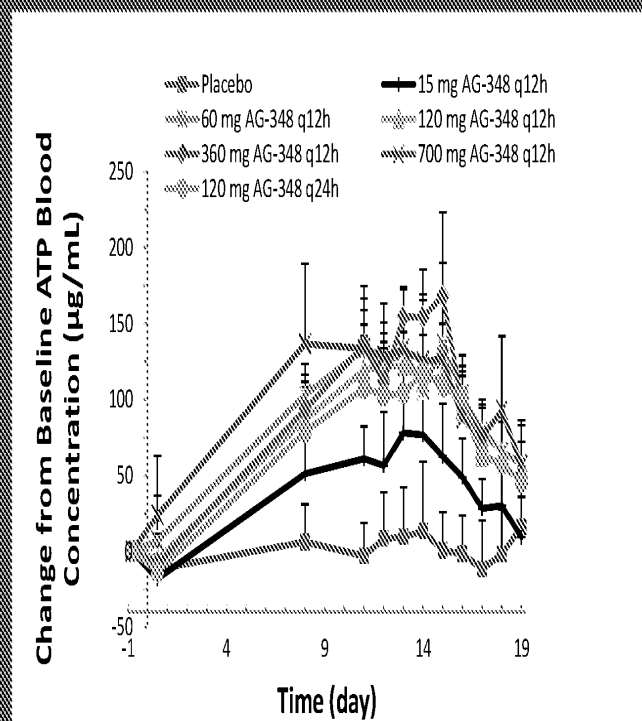
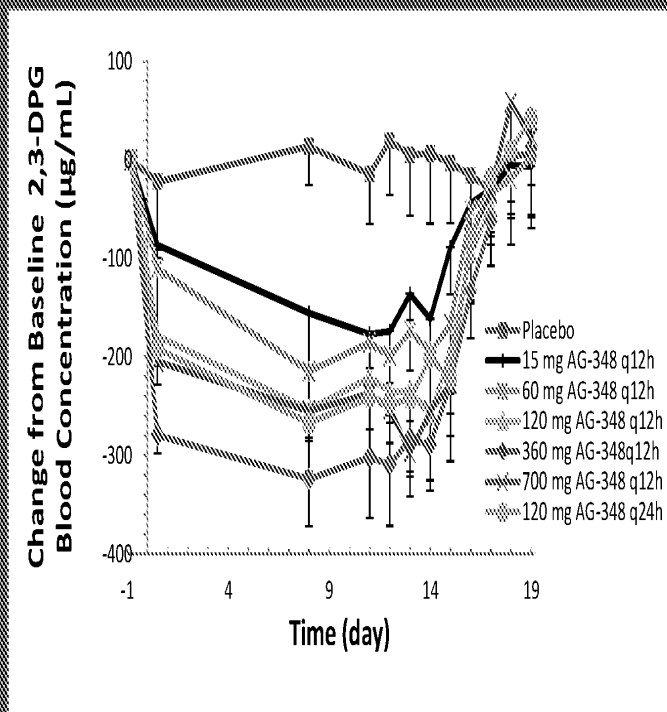


## Variations 2,3 DPG et ATP sanguins selon doses AG-348 (escalades de doses): sujets volontaires sains



- Efficacité
- Tolérance

Yang H et al. *Blood* 2014;124[21]:4007; Yang H et al. *Haematologica* 2015;100[s1]:Abs S138

# Design de l'étude



Etude ouverte de phase 2 : 14 centres US, Canada, UE

Transfusion-independent adults with PK deficiency  
(ClinicalTrials.gov NCT02476916) n=25 in each arm

## Randomization

Stratified by PK-R genotype  
(none excluded)

Arm 1  
300 mg BID

Arm 2  
50 mg BID

6 month core  
dosing period

1 2 3 6 9 12 16 20 24  
Assessment points  
(weeks)

### Primary endpoints:

- Safety and tolerability

### Secondary endpoints:

- Pharmacokinetics of AG-348
- PD response: ATP, 2,3-DPG
- Indicators of clinical activity: hemoglobin, reticulocyte count, and other hematologic parameters

Transfusion independence = no more than 3 units transfused in 12 months prior to the first day of study dosing and no transfusions within 4 months of first day of study dosing

↓  
Extension arm

# Population

- Study initiated June 2015; , All patient enrolled n=52, data cut-off September 23, 2016
- Evaluable analysis set: ≥3 weeks of data (n=32)
- Safety analysis set: received at least 1 dose of AG-348 (n=34)
- 13 patients ongoing in the core period (as of September 23, 2016)
  - Early discontinuations in the core period due to: relocation (n=1), AEs (n=3)
- Of the 17 patients who completed the core period, 15 enrolled in the extension period
- 1 patient discontinued in extension period due to physician decision (lack of efficacy)

| Characteristics                                     | 50 mg BID,<br>n=17      | 300 mg BID,<br>n=17     | Total,<br>N=34          |
|-----------------------------------------------------|-------------------------|-------------------------|-------------------------|
| Men/women, n                                        | 11/6                    | 9/8                     | 20/14                   |
| Age in years, mean (range)                          | 28.5 (19-45)            | 37.0 (20-61)            | 32.8 (19-61)            |
| Race <sup>a</sup> white, n                          | 15                      | 15                      | 30                      |
| Hemoglobin (Hb) baseline, g/dL,<br>mean (SD, range) | 9.8<br>(1.41, 7.6–12.4) | 8.7<br>(1.37, 6.5–11.8) | 9.2<br>(1.47, 6.5–12.4) |
| Duration of treatment, weeks,<br>median (range)     | 24.7 (4.7–50.4)         | 24.0 (2.4–44.4)         | 24.4 (2.4–50.4)         |
| Splenectomized, n                                   | 14                      | 14                      | 28                      |

<sup>a</sup>Not reported in 2 patients. 2 patients were Asian. AE = adverse event, SD = standard deviation

# Sécurité et Tolérance

- AG-348 was generally well tolerated; the majority of AEs were grade 1–2
  - No grade 4 AEs or deaths
  - 2 patients experienced serious AEs: Grade 2 osteoporosis; hemolysis and anemia due to discontinuation of the drug after a rapid Hb response (patient continued in the study)
  - 3 patients discontinued treatment due to AEs
  - DXA scan data (n=17) show high variability and are inconclusive

| AEs, regardless of causality<br>(occurring in >5 patients or<br>assessed as Grade ≥3) | 50 mg BID<br>n=17 |                | 300 mg BID<br>n=17 |                | Total<br>N=34 |          |
|---------------------------------------------------------------------------------------|-------------------|----------------|--------------------|----------------|---------------|----------|
|                                                                                       | All<br>grades     | Grade ≥3       | All<br>grades      | Grade ≥3       | All<br>grades | Grade ≥3 |
| Patients experiencing at least 1 AE, n                                                | 13                | 2              | 17                 | 6              | 30            | 8        |
| Headache                                                                              | 7                 | 0              | 8                  | 0              | 15            | 0        |
| Nausea                                                                                | 7                 | 0              | 7                  | 0              | 14            | 0        |
| Insomnia                                                                              | 3                 | 1              | 10                 | 1              | 13            | 2        |
| Fatigue                                                                               | 3                 | 0              | 3                  | 0              | 6             | 0        |
| Vomiting                                                                              | 2                 | 0              | 4                  | 0              | 6             | 0        |
| Hypertriglyceridemia                                                                  | 0                 | 0              | 4                  | 3              | 4             | 3        |
| Anaemia                                                                               | 1                 | 1 <sup>a</sup> | 1                  | 1 <sup>b</sup> | 2             | 2        |
| Hypertension                                                                          | 0                 | 0              | 1                  | 1              | 1             | 1        |
| Dizziness                                                                             | 2                 | 0              | 1                  | 1              | 3             | 1        |
| Haemolysis                                                                            | 0                 | 0              | 2                  | 1 <sup>b</sup> | 2             | 1        |

