENCE IN DEPENDENT FORMS.—Subject to subsection (e), a claim in dependent form shall contain a reference to a claim previously set forth and then specify a further limitation of the subject matter claimed. A claim in dependent form shall be construed to incorporate by reference all the limitations of the claim to which it refers.

The following is a quotation of pre-AIA 35 U.S.C. 112, fourth paragraph:

> Subject to the following paragraph [i.e., the fifth paragraph of pre-AIA 35 U.S.C. 112], a claim in dependent form shall contain a reference to a claim previously set forth and then specify a further limitation of the subject matter claimed. A claim in dependent form shall be construed to incorporate by reference all the limitations of the claim to which it refers.

7. Claims 53-54 are rejected under 35 U.S.C. 112(d) or pre-AIA 35 U.S.C. 112, 4th paragraph, as being of improper dependent form for failing to further limit the subject matter of the claim upon which it depends, or for failing to include all the limitations of the claim upon which it depends.

8. Claims 53-54 depend upon claim 51 and are directed to wherein the mitapivat is orally administered as part of a tablet composition. Claim 51 is directed to the method of claim 47 wherein the mitapivat is orally administered as part of a tablet composition. The scope of claims 53-54 fail to further limit the scope of claim 51 as claim 51 already requires the administration of mitapivat in tablet form. Applicant may cancel the claim(s), amend the claim(s) to place the claim(s) in proper dependent form, rewrite the claim(s) in independent form, or present a sufficient showing that the dependent claim(s) complies with the statutory requirements.

9.

Claim Rejections - 35 USC § 103

10. In the event the determination of the status of the application as subject to AIA 35 U.S.C. 102 and 103 (or as subject to pre-AIA 35 U.S.C. 102 and 103) is incorrect, any correction of the statutory basis for the rejection will not be considered a new ground of rejection if the prior art relied upon, and the rationale supporting the rejection, would be the same under either status.

11. The factual inquiries for establishing a background for determining obviousness under 35 U.S.C. 103 are summarized as follows:

1. Determining the scope and contents of the prior art.

2. Ascertaining the differences between the prior art and the claims at issue.

3. Resolving the level of ordinary skill in the pertinent art.

4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

12. Claim(s) 47-54 are rejected under 35 U.S.C. 103 as being unpatentable over Kassa (BMC Hematology pages 1-11 published 2016), in view of Chen (Annals of Clinical Microbiology and Antimicrobials Vol. 5 pages 1-11, published 2006) and Agresta (WO2016/201227 published 12/15/2016).

13. Kassa (BMC Hematology pages 1-11 published 2016) teaches the effect of anti-tuberculosis drugs on hematological profiles of tuberculosis patients. Kassa teaches tuberculosis may present significant hematological disorders and anti-TB drugs have serious side effects (abstract). Kassa teaches the anti-tuberculosis therapeutic rifampicin. Rifampicin is taught as an effective anti-tuberculosis therapeutic but has serious side effects and adverse drug reactions, such as leucopenia and thrombocytopenia (page 2 right col., page 9 right col.) As evidenced by Chen (Annals of Clinical Microbiology and Antimicrobials Vol. 5 pages 1-11, published 2006), rifampicin is art-recognized as an inducer of CYP 3A4 (page 4 right col.). Kassa teaches that rifampicin may directly cause hemolytic anemia in tuberculosis patients (page 9 right col.).

14. However, Kassa does not specifically teach treating the tuberculosis patient with hemolytic anemia being treated with rifampicin comprising administering mitapivat to the subject in need.

15. Agresta (WO2016/201227 published 12/15/2016) teaches the method of treating hemolytic anemia in a subject in need comprising administering a therapeutically effective amount of Compound 1 (page 3 lines 1-5, page 8 paragraph 4, page 11 lines 1-5). As shown in page 28 of the specification, compound 1 or N-(4-(4-(cyclopropylmethyl)piperazine-1-carbonyl)phenyl)quinoline-8-sulfonamide corresponds to the claimed mitapivat. See ([0002] of the instant specification). Agresta teaches administering mitapivat in a dose of 10-60 mg every 12 hours to treat the disclosed hematological disorders, which overlaps with the amounts of mitapivat embraced within the instant claims (page 11, page 33 paragraph 5). Thus, the administration of 20 or 25 mg/day, twice a day embraced within Agresta overlaps with the dosing cycle embodied in the presently claimed. Applicant is reminded of MPEP 2144.05 wherein the claimed ranges "overlap or lie inside ranges disclosed by the prior art" a prima facie case of obviousness exists. *In re Wertheim*, 541 F.2d 257, 191 USPQ 90 (CCPA 1976); *In re Woodruff*, 919 F.2d 1575, 16 USPQ2d 1934 (Fed. Cir. 1990). Agresta additionally teaches that mitapivat is administered in tablet form to said patients with anemia (page 28 paragraph 1, page 33 paragraphs 2-5).

16. Therefore, one of ordinary skill in the art prior to the time of the invention would have found it prima facie obvious to administer mitapivat to the tuberculosis patient with hemolytic anemia being treated with rifampicin taught by Kassa in view of Agresta. Motivation to administer mitapivat to a tuberculosis patient with hemolytic anemia being treated with rifampicin logically flows from the fact that mitapivat was taught in the art as being efficacious at treating hemolytic anemia in a subject. Accordingly, said artisan would have readily predicted that co-administration of mitapivat to the tuberculosis

patient with hemolytic anemia being treated with rifampicin would have effectively treated the hematological malignancy in the tuberculosis patient. It is further noted that the administration of mitapivat to the tuberculosis patient with hemolytic anemia being treated with rifampicin to treat hemolytic anemia in the subject results in the administration of a CYP3A4 inducer in combination with mitapivat, which reads on the presently claimed.

17. Lastly, it is noted that the combination of Kassa, Chen and Agresta do not specifically teach wherein 5 mg of mitapivat is administered twice a day (claims 47,49). However, the optimum dosing amount and frequency of administration of the hemolytic anemia treating mitapivat to the tuberculosis patient with hemolytic anemia being treated with rifampicin would have been a matter well within the insight of one of ordinary skill in the art. Such a determination would have been made in accordance with a variety of factors, such as the route of administration, pharmacological considerations, such as activity, efficacy, pharmacokinetics and toxicology profiles of the combined regimen, as well as the age, weight, sex, diet and severity of the medical condition of the patient. Thus, the dosing cycle and frequency of administration regimen that would have been employed would have varied widely and, in the absence of evidence to the contrary, the current claimed specific administration regimen is not seen to be inconsistent with one that would have been determined by the skilled artisan. Furthermore, absent and evidence demonstrating a patentable difference between the dosing amounts and the criticality of the claimed frequency and dosing cycles, the determination of the optimum or workable frequency of administration given the guidance of the prior art would have been generally prima facie obvious to the skilled

artisan. Please see MPEP 2144.05 [R-2](II)(A) and *In re Aller*, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955)("[W]here the general conditions of the claim are disclosed in the prior art, it is not inventive to discover the workable ranges by routine experimentation.")

18.