<sup>a</sup>Grade 3 anemia, not a serious AE. <sup>b</sup>Grade 3 withdrawal hemolysis and anemia in 46-year-old woman due to abrupt drug withdrawal after a very fast Hb response

AEs were graded using National Cancer Institute Common Terminology Criteria, version 4.03. Hb = hemoglobin, DXA = Dual energy X-ray absorptiometry

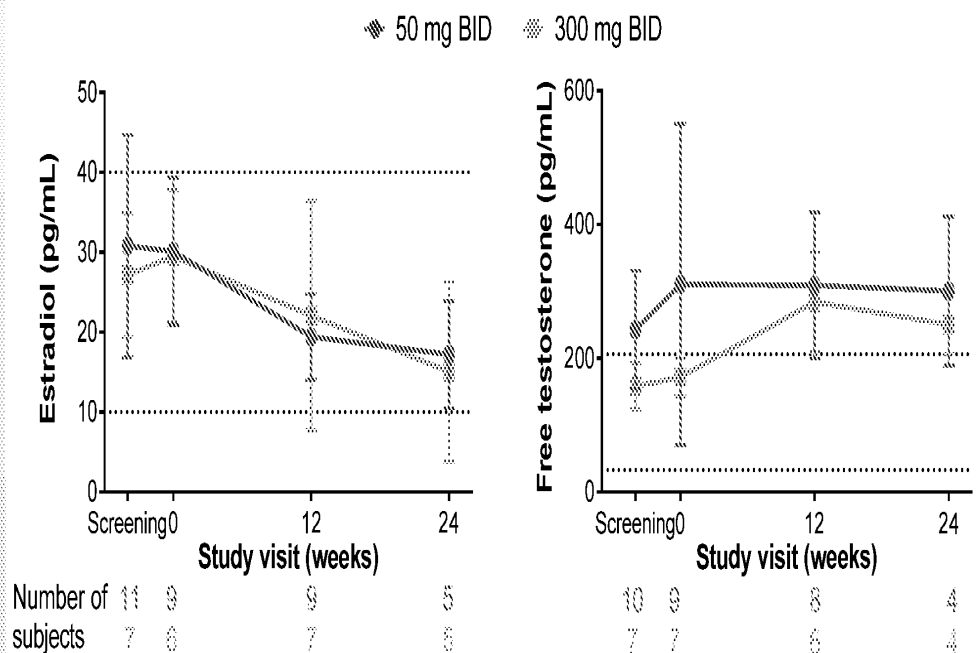
# Effet de l' AG-348 sur état hormonal

- Les résultats préliminaires sont en accord avec un effet anti aromatase

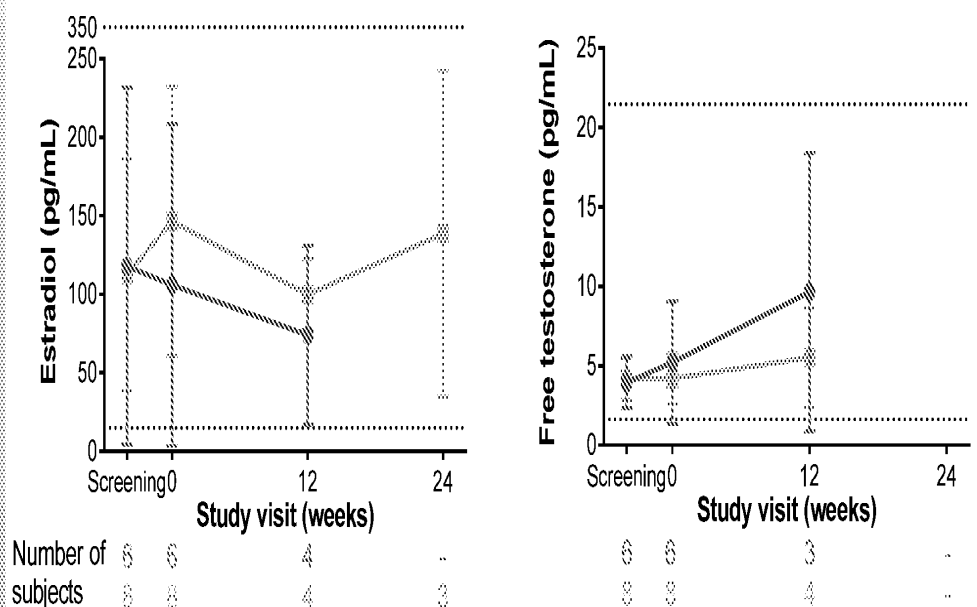
Hormone levels  
assessed at  
baseline, 12 and  
24 weeks

Patients with  
available data  
shown

Male



Female



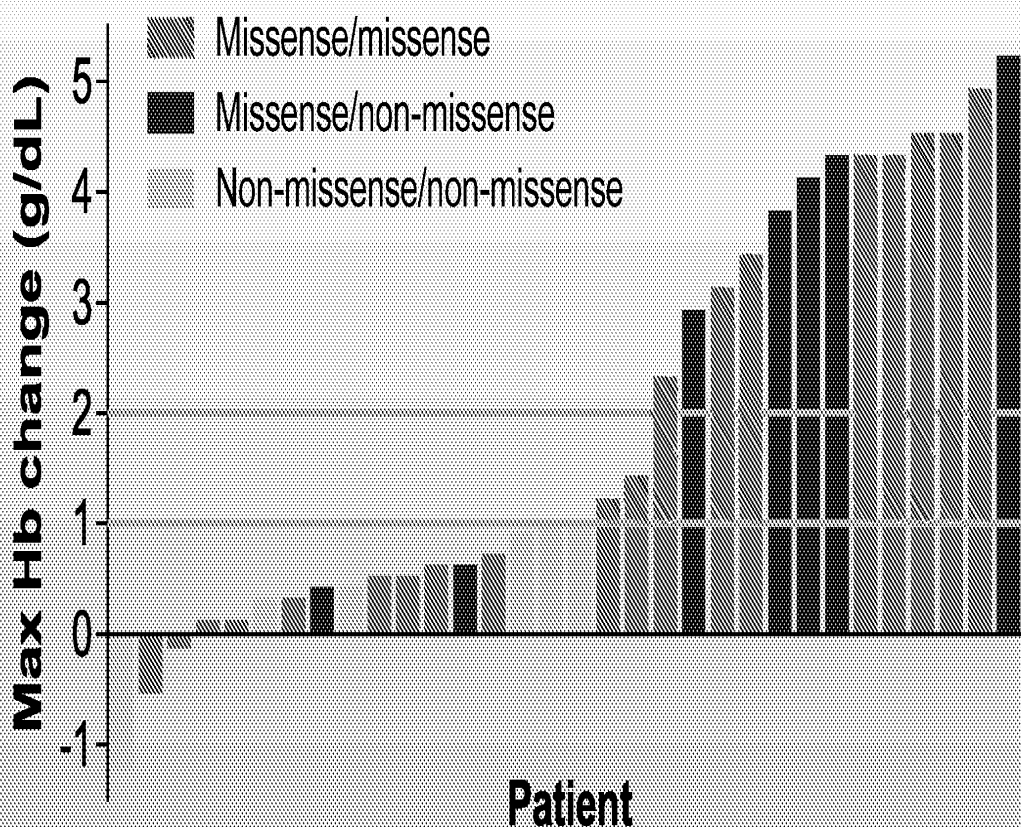
Normal reference low and high limits shown as horizontal dotted lines

# Résultats Activité clinique

## Augmentation de l'hémoglobine (Hb)

- 15 of 32 (47%) patients had a maximal increase in Hb >1.0 g/dL
  - 15 of 26 patients (58%) who had ≥1 missense mutation had a Hb response
- 5 patients homozygous for R479H (mis/mis; Amish) were non-responders
- Hb response and response maintenance are seen across a range of doses
  - Robust Hb responses led to dose decreases with maintained Hb

Maximum Hb change from baseline by genotype

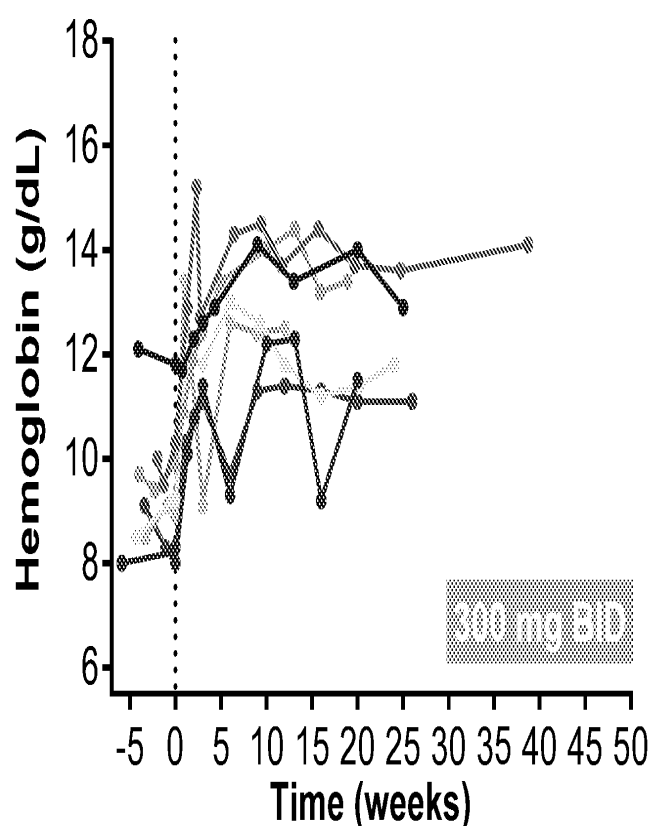
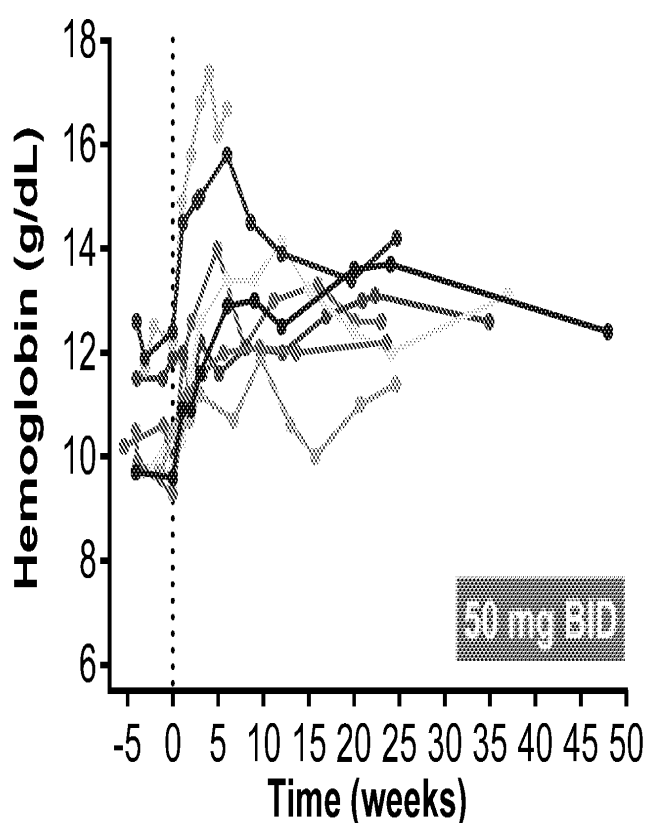