19. Claim(s) 55-58 are rejected under 35 U.S.C. 103 as being unpatentable over Kassa (BMC Hematology pages 1-11 published 2016), in view of Chen (Annals of Clinical Microbiology and Antimicrobials Vol. 5 pages 1-11, published 2006) and Agresta (WO2016/201227 published 12/15/2016) as applied to claims 47-54 in view of Sizemore (WO2019/104134 published 05/31/2019 with priority to U.S. Provisional Application 62589822 filed 11/22/2017).

20. As discussed above, the combination of Kassa, Chen and Agresta render obvious the administration of mitapivat to a tuberculosis patient being treated with rifampicin and comprising hemolytic anemia as mitapivat is art-recognized at treating hemolytic anemia in a subject in need. It is further noted that the administration of mitapivat to the tuberculosis patient with hemolytic anemia being treated with rifampicin to treat hemolytic anemia in the subject results in the administration of a CYP3A4 inducer in combination with mitapivat, which reads on the presently claimed. The combination of Kassa, Chen and Agresta additionally teach that mitapivat is administered in tablet form to said patients with anemia (page 28 paragraph 1, page 33 paragraphs 2-5).

21. However, the combination of Kassa, Chen and Agresta do not specifically teach wherein the tablet comprises microcrystalline cellulose, mannitol, croscarmellose sodium and sodium stearyl fumarate.

22. Sizemore (WO2019/104134 published 05/31/2019 with priority to U.S. Provisional Application 62589822 filed 11/22/2017) teaches mitapivat compositions. Sizemore teaches that said mitapivat compositions are formulated into tablets further comprising dispersing agents, binding agents, lubricants and granulating agents ([0094]-[0096], [0110]). Sizemore teaches that croscarmellose sodium is a suitable granulating agent, mannitol and microcrystalline cellulose are suitable binding agents, and sodium stearyl fumarate as a suitable lubricant for the mitapivat composition ([0095]-[0110]).

23. Therefore, one of ordinary skill in the art prior to the time of the invention would have found it prima facie obvious to formulate the mitapivat tablet composition administered to the tuberculosis patient with hemolytic anemia being treated with rifampicin and mitapivat as taught by the combination of Kassa, Chen and Agresta, with the formulants microcrystalline cellulose, mannitol, croscarmellose sodium and sodium stearyl fumarate in view of Sizemore.

24. MPEP 2143 provides rationale for a conclusion of obviousness including (A) Combining prior art elements according to known methods to obtain predictable results;

25. In the present case, it was known in the art that croscarmellose sodium is a suitable granulating agent, mannitol and microcrystalline cellulose are suitable binding agents, and sodium stearyl fumarate as a suitable lubricant for mitapivat tablet compositions. Accordingly, said artisan would have readily predicted that a mitapivat

tablet, formulated with microcrystalline cellulose, mannitol, croscarmellose sodium and sodium stearyl fumarate would have effectively treated hemolytic anemia in the tuberculosis patient with hemolytic anemia being treated with rifampicin and mitapivat. 26.

Conclusion

27. In view of the rejection set forth above, no claim is allowed.

28. Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL**. See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

29. 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, Winston Shen can be reached on 571-272-3157. 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 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 1628

/THEODORE R. HOWELL/
Primary Examiner, Art Unit 1628

Electronic Acknowledgement Receipt

EFS ID:	47807967
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
Filer:	Andrew Ehle
Filer Authorized By:	
Attorney Docket Number:	128748-01502
Receipt Date:	07-APR-2023
Filing Date:	12-JUN-2020
Time Stamp:	15:18:44
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Applicant Arguments/Remarks Made in an Amendment	128748_01502_Response_to_Final_OA_April_2023.PDF	161103 d1a048fb9c6b1a6c979cfb7be5968c3deee4814c	no	7

Warnings:

Information:	
Total Files Size (in bytes):	161103

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New Applications Under 35 U.S.C. 111

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.

National Stage of an International Application under 35 U.S.C. 371

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.

New International Application Filed with the USPTO as a Receiving Office

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.

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: 1628

For: MITAPIVAT THERAPY AND MODULATORS OF CYTOCHROME P450 | Examiner: George W. Kosturko

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

RESPONSE TO FINAL OFFICE ACTION AND INTERVIEW SUMMARY

In reply to the final Office Action mailed February 7, 2023, the two-month date for reply being April 7, 2023, Applicant requests favorable reconsideration of this application in view of the amendments and remarks.

Amendments to the Claims begin on page 2 of this paper.

Remarks begin on page 4 of this paper.

AMENDMENTS TO THE CLAIMS

1-58. **(Cancelled)**

59. **(New)** 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 and a moderate inducer of cytochrome P450 3A4/5 (CYP3A4/5).

60. **(New)** The method of claim 59, wherein the subject is administered about 10 mg/day of mitapivat.

61. **(New)** The method of claim 60, wherein the subject is administered about 5 mg of mitapivat twice per day.

62. **(New)** The method of claim 59, wherein the subject is administered about 40 mg/day of mitapivat.

63. **(New)** The method of claim 62, wherein the subject is administered about 20 mg of mitapivat twice per day.

64. **(New)** The method of claim 59, wherein the subject is administered about 100 mg/day of mitapivat.

65. **(New)** The method of claim 64, wherein the subject is administered about 50 mg of mitapivat once twice per day.

66. **(New)** The method of claim 59, wherein the subject is administered about 200 mg/day of mitapivat.

67. (**New**) The method of claim 66, wherein the subject is administered about 100 mg of mitapivat once twice per day.

68. (**New**) The method of claim 59, wherein the mitapivat is orally administered as part of a tablet composition.

69. (**New**) The method of claim 61, wherein the mitapivat is orally administered as part of a tablet composition.

70. (**New**) The method of claim 63, wherein the mitapivat is orally administered as part of a tablet composition.

71. (**New**) The method of claim 65, wherein the mitapivat is orally administered as part of a tablet composition.

72. (**New**) The method of claim 67, wherein the mitapivat is orally administered as part of a tablet composition.

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

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

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

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

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

REMARKS

I. Status of Claims

Applicant noticed that the claims filed with the prior response inadvertently contained two sets of claims for claim numbers 51-53, i.e., there were two claims for Claim 51, two claims for Claim 52, and two claim for Claim 53. To avoid any confusion, all of the pending claims are cancelled and replaced by new Claims 59-77. But for 1) the replacing of 100 mg/day for 50 mg/day in prior Claim 47 and the first Claim 52, 2) replacing once per day with twice per day for the first Claim 53, 3) updating the dependencies for the second Claim 53 and Claim 54, 4) adding 200 mg/day in prior claim 47, and 5) adding new claims 66, 67, 72, and 77 specifying administration of 200 mg/day, 100 mg twice per day, and tablet compositions, these new claims are identical to those prior pending. Support for these amendments can be found, at least, for example, in paragraph 0090 to 0093 of the specification as filed. No new matter has been added. Upon entry of this amendment, Claims 59-77 are pending.

II. Examiner Interviews

Applicant thanks the Examiner for his courtesy extended to Applicant's representative during the phone interviews of March 20, 2023 and April 6, 2023. Upon review of the final Office Action, Applicant noticed that prior art relied upon is directed to a strong inducer of CYP 3A4 (rifampicin), whereas the pending claims are directed to a moderate inducer of CYP 3A4. Since there are no comments in the Office Action addressing this distinction, Applicant assumed this claim feature (i.e., moderate inducer of CYP 3A4) was overlooked, or that rifampicin was inadvertently considered to be something other than a strong inducer of CYP 3A4. Applicant telephoned the Examiner on March 20, 2023 to obtain clarification. Based on that discussion, the Examiner indicated that he would reconsider the cited art, and should it fail to disclose a moderate inhibitor of CYP 3A4, he would issue a communication before the April 7th two-month date for responding to the subject Office Action. Nothing was issued by April 5th so Applicant's representative telephoned the Examiner on April 6, 2023, and he requested that a paper response be filed.

III. Rejection Under 35 U.S.C. § 112(d)

Claims 53 and 54 have been rejected under 35 U.S.C. § 112(d) as allegedly being of improper dependent form. See Office Action at 2-3. As explained above, Claims 51-53 were duplicative, hence causing the dependency issues. Nonetheless, this issue no longer applied in view of the replacement claims submitted herewith.

IV. 35 U.S.C. § 103(a) – Obviousness

Claims 47-54 have been rejected under 35 U.S.C. §103(a) for allegedly being obvious in view of Kassa (BMC Hematology pages 1-11, published 2016) in combination with Chen (Annals of Clinical Microbiology and Antimicrobials Vol. 5 pages 1-11, published 2006) and Agresta (WO 2016/201227). *See Office Action at 4-7*. In short, the Examiner contends that Kassa teaches all but treating a tuberculosis patient with hemolytic anemia being treated with rifampicin (an inducer of CYP 3A4; evidenced by Chen) comprising further administering mitapivat. *See id.* The Examiner tries to cure this defect with Agresta, which teaches the administration of mitapivat for treating hemolytic anemia. The Examiner contends that it would have been obvious to combine these references to arrive at the instant claims. Applicant respectfully disagrees.

As stated above, rifampicin is a strong inducer of CYP 3A4 (see specification at [00159]). The pending claims, however, are directed to methods for treating hemolytic anemia comprising administering mitapivat and a moderate inducer of cytochrome P450 3A4/5 (CYP3A4/5). There is nothing in the Office Action to establish why one of skill in the art would have been motivated to use a strong inducer of CYP 3A4, much less that there would be a reasonable expectation of success in doing so. The Examiner bears the initial burden to establish a *prima facie* case of obviousness. *See MPEP § 2142*. Here that has not been done and, therefore, the present rejection is not proper. Reconsideration and withdrawal of this rejection is respectfully requested.

The Examiner further alleges that Claims 55-58 would be obvious in view of Kassa, Chen, and Agresta in combination with Sizemore (WO 2019/104134) because Sizemore teaches tablets comprising microcrystalline cellulose, mannitol, croscarmellose sodium, and sodium stearyl fumarate. *See Office Action at 7-8*. Applicant respectfully disagrees, at least for the reasons stated above. A *prima facie* of obviousness has not been established using the prior art of record. Reconsideration and withdrawal of this rejection is respectfully requested.

V. Final Comments

Favorable reconsideration of the present application and timely allowance of the pending claims is respectfully requested. If a telephone conversation with Applicant's attorney would help expedite the prosecution of the above-identified application, the Examiner is respectfully invited to call the undersigned attorney at (617) 449-6585. Please charge any deficiency in the fees related to the filing of this paper to our Deposit Account No. 50-4876, under Order No. 128748-01502.

Dated: April 7, 2023

Respectfully submitted,

By /Andrew R. Ehle/
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PTO/SB/06 (09-11)
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PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 16/900,610	Filing Date 06/12/2020	☐ To be Mailed

ENTITY: ☑ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA		RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A		N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A		N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*		x $100 =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*		x $460 =	
☐ 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).				
☐ 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 (37 CFR 1.16(i))	* 19	Minus	** 20	= 0		x $100 =	0
	Independent (37 CFR 1.16(h))	* 1	Minus	*** 6	= 0		x $480 =	0
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ 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 (37 CFR 1.16(i))	*	Minus	**	=		x $0 =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=		x $0 =	
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ 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.

Examiner-Initiated Interview Summary	**Application No.** 16/900,610	**Applicant(s)** Iyer et al.		
	Examiner GEORGE W KOSTURKO	**Art Unit** 1621	**AIA (First Inventor to File) Status** Yes	**Page** 1 of 1

All Participants (applicant, applicants representative, PTO personnel)	**Title**	**Type**
GEORGE W KOSTURKO	Examiner	Telephonic
ANDREW EHLE	Attorney of Record	

Date of Interview: 18 April 2023

Issues Discussed:

Objections

Discussed grammatical errors of "once twice a day" in claims 65 and 67. Discussed that 50 mg once a day does not get to the about 100 mg dose required in claim 65 and 100 mg once a day does not get ot the required dose of 200 mg in claim 67. Applicant agreed to cancel the phrase "once" in claims 65 and 67.

/GEORGE W KOSTURKO/ Examiner, Art Unit 1621	/THEODORE R. HOWELL/ Primary Examiner, Art Unit 1628

Applicant is reminded that a complete written statement as to the substance of the interview must be made of record in the application file. It is the applicants responsibility to provide the written statement, unless the interview was initiated by the Examiner and the Examiner has indicated that a written summary will be provided. See MPEP 713.04
Please further see:
MPEP 713.04
Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews, paragraph (b)
37 CFR § 1.2 Business to be transacted in writing

Applicant recordation instructions: It is not necessary for applicant to provide a separate record of the substance of interview.

Examiner recordation instructions: Examiners must summarize the substance of any interview of record. A complete and proper recordation of the substance of an interview should include the items listed in MPEP 713.04 for complete and proper recordation including the identification of the general thrust of each argument or issue discussed, a general indication of any other pertinent matters discussed regarding patentability and the general results or outcome of the interview, to include an indication as to whether or not agreement was reached on the issues raised.

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NOTICE OF ALLOWANCE AND FEE(S) DUE

190109 7590 05/03/2023

McCarter & English / Agios Pharmaceuticals
265 Franklin Street
Boston, MA 02110

EXAMINER
KOSTURKO, GEORGE W

ART UNIT	PAPER NUMBER
1621	

DATE MAILED: 05/03/2023

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	$0.00	$1200	08/03/2023

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.

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 05/03/2023
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	$0.00	$1200	08/03/2023

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).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/AIA/122 or PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/AIA/47 or PTO/SB/47; Rev 03-02 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 _____
2 _____
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

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.

❑ Applicant asserting small entity status. See 37 CFR 1.27

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.

❑ Applicant changing to regular undiscounted fee 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. _____

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

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 05/03/2023

McCarter & English / Agios Pharmaceuticals
265 Franklin Street
Boston, MA 02110

EXAMINER	
KOSTURKO, GEORGE W	
ART UNIT	PAPER NUMBER
1621	

DATE MAILED: 05/03/2023

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.