Evaluable analysis set, ≥3 weeks of data

10

## Une augmentation de l'Hb rapide et maintenue

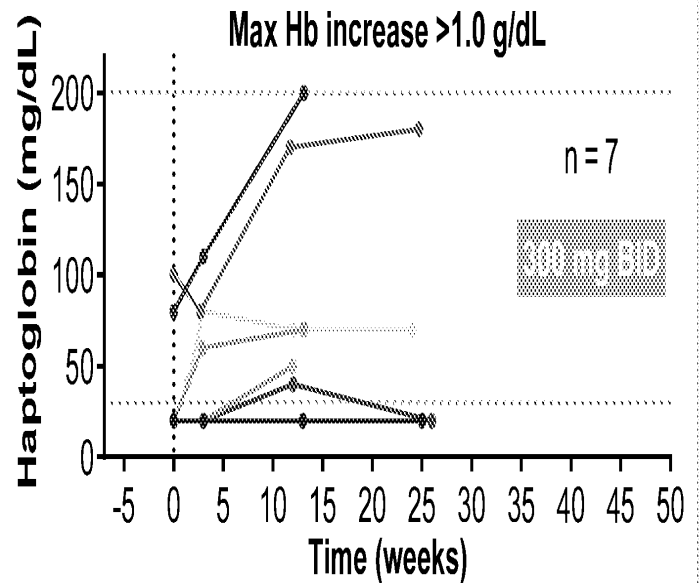
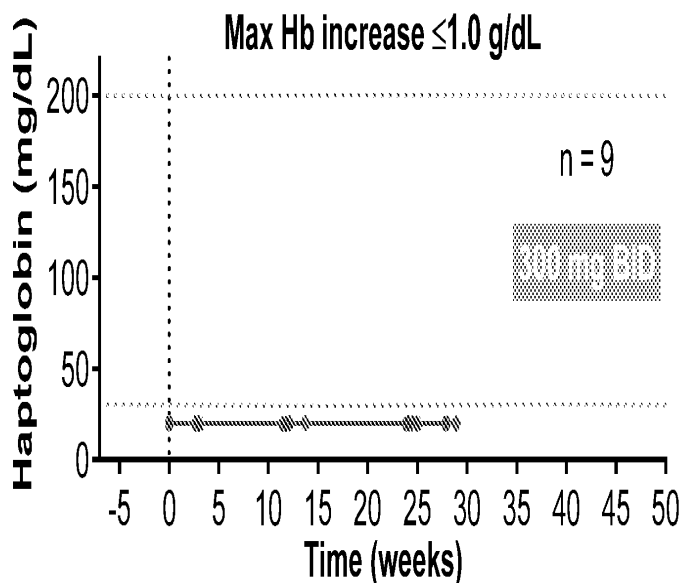
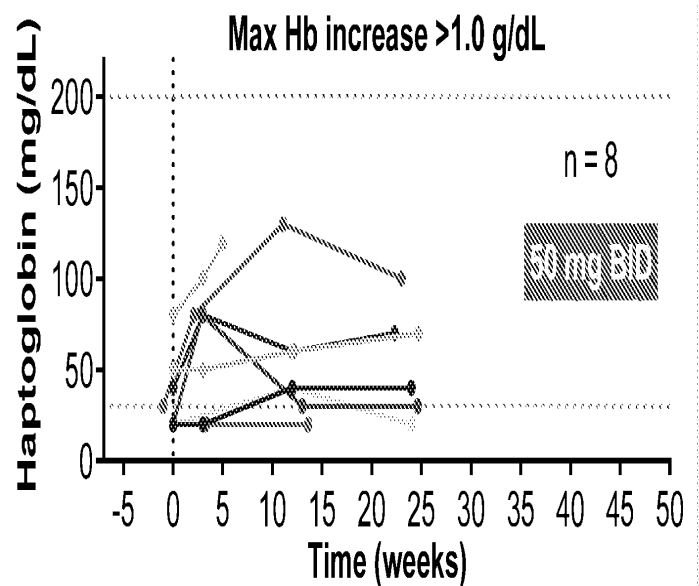
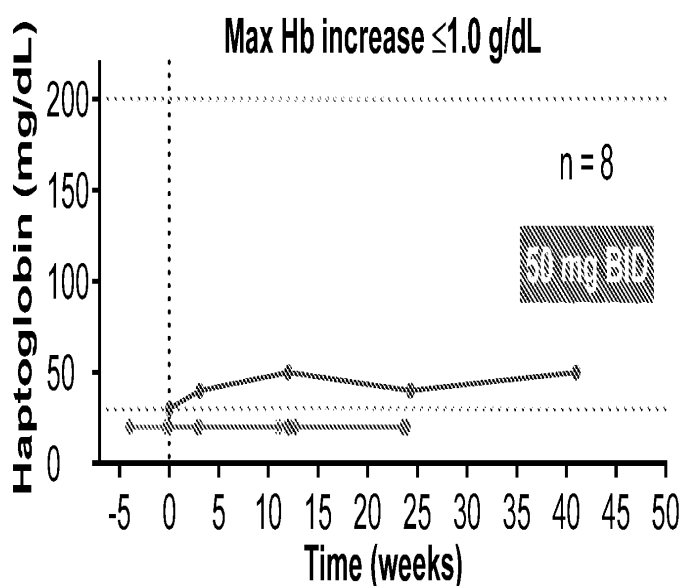
- In patients who had Hb increases  $>1.0$  g/dL (n=15):
  - Median time to Hb increase  $>1.0$  g/dL was 1.4 weeks (range, 1.1–21.0)
  - The mean maximum increase was 3.6 g/dL (range, 1.2–5.2)
- 10 patients had dose reductions: 5 due to rapid Hb increase<sup>a</sup>



Responders n=15, each line represents an individual patient

<sup>a</sup>Other dose reductions due to AEs (n=3), self-reduction due to fatigue (n=1), taper prior to discontinuation (n=1)

## Le taux Haptoglobine augmente chez les répondeurs indiquant une diminution de l'hémolyse



Normal reference low and high limits shown as horizontal dotted lines

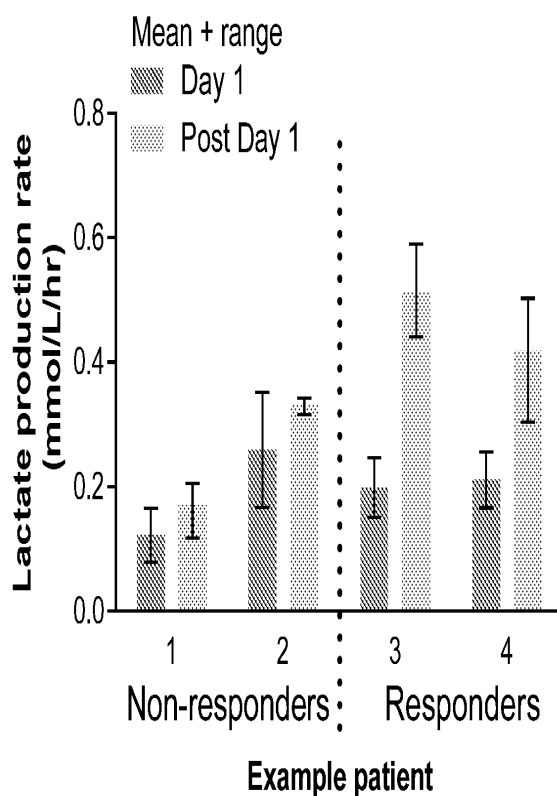
# Résultats Pharmacodynamique

## Une augmentation de l'hémoglobine corrélée à amélioration de la voie de la glycolyse

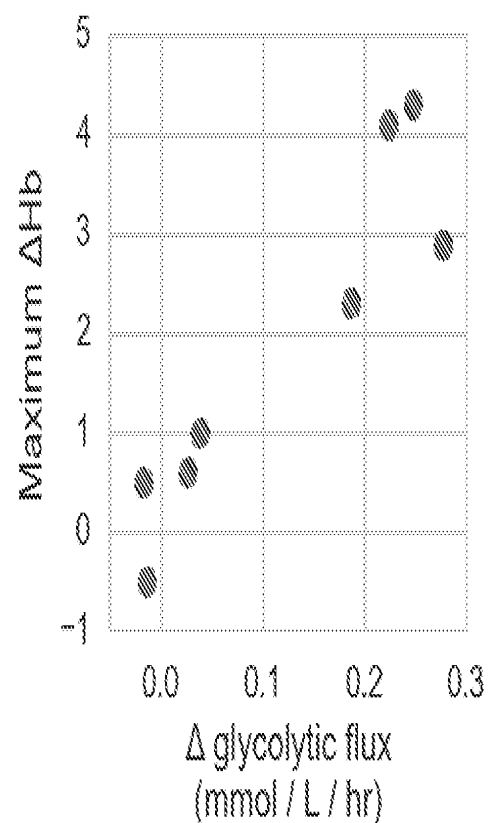
- Rate of metabolism of PK-R pathway was assessed in blood samples from a subset of patients pre and post treatment
- These data suggest a positive correlation between Hb change and change in glycolytic flux

Detailed metabolic characterization in blood from 8 patients

Glycolytic flux assessed *ex vivo* via incorporation of labelled glucose into the end product, lactate



Representative 4 patients shown here



N=8

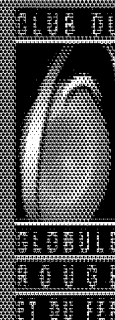
## DRIVE-PK Conclusions

- L'AG-348 est une nouvelle molécule, première dans sa catégorie , capable d'activer l'enzyme de la pyruvate kinase chez les patients déficients
- Une dose quotidienne jusque 6 mois est bien tolérée
  - La signification de l'effet anti aromatase n'est pas précis à ce jour
- AG-438 est capable d'augmenter l'hémoglobine de manière rapide et durable et significative (>1g/dl) chez près de 47% des patients.
  - L'augmentation est liée à une action sur la voie de la glycolyse
  - Corrélation préliminaires entre réponse et génotype
- Ces données illustrent le potentiel et l'intérêt des agents activateurs de l'enzyme comme traitement modifiant l'évolution de la maladie .

# Remerciements

Patients qui ont accepté de participer

Tous les cliniciens Code PK, filière MCGRE, Club Du Globule Rouge et du Fer



\* ,

## \* Sites Participants :

- Hôpital Henri Mondor, Creteil ; Frédéric Galacteros
- Hôpital Saint Vincent de Paul, Lille ; Christian Rose
- Hôpital de la Timone; Marseille , Emmanuelle Bernit
- ( L Garcon, M Micheau, L Pascal)
- Hammersmith Hospital; Mark Layton
- Universitair Medisch Centrum Utrecht, Eduard van Beers
- Fondazione IRCCS Ca' Granda, Ospedale Maggiore Policlinico; Wilma Barcellini
- Boston Children's Hospital; Rachael Grace
- Stanford University Medical Centre; Bertil Glader
- University of Utah; Hassan Yaish
- Weill Cornell – New York Presbyterian Hospital; Sujit Sheth
- The Children's Hospital of Philadelphia; Janet Kwiatkowski
- Central Pennsylvania Clinic; Holmes Morton
- Wayne State University School of Medicine; Yaddanapudi Ravindranath
- University of Toronto – University Health Network; Kevin Kuo

\* Agios Pharmaceuticals

## 8.1. Acids for Salts of Organic Bases

### 8.1.1. Hydrochloric Acid

This acid is the most widely used acid for formation of salts both for the isolation of synthetic intermediates and for the final pharmaceutical dosage form. It is a very strong acid and so forms salts with most bases. However, loss of the volatile HCl can give rise to long-term stability concerns with very weak bases.

Chlorides and hydrochloric acid can cause stress corrosion/pitting in stainless steel equipment particularly at low pH. On a large scale, this necessitates use of glass lined vessels or equipment such as centrifuges and pressure filters being fabricated from special resistant alloys (e.g., Hastelloy).

Hydrochloric acid is conveniently used as the 36% (w/w) solution in water. If, however, water interferes with the formation and isolation of a solid crystalline product, it is possible to use the anhydrous gas from cylinders.

### 8.1.2. Sulfuric Acid

This strong acid has also been widely used both for isolation of intermediates and as the final pharmaceutical salt form. The dibasic acid can give either neutral (i.e., two moles of base to one mole of sulfuric acid) or acid salts. It is important, therefore, to ensure that the correct stoichiometry is used in the formation of a sulfate salt.

### 8.1.3. Hydrobromic Acid

Retrospectively, hydrobromic acid is the third most frequently used acid for final pharmaceutical products, but, because of the potential of adverse reactions to bromide ions, it is becoming less favored for new products.

It is still widely used for isolation of intermediates, since hydrobromides are often of lower solubility and better crystallinity than the corresponding hydrochlorides.

Hydrobromic acid is also volatile and attacks stainless steel. It is used both as the anhydrous gas and as a 48% (w/w) solution in water.

### 8.1.4. Maleic Acid

Many pharmaceutical final products are isolated as maleates because of high crystallinity of the salts. Its two carboxyl groups may form both neutral

and acid salts. A potential disadvantage is that it can isomerize to the thermodynamically more stable (*E*)-isomer, fumaric acid. For this reason, high temperatures must be avoided.

The solid-state nature of maleic acid requires it to be dissolved in a suitable solvent when used for salt formation reactions.

#### 8.1.5. *Methanesulfonic Acid*

This is a strong acid and so forms stable salts with many weak bases. It is frequently used for the salt form of the final pharmaceutical product. In addition, it finds considerable utility for isolation of synthetic intermediates. Operationally, it is very convenient to use on a large scale, since it is a liquid containing no water.

#### 8.1.6. *Phosphoric Acid*

Phosphoric acid is especially used to enhance water solubility of final products. It is an involatile acid and so tends to form thermally stable salts. There is, however, a tendency to form hydrates.

Stainless steel processing equipment is relatively inert to attack from phosphoric acid. Anhydrous phosphoric acid is very viscous liquid and so is operationally difficult to use.

#### 8.1.7. *para-Toluenesulfonic Acid*

This strong acid is relatively cheap. Because of its high molecular weight, it forms insoluble salts with many organic bases. For this reason, it is widely used to isolate and purify synthetic intermediates. It is occasionally also used as a salt of a pharmaceutical product.

#### 8.1.8. *Carbonic Acid*

This is a very weak acid and only forms salts with the strongest of organic bases such as guanidines. This special property can be used to separate guanidines from weaker bases.

### 8.1.9. *Other Organic Acids*

These include tartaric, citric, fumaric, succinic, acetic, formic, lactic, malic, malonic, and many other acids. The primary choice of salt is often made on the empirical basis of laboratory studies. This takes into account issues such as the stability, bioavailability, pharmaceutical acceptability, and ease of manufacture. Readily crystallizing salts with a high degree of crystallinity are preferred from a manufacturing viewpoint, since they offer good purification and chemical stability properties.

## 8.2. *Bases for Salts of Organic Acids*

### 8.2.1. *Sodium*

This is the most commonly used base both for final pharmaceutical products and for intermediates. Sodium salts are often crystalline, but often have a tendency to form hydrates of variable water content. Some of the salts are very hygroscopic, which can give rise to handling issues. Those may be prone to support microbial growth because of the water content, and special precautions are needed to ensure freedom from contamination.

There are several techniques used for formation of sodium salts on a large scale. The simplest way is to neutralize an aqueous solution of the acid with sodium hydroxide or sodium carbonate/bicarbonate. The water is then removed by distillation or freeze-drying, or the sodium salt can be precipitated by addition of a miscible organic solvent such as acetone. If the acid is insoluble in water, methanol can be used as solvent and one equivalent of sodium methoxide is used to form the sodium salt, followed by a suitable workup.

### 8.2.2. *Potassium*

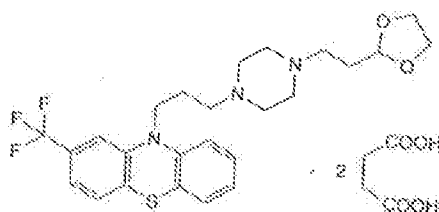
The comments about sodium salts referred to above are in general equally applicable to potassium salts.

### 8.2.3. *Calcium*

The simplest method of formation is by addition of an aqueous solution of calcium hydroxide to a solution of the acid in water, followed by evaporation/concentration.