Notice of Allowability	Application No. 16/900,610	Applicant(s) Iyer et al.	
	Examiner GEORGE W KOSTURKO	Art Unit 1621	AIA (FITF) Status 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 04/07/2023.
   ☐ 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** or send an inquiry to **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. ☐ Notice of References Cited (PTO-892)
2. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____.
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material _____.
4. ☑ Interview Summary (PTO-413), Paper No./Mail Date. _____.
5. ☑ Examiner's Amendment/Comment
6. ☑ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/GEORGE W KOSTURKO/ Examiner, Art Unit 1621	/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 April 07, 2023 are currently pending.

2. EXAMINER'S AMENDMENT

3. An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in an interview with Attorney Andrew Ehle on 04/18/2023.

The application has been amended as follows:

Claim 65 is **AMENDED** as follows: --"The method of claim 64, wherein the subject is administered about 50 mg of mitapivat ~~once~~ twice per day"—.

Claim 67 is **AMENDED** as follows: --" The method of claim 64, wherein the subject is administered about 100 mg of mitapivat ~~once~~ twice per day"--.

Reasons for Allowance

4. The following is an examiner's statement of reasons for allowance: Applicant's arguments submitted 04/07/2023 are sufficient to overcome the rejection of record.

5. 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."

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 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

6.

/THEODORE R. HOWELL/
Primary Examiner, Art Unit 1628

Electronic Acknowledgement Receipt

EFS ID:	48387141
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
Filer:	Andrew Ehle
Filer Authorized By:	
Attorney Docket Number:	128748-01502
Receipt Date:	02-AUG-2023
Filing Date:	12-JUN-2020
Time Stamp:	18:44:32
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	no

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Specification	Application_Clean.pdf	252760 5d42a58e31f4570fade6986ef18d8c58700a85f7	no	63

Warnings:

Information:					
2	Specification	Application_Marked.pdf	257360 d774b89b97d89718b0a93f0fa990f2ed8269afc4	no	63
Warnings:					
Information:					
3	Amendment after Notice of Allowance (Rule 312)	128748_01502_312_Amendment_Aug_2023_F.pdf	24094 c548b2fa11cc599e2312e8e681c23390d695f04c	no	3
Warnings:					
Information:					
		Total Files Size (in bytes):	534214		

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.

New Applications Under 35 U.S.C. 111

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.

National Stage of an International Application under 35 U.S.C. 371

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.

New International Application Filed with the USPTO as a Receiving Office

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.

MITAPIVAT THERAPY AND MODULATORS OF CYTOCHROME P450

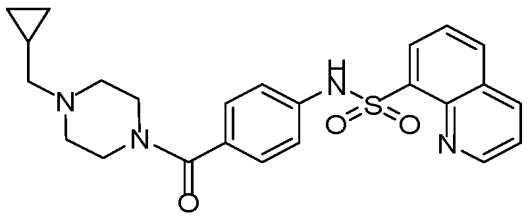
RELATED APPLICATIONS

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

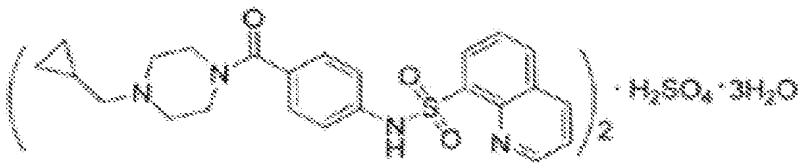
[0001] Pyruvate kinase deficiency (PKD) is a disease of the red blood cells caused by a deficiency of the pyruvate kinase R (PKR) enzyme due to recessive mutations of PKLR gene. PKR activators can be beneficial to treat PKD, thalassemia (*e.g.*, beta-thalassemia), abetalipoproteinemia or Bassen-Kornzweig 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.*, 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 hemylotic 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 hemylotic 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]

"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.*, 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_2 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 ≥33% reduction in the number of RBC units transfused within at least 5 weeks of treatment. In certain embodiments, reduction of transfusion burden is ≥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 ±10%. In one embodiment, "about" means ±5%. When "about" is present before a series of numbers of 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]

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

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 equivalent to about 5 mg to about 200 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 5 mg to about 100 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 5 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 10 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 15 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 20 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 25 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 30 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 35 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 40 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 45 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 50 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 55 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 60 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 65 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 70 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 75 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 80 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 85 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 90 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 95 mg of mitapivat. In certain embodiments,

mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 100 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 110 mg of mitapivat. In certain embodiments, 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 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 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 (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 $AUC_{0\text{-}24}$, $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, $AUC_{\%estrap}$) and C_{max} 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 administrated 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) 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 here 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 embodiments, for the method of any one of the embodiments, the CYP3A4/5 inducer is a mild CYP3A4/5 inducer. In another embodiments, for the method of any one of the embodiments, the CYP3A4/5 inhibitor is a mild CYP3A4/5 inhibitor. In another embodiments, 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 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, armodafninil, 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) ≤12.0 g/dL (if male) or ≤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) ≤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 α- to β-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 β-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 α-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 β-thalassemia intermedia, Hb E β-thalassemia, α-thalassemia (Hb H disease), or β-thalassemia with mutations of 1 or more α 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-liproteinemia (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 bisphosphate (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 ^{13}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

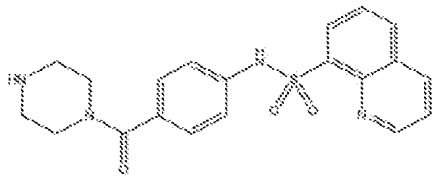
[00136] CYP-enzyme phenotyping using human liver microsomes and recombinant CYP enzymes revealed that mitapivat sulfate was primarily metabolized by CYP3A4/5 (>90%), with minor contributions from CYP2C9, CYP2C8, and CYP1A2. There was evidence of metabolism-dependent inhibition of CYP2C19 (largely reversible) 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 6β-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: $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, $AUC_{0\text{-}24}$, $AUC_{\%extrap}$, Cmax, CL/F (mitapivat only), Tlast, Tmax, t½, and Vz/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 t½) 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, $AUC_{0\text{-}t}$, and $AUC_{0\text{-}inf}$) 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_{0-t} (ng*hr/mL)	8860	14	1885	14	4.6998	4.2519 - 5.1949	15.05
AUC_{0-inf} (ng*hr/mL)	9475	14	1942	14	4.8780	4.4154 - 5.3890	14.97
C_{max} (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)
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)
Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the linear mixed effect model.
Geometric Mean Ratio (GMR) = test / reference
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_{0-t} and AUC_{0-inf} 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_{0-t} (ng*hr/mL)	300.0	14	197.1	14	1.5218	1.4268 - 1.6231	9.65
AUC_{0-inf} (ng*hr/mL)	338.6	14	216.2	14	1.5661	1.4784 - 1.6590	8.63
C_{max} (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) 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) Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the linear mixed effect model. Geometric Mean Ratio (GMR) = test / reference 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_{0-t} and AUC_{0-inf} 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_{0-t} (ng*hr/mL)	486.4	14	5565	14	0.0874	0.0719 - 0.1062	29.73
AUC_{0-inf} (ng*hr/mL)	496.4	14	5681	14	0.0874	0.0719 - 0.1062	29.84
C_{max} (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) 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) Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the linear mixed effect model. Geometric Mean Ratio (GMR) = test / reference 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_{0-t} and AUC_{0-inf} 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_{0-t} (ng*hr/mL)	230.5	14	623.0	14	0.3700	0.3316 - 0.4129	16.50
AUC_{0-inf} (ng*hr/mL)	244.9	14	662.6	14	0.3696	0.3321 - 0.4113	16.08
C_{max} (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) 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) Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the linear mixed effect model. Geometric Mean Ratio (GMR) = test / reference 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 (Emax and EC50) 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