## 2.4. Succinates

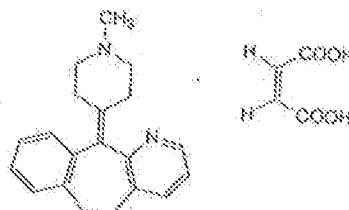
*Oxaflumazine Succinate*  
*Oxaflumine*® (Diamant)

*Procedure* [8]

A solution of 257 g oxaflumazine (= 10-(3-[4-[2-(1,3-dioxan-2-yl)ethyl]-piperazin-1-yl]propyl)-2-(trifluoromethyl)-10*H*-phenothiazine) base in 500 ml of acetone is added with stirring to a solution of 118 g of succinic acid in 4000 ml of acetone. The agitation is continued at room temperature until the desired salt crystallizes. The mixture is allowed to stand overnight at 0 °C. The weight of the collected crystals is 256 g (yield, 70%). After recrystallization in 1000 ml of acetonitrile (MeCN) the acid disuccinate melts at 136–138 °C.

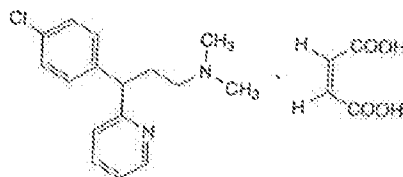
## 2.5. Maleates

*Azatadine Maleate*  
*Optimine*® (Schering)

*Procedure* [9]

To a solution containing 4.3 g of 6,11-dihydro-11-(1-methylpiperidin-4-ylidene)-5*H*-benzo[5,6]cyclohepta[1,2-*b*]pyridine in 55 ml of AcOEt, add a solution of 3.45 g of maleic acid dissolved in AcOEt. Filter the resulting precipitate and recrystallize the desired product from an AcOEt/MeOH mixture to yield the dimaleate salt. M.p. 152 – 154 °C.

*Dexchlorpheniramine Maleate*  
*Polaramine*® (Schering)

*Procedure* [10]

(+)-Dexchlorpheniramine (= γ-(4-chlorophenyl)-*N,N*-dimethylpyridine-2-propanamine) (4.3 g) and maleic acid (1.8 g) are dissolved in 20 ml of isopropyl acetate (AcO(*i*-Pr)) and kept at room temperature until crystallization

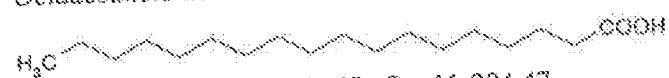
*Disodium salt*: LD: rat oral, > 6 g/kg; rabbit oral, > 6 g/kg; LD<sub>50</sub>: rat i.p., 5500 mg/kg; i.v., 560 mg/kg; rabbit i.p., 6 g/kg; i.v., 1440 mg/kg. Toxic effects were found to be due to the sodium content [25].

Salt                      Sebacate

Use and Examples      Piperazine.  
Proposed as substrate for parenteral nutrition [26–29].

## Stearic Acid

### Octadecanoic acid



$\text{C}_{18}\text{H}_{36}\text{O}_2$ ,  $M_r$  284.47

pK<sub>a</sub>                      ~4.9 (A)

Properties                M.p. 70–71 °C.  
*Solubility*: 1 g dissolves in 26 ml of acetone, 21 ml of EtOH, 5 ml of benzene, 2 ml of CHCl<sub>3</sub>; soluble in amyl acetate, toluene.

Safety, Toxicology      *Acid*: LD<sub>50</sub>: rat i.v., 21.5 ± 1.8 mg/kg; mouse i.v., 23 ± 0.7 mg/kg [6]; rabbit skin, > 5 g/kg; LDLo: rat oral, 4640 mg/kg.

Salt                        Stearate

Use and Examples      For formation of liposoluble esters: erythromycin.

Remarks                May be considered also for formation of drug salts to achieve liposolubility.

## Succinic Acid

### Butanedioic acid



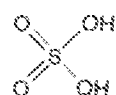
$\text{C}_4\text{H}_6\text{O}_4$ ,  $M_r$  118.09

pK<sub>a</sub>                      (1) 4.21 (A)      (2) 5.64 (A) (25 °C)

Properties                M.p. 185–187 °C

|                    |                                                                                                                                                                                                                                                                                          |
|--------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
|                    | <i>Solubility:</i> sparingly soluble in H <sub>2</sub> O (g/100 g solution (temp. [°C])): 2.72 (0), 4.32 (10), 6.45 (20), 7.46 (25), 9.57 (30), 13.95 (40), 26.38 (60), 41.45 (80).                                                                                                      |
| Safety, Toxicology | <i>Acid:</i> LD <sub>50</sub> : rat oral, 2260 mg/kg; mouse i.p., 2702 mg/kg; i.v., 1400 mg/kg.<br><i>Disodium salt:</i> LD <sub>50</sub> : mouse i.v., 4500 mg/kg.<br><i>Monosodium salt:</i> LD <sub>50</sub> : rat oral, >8 g/kg;<br>LDLo: cat i.v., 2 g/kg; guinea pig i.m., 3 g/kg. |
| Salt               | Succinate; hydrogensuccinate, acid succinate.                                                                                                                                                                                                                                            |
| Examples           | Benfurodil, bamethan, chloramphenicol, cibenzone, deanol, doxylamine, ergotamine, loxapine, metoprolol, oxafloxazine, sumatriptan; Fe <sup>II</sup> .                                                                                                                                    |

## Sulfuric Acid

pK<sub>a</sub>H<sub>2</sub>O<sub>4</sub>S, M<sub>r</sub> 98.08

(1) -3 (A) (2) 1.92 (A)

Properties

Colorless, odorless, highly hygroscopic liquid, *d* = 1.83, b.p. ~290 °C.  
Supplied as aq. solution in various concentrations up to ~98%.  
Miscible with H<sub>2</sub>O and EtOH with generation of heat.

Safety, Toxicology

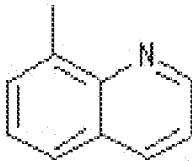
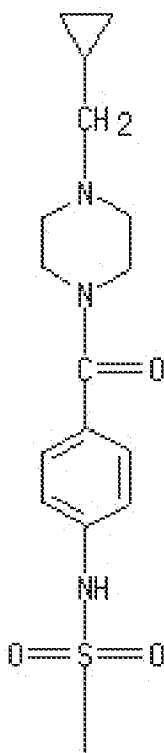
LD<sub>50</sub>: rat oral, 2140 mg/kg.

Salt

Sulfate; hydrogensulfate.

Examples

Amantadine, amphetamine, amikacin, ampicillin, atropine, berberine, betanidine, bleomycin, butacaine, capreomycin, cefpirome, clopidogrel, cyclomethycaine, debrisoquine, dibekacin, dihydralazine, dihydrostreptomycin, ephedrine, gentamicin, guanoxan, indinavir, isoprenaline, kanamycin, lividomycin, mefenbermine, morphine, neomycin, oriprenaline, paromomycin, polymyxin B, quinine, salbutamol, sparteine, streptomycin, terbutaline, tuaminoheptane, vinblastine, vincristine, viomycin.