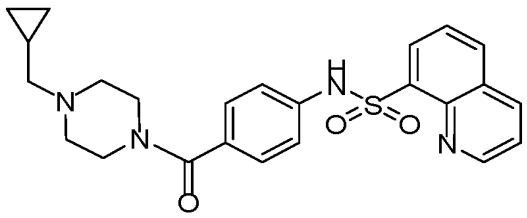
RELATED APPLICATIONS

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

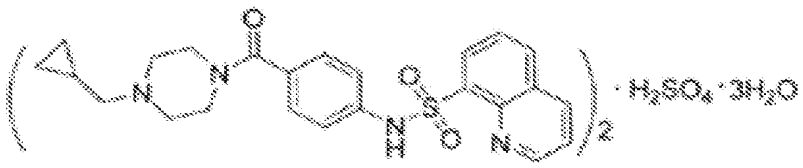
[0001] Pyruvate kinase deficiency (PKD) is a disease of the red blood cells caused by a deficiency of the pyruvate kinase R (PKR) enzyme due to recessive mutations of PKLR gene. PKR activators can be beneficial to treat PKD, thalassemia (*e.g.*, beta-thalassemia), abetalipoproteinemia or Bassen-Kornzweig 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 hemylotic 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 hemylotic 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 ≤ 1000 Da, or ≤ 750 Da, or ≤ 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 ≥ 80 percent.

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

[0018] A mild inducer decreases the AUC of a sensitive index CYP substrate by ≥ 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 ≥ 5-fold. A moderate inhibitor increases the AUC of a sensitive index CYP substrate by ≥ 2- to < 5-fold. A mild inhibitor increases the AUC of a sensitive index CYP substrate by ≥ 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 ≤ 1000 Da, or ≤ 750 Da, or ≤ 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 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_2 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 ≥33% reduction in the number of RBC units transfused within at least 5 weeks of treatment. In certain embodiments, reduction of transfusion burden is ≥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 ±10%. In one embodiment, "about" means ±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]

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

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 equivalent to about 5 mg to about 200 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 5 mg to about 100 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 5 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 10 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 15 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 20 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 25 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 30 mg of [[ ]]mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 35 mg of [[ ]]mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 40 mg of [[ ]]mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 45 mg of [[ ]]mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 50 mg of [[ ]]mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 55 mg of [[ ]]mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 60 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 65 mg of [[ ]]mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 70 mg of [[ ]]mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 75 mg of [[ ]]mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 80 mg of [[ ]]mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 85 mg of [[ ]]mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 90 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 95 mg of [[ ]]mitapivat. In certain embodiments,

mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 100 mg of [[ ]]mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 110 mg of [[ ]]mitapivat. In certain embodiments, 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 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 ~~thereofis~~ 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 $AUC_{0\text{-}24}$, $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, $AUC_{\%estrap}$) and C_{max} 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 administrated 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 here 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 embodiments, for the method of any one of the embodiments, the CYP3A4/5 inducer is a mild CYP3A4/5 inducer. In another embodiments, for the method of any one of the embodiments, the CYP3A4/5 inhibitor is a mild CYP3A4/5 inhibitor. In another embodiments, 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, armodafninil, 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) ≤12.0 g/dL (if male) or ≤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) ≤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 α- to β-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 β-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 α-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 β-thalassemia intermedia, Hb E β-thalassemia, α-thalassemia (Hb H disease), or β-thalassemia with mutations of 1 or more α 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-liproteinemia (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 bisphosphate (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 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 ^{13}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

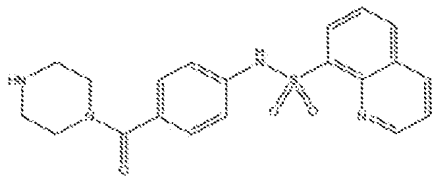
[00136] CYP-enzyme phenotyping using human liver microsomes and recombinant CYP enzymes revealed that mitapivat sulfate was primarily metabolized by CYP3A4/5 (>90%), with minor contributions from CYP2C9, CYP2C8, and CYP1A2. There was evidence of metabolism-dependent inhibition of CYP2C19 (largely reversible) 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 6β-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}, $AUC_{\%extrap}$, Cmax, CL/F (mitapivat only), Tlast, Tmax, t½, and Vz/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 t½) 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_{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_{0-t} (ng*hr/mL)	8860	14	1885	14	4.6998	4.2519 - 5.1949	15.05
AUC_{0-inf} (ng*hr/mL)	9475	14	1942	14	4.8780	4.4154 - 5.3890	14.97
C_{max} (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)
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)
Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the linear mixed effect model.
Geometric Mean Ratio (GMR) = test / reference
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_{0-t} and AUC_{0-inf} 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_{0-t} (ng*hr/mL)	300.0	14	197.1	14	1.5218	1.4268 - 1.6231	9.65
AUC_{0-inf} (ng*hr/mL)	338.6	14	216.2	14	1.5661	1.4784 - 1.6590	8.63
C_{max} (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)
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)
Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the linear mixed effect model.
Geometric Mean Ratio (GMR) = test / reference
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_{0-t} and AUC_{0-inf} 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_{0-t} (ng*hr/mL)	486.4	14	5565	14	0.0874	0.0719 - 0.1062	29.73
AUC_{0-inf} (ng*hr/mL)	496.4	14	5681	14	0.0874	0.0719 - 0.1062	29.84
C_{max} (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) 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) Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the linear mixed effect model. Geometric Mean Ratio (GMR) = test / reference 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_{0-t} and AUC_{0-inf} 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_{0-t} (ng*hr/mL)	230.5	14	623.0	14	0.3700	0.3316 - 0.4129	16.50
AUC_{0-inf} (ng*hr/mL)	244.9	14	662.6	14	0.3696	0.3321 - 0.4113	16.08
C_{max} (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) 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) Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the linear mixed effect model. Geometric Mean Ratio (GMR) = test / reference 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 (Emax and EC50) 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

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

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.

AMENDMENTS TO THE SPECIFICATION

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

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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(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	$1,200.00			$1,200.00	

EXAMINER	ART UNIT	CLASS-SUBCLASS
George W. Kosturko	1621	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. Steven G. Davis, 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. 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.

☐ Applicant asserting small entity status. See 37 CFR 1.27. 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.