CC A16A Other Alimentary Tract And Metabolism Products; B3X Other Anti-anaemic Products

CT Indication: anemia; cardiovascular disease; endocrine and metabolic disorders; enzyme deficiency; hematologic disease; metabolic disease; thalassemia  
Pharmacology: small molecule; metabolism regulator; enzyme modulator; transferase activator; pyruvate kinase-R activator; pyruvate kinase activator  
Administration: oral

HDP Phase III (50)

ED Entered STN: 3 Jan 2014  
Last Updated on STN: 21 Feb 2020

LI 28 January 2019 : In December 2018 Agios initiated a phase II trial to evaluate the efficacy, safety, pharmacokinetics and pharmacodynamics of mitapivat (AG 348) in adult participants with non-transfusion-dependent thalassemia. Mitapivat, an orally available, small molecule activator of pyruvate kinase-R enzyme, is also under phase III evaluation for pyruvate kinase deficiency.

| DSTA          |           |       |               |                   |
|---------------|-----------|-------|---------------|-------------------|
| Type          | Status    | Stage | Region        | Indication        |
| Highest Phase | Phase III | 50    |               |                   |
| Phase         | Phase III | 50    | United States | enzyme deficiency |

|       |           |    |               |                   |
|-------|-----------|----|---------------|-------------------|
| Phase | Phase II  | 40 | United States | thalassemia       |
| Phase | Phase III | 50 | UK            | enzyme deficiency |
| Phase | Phase II  | 40 | UK            | thalassemia       |
| Phase | Phase III | 50 | Switzerland   | enzyme deficiency |
| Phase | Phase III | 50 | Spain         | enzyme deficiency |
| Phase | Phase III | 50 | South Korea   | enzyme deficiency |
| Phase | Phase III | 50 | Japan         | enzyme deficiency |
| Phase | Phase III | 50 | Italy         | enzyme deficiency |
| Phase | Phase III | 50 | France        | enzyme deficiency |
| Phase | Phase III | 50 | Europe        | enzyme deficiency |
| Phase | Phase III | 50 | Canada        | enzyme deficiency |
| Phase | Phase II  | 40 | Canada        | thalassemia       |
| Phase | Phase III | 50 | Ireland       | enzyme deficiency |
| Phase | Phase III | 50 | Netherlands   | enzyme deficiency |
| Phase | Phase III | 50 | Thailand      | enzyme deficiency |

CO

| Type       | Company | Nationality | Corporation           |
|------------|---------|-------------|-----------------------|
| Originator | Agios   | USA         | Agios (United States) |

TX Patent Summary: Product (Agios Pharmaceuticals Inc): US8785450 2010, priority US20090221430P 2009. This patent claims mitapivat specifically.

TX Commercial Summary: Commercial overview. OverviewAgios is developing mitapivat (AG 348), an orally available, small molecule activator of pyruvate kinase-R (PKR) enzyme, for the treatment of pyruvate kinase deficiency and thalassemia. Mutations in PKR cause defects in red cell glycolysis and lead to hematologic inborn errors of metabolism known as pyruvate kinase deficiency, or PK deficiency that present as hemolytic anemia. Mitapivat is in phase III evaluation for pyruvate kinase deficiency and phase II evaluation for thalassemia.Regulatory progress. \*\*Enzyme deficiency\*\*USAAgios pyruvate kinase deficiency.---Fast Track - Apr 2017.Agios pyruvate kinase deficiency.---Orphan Drug - Mar 2017.R&D progress. \*\*Enzyme deficiency\*\*Phase III, "ACTIVATE-T; NCT03559699" (pyruvate kinase deficiency), Agios, Canada, France, Ireland, Italy, Netherlands, Spain, Switzerland, Thailand, UK, USA.---DESIGN: Trial started - Jun 2018. An open-label, single-group phase III trial to evaluate the efficacy and safety of AG 348 in regularly transfused adults with pyruvate kinase deficiency has been initiated. The target enrollment is 20 patients. The primary outcome measure is percentage of patients achieving a reduction in transfusion burden (ClinicalTrials.gov).---DESIGN: Estimated Completion Date - May 2021.Phase III, "ACTIVATE; NCT03548220" (pyruvate kinase deficiency), Agios, Canada, Europe, Japan, South Korea, USA.---DESIGN: Trial started - Jun 2018. A randomized, double-blind, parallel-group, placebo-controlled phase III trial to evaluate the efficacy and safety of AG 348 in not regularly transfused adults with pyruvate kinase deficiency has been initiated. The target enrollment is 80 patients. The primary endpoint is proportion of patients who achieve at least a 1.5 g/dL increase in hemoglobin sustained over multiple visits in part 2 of the trial. Source-<http://bit.ly/2tatDnw> ---DESIGN: Estimated Completion Date - Jan 2021.Phase II, "DRIVE PK", Agios, USA.---DESIGN: Trial started - 2015. Agios has initiated a randomized, multicenter, open-label phase II trial to evaluate the safety and efficacy of AG 348 in adult, transfusion-independent patients with pyruvate kinase deficiency.