☐ Applicant changing to regular undiscounted fee 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/ Date August 2, 2023

Typed or printed name Andrew R. Ehle Registration No. 78,384

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/Lauren Margolis
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:				
Pages:				
Claims:				
Miscellaneous-Filing:				
Petition:				
Patent-Appeals-and-Interference:				
Post-Allowance-and-Post-Issuance:				
UTILITY APPL ISSUE FEE	1501	1	1200	1200

Description	Fee Code	Quantity	Amount	Sub-Total in USD($)
Extension-of-Time:				
Miscellaneous:				
			Total in USD ($)	**1200**

Electronic Acknowledgement Receipt

EFS ID:	48384040
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
Filer:	Andrew Ehle
Filer Authorized By:	
Attorney Docket Number:	128748-01502
Receipt Date:	02-AUG-2023
Filing Date:	12-JUN-2020
Time Stamp:	13:05:15
Application Type:	Utility under 35 USC 111(a)

Payment information:

Submitted with Payment	yes
Payment Type	DA
Payment was successfully received in RAM	$1200
RAM confirmation Number	E202382D09238382
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

Document Number	Document Description	File Name	File Size(Bytes)/ Message Digest	Multi Part /.zip	Pages (if appl.)
1	Issue Fee Payment (PTO-85B)	128748_01502_Issue_Fee_Aug_2023.PDF	185434 139968079552245cb6fc52384065a8ec5064bbcd	no	1
Warnings:					
Information:					
2	Fee Worksheet (SB06)	fee-info.pdf	37934 77850b6f2be52dabcc832ca42344cf7fb2f9661f	no	2
Warnings:					
Information:					
		Total Files Size (in bytes):	223368		

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.

New Applications Under 35 U.S.C. 111

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.

National Stage of an International Application under 35 U.S.C. 371

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.

New International Application Filed with the USPTO as a Receiving Office

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.

PTO/SB/30EFS (01-22)
Approved for use through 05/31/2024. OMB 0651-0031
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REQUEST FOR CONTINUED EXAMINATION(RCE)TRANSMITTAL
(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		

This is a Request for Continued Examination (RCE) under 37 CFR 1.114 of the above-identified application.
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

SUBMISSION REQUIRED UNDER 37 CFR 1.114

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).

☐ 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.

- ☐ Consider the arguments in the Appeal Brief or Reply Brief previously filed on _____
- ☐ Other _____

☒ Enclosed

- ☐ Amendment/Reply
- ☐ Information Disclosure Statement (IDS)
- ☐ Affidavit(s)/ Declaration(s)
- ☒ Other e-Petition - withdrawal from issue after payment of the issue fee

MISCELLANEOUS

☐ Suspension of action on the above-identified application is requested under 37 CFR 1.103(c) for a period of months _____ (Period of suspension shall not exceed 3 months; Fee under 37 CFR 1.17(i) required)

☐ Other _____

FEES

The RCE fee under 37 CFR 1.17(e) is required by 37 CFR 1.114 when the RCE is filed.
☒ The Director is hereby authorized to charge any underpayment of fees, or credit any overpayments, to Deposit Account No 50-4876

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT REQUIRED

☒ Patent Practitioner Signature

Applicant Signature

PTO/SB/30EFS (01-22)
Approved for use through 05/31/2024. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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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.

MITAPIVAT THERAPY AND MODULATORS OF CYTOCHROME P450

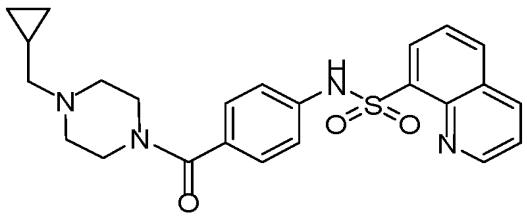
RELATED APPLICATIONS

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.

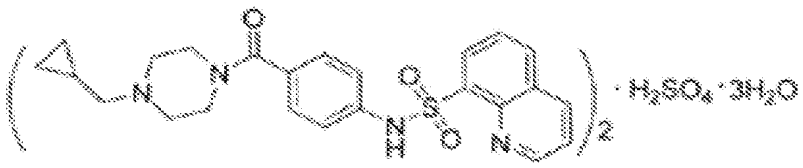
[0002] Mitapivat, also known as AG-348 or by its chemical name *N*-(4-(4-(cyclopropylmethyl)piperazine-1-carbonyl)phenyl)quinoline-8-sulfonamide, and is represented by the following structural formula:



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

Apotex Ex. 1002 Page 305

[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 ~~hemylotic~~ 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 ~~hemylotic~~ 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 ≥ 80 percent.

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

[0018] A mild inducer decreases the AUC of a sensitive index CYP substrate by ≥ 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 ≥ 5-fold. A moderate inhibitor increases the AUC of a sensitive index CYP substrate by ≥ 2- to < 5-fold. A mild inhibitor increases the AUC of a sensitive index CYP substrate by ≥ 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 ≤ 1000 Da, or ≤ 750 Da, or ≤ 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 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_2 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 ≥33% reduction in the number of RBC units transfused within at least 5 weeks of treatment. In certain embodiments, reduction of transfusion burden is ≥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 ±10%. In one embodiment, "about" means ±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

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 equivalent to about 5 mg to about 200 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 5 mg to about 100 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 5 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 10 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 15 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 20 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 25 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 30 mg of [[ ]]mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 35 mg of [[ ]]mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 40 mg of [[ ]]mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 45 mg of [[ ]]mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 50 mg of [[ ]]mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 55 mg of [[ ]]mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 60 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 65 mg of [[ ]]mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 70 mg of [[ ]]mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 75 mg of [[ ]]mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 80 mg of [[ ]]mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 85 mg of [[ ]]mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 90 mg of [[ ]]mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 95 mg of [[ ]]mitapivat. In certain embodiments,

mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 100 mg of [[ ]]mitapivat. In certain embodiments, [[ ]]mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 110 mg of [[ ]]mitapivat. In certain embodiments, [[ ]]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 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 ~~thereofis~~ 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 (~~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 $AUC_{0\text{-}24}$, $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, $AUC_{\%estrap}$) and C_{max} 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 administrated 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~~ 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 here 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 embodiments, for the method of any one of the embodiments, the CYP3A4/5 inducer is a mild CYP3A4/5 inducer. In another embodiments, for the method of any one of the embodiments, the CYP3A4/5 inhibitor is a mild CYP3A4/5 inhibitor. In another embodiments, 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, armodafninil, 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) ≤12.0 g/dL (if male) or ≤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) ≤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 α- to β-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 β-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 α-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 β-thalassemia intermedia, Hb E β-thalassemia, α-thalassemia (Hb H disease), or β-thalassemia with mutations of 1 or more α 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-liproteinemia (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 bisphosphate (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 ^{13}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

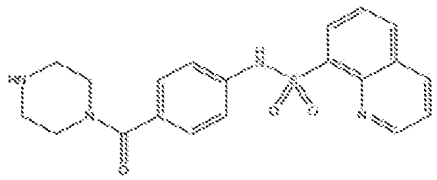
[00136] CYP-enzyme phenotyping using human liver microsomes and recombinant CYP enzymes revealed that mitapivat sulfate was primarily metabolized by CYP3A4/5 (>90%), with minor contributions from CYP2C9, CYP2C8, and CYP1A2. There was evidence of metabolism-dependent inhibition of CYP2C19 (largely reversible) 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 6β-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: $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, $AUC_{0\text{-}24}$, $AUC_{\%extrap}$, Cmax, CL/F (mitapivat only), Tlast, Tmax, t½, and Vz/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.*, Tmax and t½) 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]~~ **[00151]** To evaluate the effect of itraconazole and rifampin on mitapivat and the Metabolite [[ ]](primary objective), plasma mitapivat and the Metabolite PK parameters (Cmax, $AUC_{0\text{-}t}$, and $AUC_{0\text{-}inf}$) 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_{0-t} (ng*hr/mL)	8860	14	1885	14	4.6998	4.2519 - 5.1949	15.05
AUC_{0-inf} (ng*hr/mL)	9475	14	1942	14	4.8780	4.4154 - 5.3890	14.97
C_{max} (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)
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)
Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the linear mixed effect model.
Geometric Mean Ratio (GMR) = test / reference
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_{0-t} and AUC_{0-inf} 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]~~ **[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_{0-t} (ng*hr/mL)	300.0	14	197.1	14	1.5218	1.4268 - 1.6231	9.65
AUC_{0-inf} (ng*hr/mL)	338.6	14	216.2	14	1.5661	1.4784 - 1.6590	8.63
C_{max} (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)
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)
Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the linear mixed effect model.
Geometric Mean Ratio (GMR) = test / reference
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_{0-t} and AUC_{0-inf} 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]~~ **[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_{0-t} (ng*hr/mL)	486.4	14	5565	14	0.0874	0.0719 - 0.1062	29.73
AUC_{0-inf} (ng*hr/mL)	496.4	14	5681	14	0.0874	0.0719 - 0.1062	29.84
C_{max} (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)
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)
Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the linear mixed effect model.
Geometric Mean Ratio (GMR) = test / reference
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_{0-t} and AUC_{0-inf} 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_{0-t} (ng*hr/mL)	230.5	14	623.0	14	0.3700	0.3316 - 0.4129	16.50
AUC_{0-inf} (ng*hr/mL)	244.9	14	662.6	14	0.3696	0.3321 - 0.4113	16.08
C_{max} (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)
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)
Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the linear mixed effect model.
Geometric Mean Ratio (GMR) = test / reference
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 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]~~ **[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 (Emax and EC50) 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

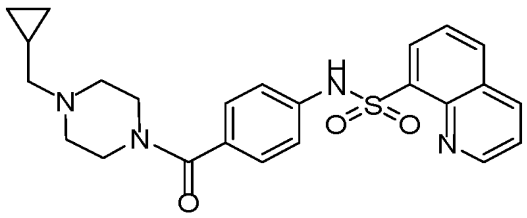
RELATED APPLICATIONS

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

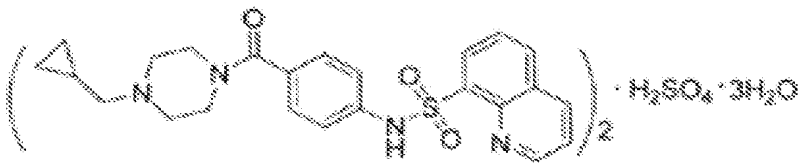
[0001] Pyruvate kinase deficiency (PKD) is a disease of the red blood cells caused by a deficiency of the pyruvate kinase R (PKR) enzyme due to recessive mutations of PKLR gene. PKR activators can be beneficial to treat PKD, thalassemia (*e.g.*, beta-thalassemia), abetalipoproteinemia or Bassen-Kornzweig 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_2 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 ≥33% reduction in the number of RBC units transfused within at least 5 weeks of treatment. In certain embodiments, reduction of transfusion burden is ≥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 ±10%. In one embodiment, "about" means ±5%. When "about" is present before a series of numbers of 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

equivalent to about 5 mg to about 200 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 5 mg to about 100 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 5 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 10 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 15 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 20 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 25 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 30 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 35 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 40 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 45 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 50 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 55 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 60 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 65 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 70 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 75 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 80 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 85 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 90 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 95 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 100 mg of mitapivat. In certain embodiments, mitapivat or a pharmaceutically acceptable salt thereof is formulated equivalent to about 110 mg of mitapivat. In certain embodiments,

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

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 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 $AUC_{0\text{-}24}$, $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, $AUC_{\%estrap}$) and C_{max} 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 administrated 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) 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 here 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 embodiments, for the method of any one of the embodiments, the CYP3A4/5 inducer is a mild CYP3A4/5 inducer. In another embodiments, for the method of any one of the embodiments, the CYP3A4/5 inhibitor is a mild CYP3A4/5 inhibitor. In another embodiments, 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, 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, armodafninil, 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) ≤12.0 g/dL (if male) or ≤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) ≤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 α- to β-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 β-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 α-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 β-thalassemia intermedia, Hb E β-thalassemia, α-thalassemia (Hb H disease), or β-thalassemia with mutations of 1 or more α 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-liproteinemia (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 bisphosphate (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 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 ^{13}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

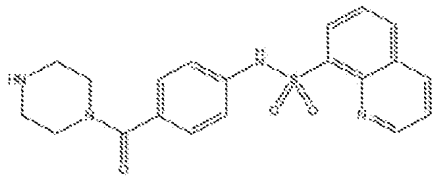
[00136] CYP-enzyme phenotyping using human liver microsomes and recombinant CYP enzymes revealed that mitapivat sulfate was primarily metabolized by CYP3A4/5 (>90%), with minor contributions from CYP2C9, CYP2C8, and CYP1A2. There was evidence of metabolism-dependent inhibition of CYP2C19 (largely reversible) 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 6β-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: $AUC_{0\text{-}t}$, $AUC_{0\text{-}inf}$, $AUC_{0\text{-}24}$, $AUC_{\%extrap}$, Cmax, CL/F (mitapivat only), Tlast, Tmax, t½, and Vz/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 t½) 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, $AUC_{0\text{-}t}$, and $AUC_{0\text{-}inf}$) 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.

[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 Cmax, 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_{0-t} (ng*hr/mL)	8860	14	1885	14	4.6998	4.2519 - 5.1949	15.05
AUC_{0-inf} (ng*hr/mL)	9475	14	1942	14	4.8780	4.4154 - 5.3890	14.97
C_{max} (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) 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) Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the linear mixed effect model. Geometric Mean Ratio (GMR) = test / reference 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_{0-t} and AUC_{0-inf} 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_{0-t} (ng*hr/mL)	300.0	14	197.1	14	1.5218	1.4268 - 1.6231	9.65
AUC_{0-inf} (ng*hr/mL)	338.6	14	216.2	14	1.5661	1.4784 - 1.6590	8.63
C_{max} (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) 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) Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the linear mixed effect model. Geometric Mean Ratio (GMR) = test / reference 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_{0-t} and AUC_{0-inf} 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

[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_{0-t} (ng*hr/mL)	486.4	14	5565	14	0.0874	0.0719 - 0.1062	29.73
AUC_{0-inf} (ng*hr/mL)	496.4	14	5681	14	0.0874	0.0719 - 0.1062	29.84
C_{max} (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) 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) Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the linear mixed effect model. Geometric Mean Ratio (GMR) = test / reference 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_{0-t} and AUC_{0-inf} 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.

[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_{0-t} (ng*hr/mL)	230.5	14	623.0	14	0.3700	0.3316 - 0.4129	16.50
AUC_{0-inf} (ng*hr/mL)	244.9	14	662.6	14	0.3696	0.3321 - 0.4113	16.08
C_{max} (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) 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) Geometric least-squares means (LSMs) are calculated by exponentiating the LSMs from the linear mixed effect model. Geometric Mean Ratio (GMR) = test / reference 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 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

[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 (Emax and EC50) 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.

Doc Code: PET.AUTO Document Description: Petition automatically granted by EFS-Web	PTO/SB/140 U.S. Patent and Trademark Office 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)**
Application Number	16900610
Filing Date	12-Jun-2020
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

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.

APPLICANT HEREBY PETITIONS TO WITHDRAW THIS APPLICATION FROM ISSUE UNDER 37 CFR 1.313(c).

A grantable petition requires the following items:
(1) Petition fee; and
(2) One of the following reasons:
(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;
(b) Consideration of a request for continued examination in compliance with § 1.114 (for a utility or plant application only); or
(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).

Petition Fee

- ◯ Small Entity
- ◯ Micro Entity
- ◉ Regular Undiscounted

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**

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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

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
Filer:	Andrew Ehle
Filer Authorized By:	
Attorney Docket Number:	128748-01502
Receipt Date:	04-AUG-2023
Filing Date:	12-JUN-2020
Time Stamp:	16:29:12
Application Type:	Utility under 35 USC 111(a)

Payment information:

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Payment was successfully received in RAM	$1500
RAM confirmation Number	E202384G29096823
Deposit Account	
Authorized User	
The Director of the USPTO is hereby authorized to charge indicated fees and credit any overpayment as follows:	

File Listing:

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 3e0929f5b4fbe6d5b201dc8798812c457b02deab	no	3
Warnings:					
Information:					
2	Amendment Submitted/Entered with Filing of Continued Prosecution Application (CPA)/Request for Continued Examination(RCE)	128748_01502_Aug_Amendment.pdf	35399 22bc5f8b989ac8835d5f7233001bfbdb451fdba4	no	6
Warnings:					
Information:					
3	Amendment Submitted/Entered with Filing of Continued Prosecution Application (CPA)/Request for Continued Examination(RCE)	128748_01502_Application_Aug_Marked.pdf	254393 4f0faab1cf779edc0b7cb2a322b1aaf7300904d2	no	63
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 49ca0762f8d7efddf9ac597a42607153875fcab4	no	63
Warnings:					
Information:					
5	ePetition Request Form	petition-request.pdf	39763 9de7e88db085f6eebae4a2334440d04c4f72f08c	no	2
Warnings:					
Information:					
6	Fee Worksheet (SB06)	fee-info.pdf	40343 136c218e7f03935e1a2d1b0d0f33dd192a83a939	no	2
Warnings:					
Information:					

Total Files Size (in bytes):	2289336

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.

New Applications Under 35 U.S.C. 111

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.

National Stage of an International Application under 35 U.S.C. 371

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.

New International Application Filed with the USPTO as a Receiving Office

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.

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
Registration No.: 78,384
MCCARTER & ENGLISH, LLP
265 Franklin Street
Boston, Massachusetts 02110
(617) 449-6500
(617) 607-9200 (Fax)
Attorney/Agent For Applicant

PTO/SB/06 (09-11)
Approved for use through 1/31/2014. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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.

PATENT APPLICATION FEE DETERMINATION RECORD Substitute for Form PTO-875	Application or Docket Number 16/900,610	Filing Date 06/12/2020	☐ To be Mailed

ENTITY: ☑ LARGE ☐ SMALL ☐ MICRO

APPLICATION AS FILED - PART I

FOR	(Column 1) NUMBER FILED	(Column 2) NUMBER EXTRA		RATE ($)	FEE ($)
☐ BASIC FEE (37 CFR 1.16(a), (b), or (c))	N/A	N/A		N/A	
☐ SEARCH FEE (37 CFR 1.16(k), (i), or (m))	N/A	N/A		N/A	
☐ EXAMINATION FEE (37 CFR 1.16(o), (p), or (q))	N/A	N/A		N/A	
TOTAL CLAIMS (37 CFR 1.16(i))	minus 20 =	*		x $100 =	
INDEPENDENT CLAIMS (37 CFR 1.16(h))	minus 3 =	*		x $460 =	
☐ 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).				
☐ 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 (37 CFR 1.16(i))	* 19	Minus	** 20	= 0		x $100 =	0
	Independent (37 CFR 1.16(h))	* 1	Minus	*** 6	= 0		x $480 =	0
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ 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 (37 CFR 1.16(i))	*	Minus	**	=		x $0 =	
	Independent (37 CFR 1.16(h))	*	Minus	***	=		x $0 =	
	☐ Application Size Fee (37 CFR 1.16(s))							
	☐ 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.

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.

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).

❑ Change of correspondence address (or Change of Correspondence Address form PTO/AIA/122 or PTO/SB/122) attached.

❑ "Fee Address" indication (or "Fee Address" Indication form PTO/AIA/47 or PTO/SB/47; Rev 03-02 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 _____
2 _____
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

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.

❑ Applicant asserting small entity status. See 37 CFR 1.27

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.

❑ Applicant changing to regular undiscounted fee 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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UNITED STATES DEPARTMENT OF COMMERCE
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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 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

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.

Notice of Allowability	Application No. 16/900,610	Applicant(s) Iyer et al.	
	Examiner GEORGE W KOSTURKO	Art Unit 1621	AIA (FITF) Status 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** or send an inquiry to **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. ☐ Notice of References Cited (PTO-892)
2. ☐ Information Disclosure Statements (PTO/SB/08), Paper No./Mail Date _____.
3. ☐ Examiner's Comment Regarding Requirement for Deposit of Biological Material _____.
4. ☐ Interview Summary (PTO-413), Paper No./Mail Date. _____.
5. ☐ Examiner's Amendment/Comment
6. ☑ Examiner's Statement of Reasons for Allowance
7. ☐ Other _____.

/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

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

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
265 Franklin Street
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;

The United States represents the largest, most dynamic marketplace in the world and is an unparalleled location for business investment, innovation, and commercialization of new technologies. The USA offers tremendous resources and advantages for those who invest and manufacture goods here. Through SelectUSA, our nation works to encourage and facilitate business investment. To learn more about why the USA is the best country in the world to develop technology, manufacture products, and grow your business, visit SelectUSA.gov.

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.

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

PTO/SB/44 (09-07)
Approved for use through 03/31/2026. OMB 0651-0033
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
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.
(Also Form PTO-1050)

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

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

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.

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

Katherine Kelly Vidal
Director of the United States Patent and Trademark Office