Patients (n=50) will be randomized to receive 50 or 300 mg AG 348 twice daily. The trial will include a six-month dosing period and based on safety and clinical activity, the treatment can be continued beyond six months. Source-<http://bit.ly/1KRSrDB> (Agiros, JUN 2015).---PROGRESS: Progress update - Jun 2016. As of March 2016, 18 transfusion-independent patients were treated with twice-daily dosing of AG 348 for up to nine months (Agiros, JUN 2016).---RESULTS: Clinical data - Jun 2016. AG 348 achieved proof-of-concept in this trial; both doses of the agent demonstrated clinical activity. Treatment with AG 348 resulted in rapid and sustained hemoglobin increases of more than 1 g/dL in nine of 18 patients ranging from 2.3-4.9 g/dL with a mean maximum hemoglobin increase of 3.4 g/dL. Four patients in the 50 mg group and five patients in the 300 mg group experienced increases of more than 1 g/dL. The median time to a hemoglobin increase of more than 1 g/dL was 1.9 weeks. No patients with two non-missense mutations and five patients with one missense mutation had increases in hemoglobin of more than 1 g/dL. Pharmacokinetics were favorable and similar to that observed in healthy volunteers. Pharmacodynamic data did not demonstrate a correlation between hemoglobin increases and ATP elevation. AG 348 was well tolerated and no patients discontinued treatment early. Most adverse events were mild-to-moderate and transient. The most frequent adverse events included nausea, headache, hot flush and insomnia. One patient received a dose reduction (300 to 50 mg) due to rapidly increasing hemoglobin. One Grade 2 adverse event of osteoporosis was reported since March 2016 (this patient had osteopenia at baseline assessment). An upward trend in free testosterone and a downward trend in estradiol were observed. Source-<http://bit.ly/2dgYdEe> (Agiros, JUN 2016).---PROGRESS: Progress update - Dec 2016. As of September 2016, 34 patients had been treated and are included in the safety analysis and 32 patients with at least 3 weeks of data are included in the efficacy analysis. 17 patients completed the initial 24-week treatment period. 82% patients (28 of the 34) had been splenectomized before entry into the trial. Source-<http://bit.ly/2lxSl0f> ---RESULTS: results update - Dec 2016. In the 32 patients evaluable for efficacy, the mean baseline Hb was 9.2 g/dL. 15 of 32 total evaluable patients and 15 of 26 evaluable patients with at least one missense mutation achieved rapid, robust and sustained hemoglobin increases from baseline of more than 1.0 g/dL. In patients with more than 1.0 g/dL hemoglobin increases, the mean maximum hemoglobin increase was 3.6 g/dL and the median time to a hemoglobin increase was 1.4 weeks. In patients with at least one missense mutation, 26 were evaluable out of which 15 showed an increase in hemoglobin of more than 1.0 g/dL. None of the six patients with two non-missense mutations showed increases in hemoglobin of more than 1.0 g/dL. Additional data showed that out of eight AG 348-treated patients, who had hemoglobin level increases more than 1.0 g/dL, four patients experienced a greater than 50% average increase in the rate of metabolism of the PKR pathway. Source-<http://bit.ly/2lxSl0f> ---PROGRESS: Progress update - Dec 2016. A total of 52 patients have been enrolled. Source-<http://bit.ly/2klPMfL> Phase I, "NCT02149966", Agiros, USA.---DESIGN: Trial started - Jun 2014. Agiros has initiated a single-center, randomized, double-blind, placebo-controlled phase I trial to assess the safety and tolerability of multiple ascending doses of AG 348 administered to healthy volunteers for 14 days. Evaluation of the safety, pharmacokinetics and pharmacodynamics of AG 348, and assessment of select metabolic biomarkers, are the key objectives of this trial. Source-<http://bit.ly/1oPpevT> (Agiros, JUN 2014).---PROGRESS: Progress update - Jun 2015. The trial enrolled healthy volunteers (n=48) who were randomized to receive either placebo or AG 348 (15, 50, 120, 360 or 700 mg twice daily, or 120 mg once daily) for 14 days (Agiros, JUN 2015).---RESULTS: Clinical data - Jun 2015. 32 of 36 participants in AG 348-treated group completed the trial. The agent was well tolerated, with most adverse events occurring in the highest dose group (700 mg), with all but one being mild-to-moderate. Two volunteers withdrew from the AG 348-treated group due to adverse events, including drug eruption (60 mg) and Grade 3 liver function test abnormalities (700 mg), which were resolved after discontinuation of the treatment. Two volunteers from 700 mg AG 348-treated group withdrew consent due to nausea or vomiting. Serum hormone changes consistent with reversible aromatase inhibition were observed. The agent also showed a favorable pharmacokinetic profile with rapid absorption, low-to-moderate variability and a dose-proportional increase in exposure following multiple doses. A decrease in 2,3-DPG (2,3-diphosphoglycerate) and an increase in adenosine triphosphate (ATP) in blood was observed; a 50% decrease in 2,3-DPG was observed with doses

120 mg and higher, with levels returning back to baseline approximately 72 h after AG 348 was discontinued. AG 348 at doses 60 mg and higher showed approximately 50% increase in ATP in blood.

Source-<http://bit.ly/1KRSrDB> (AgiOS, JUN 2015).Phase I, "NCT02109106" (pyruvate kinase deficiency), Agios, USA.---DESIGN: Trial started - Apr 2014. Agios has initiated dosing in a randomized, double-blind, placebo-controlled phase I trial of AG 348 in healthy volunteers. Approximately 48 participants will be enrolled and randomized to receive a single oral dose of AG 348 or placebo. The primary outcome measures include safety and tolerability assessment. The secondary outcome measures include the evaluation of pharmacokinetic and pharmacodynamic relationships of AG 348 and certain metabolic biomarkers.

Source-<http://investor.agios.com/phoenix.zhtml?c=251862&p=irol-newsArticle&ID=1919588&highlight=> (AgiOS; ClinicalTrials.gov, APR 2014).---PROGRESS: Progress update - Jun 2014. Dosing has been completed in more than half of the planned cohorts. Source-<http://bit.ly/1oPpevT> (AgiOS, JUN 2014).---RESULTS: Clinical data - Jun 2014. Treatment was well tolerated. Source-<http://bit.ly/1oPpevT> (AgiOS, JUN 2014).Clinicals, Agios, USA.---DESIGN: Trial planned - Aug 2017. Agios plans to initiate a randomized, placebo-controlled trial with a 1: 1 randomization to evaluate AG 348 in the treatment of pyruvate kinase deficiency. The expected enrollemnt is approximately 80 to 100 non-transfusion dependent patients. The primary endpoint of the trial is the proportion of patients who achieve at least a 1.5 g/dL increase in hemoglobin. The trial is expected to initiate in first half 2018 (AgiOS second quarter results, AUG 2017).Clinicals, Agios, USA.---DESIGN: Trial planned - Aug 2017. Agios plans to initiate a single arm trial to evaluate AG 348 in approximately 20 regularly transfused patients with a primary endpoint of reduction in transfusion burden over six months. The trial is expected to initiate in first half 2018 (AgiOS second quarter results, AUG 2017).\*\*Thalassemia\*\*Phase II, "NCT03692052", Agios, Canada, UK, USA.---DESIGN: Trial started - Dec 2018. Agios has initiated a phase II trial to evaluate the efficacy, safety, pharmacokinetics and pharmacodynamics of mitapivat in adult participants with non-transfusion-dependent thalassemia (NTDT). This trial will include a 24-week core period followed by a 2-year extension period for eligible participants. The enrollment target is 17 participants, who will receive an initial dose of be 50 mg twice daily with one potential dose-level increase to 100 mg twice daily, at the Week-6 visit, based on the participant's safety and hemoglobin concentrations. The primary endpoint is percentage of participants achieving a hemoglobin response (ClinicalTrials.gov).---DESIGN: Estimated Completion Date - Dec 2021. Preclinical, (pyruvate kinase deficiency), Agios, USA.---DESIGN: Study started - 2013. Preclinical studies of AG 348 are being conducted (AgiOS, DEC 2013).---RESULTS: Preclinical data - Dec 2013. In blood samples of patients with pyruvate kinase deficiency-associated hemolytic anemia, AG 348 activated a spectrum of pyruvate kinase-R mutant proteins leading to a normalization of metabolic balance (AgiOS, DEC 2013).Company predictions. Agios - Feb 2019.---Enrollment completion in ACTIVATE-T and ACTIVATE trials anticipated by year end 2019

Source-<http://bit.ly/2QtNs5r>AgiOS - Nov 2017.---Initiation of two global pivotal trials in pyruvate kinase deficiency anticipated in first half 2018. Source-<https://bit.ly/2GaNziV>AgiOS - Nov 2017.---Updated data from a phase II trial (DRIVE PK) in pyruvate kinase deficiency anticipated in fourth quarter 2017.

|                   |                                                                                                                                                                       |
|-------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| RDAT: 13 Jan 2020 | RNTE: See Company predictions.                                                                                                                                        |
| DEC 2018          | Agios trial started (Phase II), Canada, UK, USA (thalassemia) .                                                                                                       |
| JUN 2018          | Agios trial started (Phase III), Canada, Europe, Japan, South Korea, USA (enzyme deficiency) (pyruvate kinase deficiency).                                            |
| JUN 2018          | Agios trial started (Phase III), Canada, France, Ireland, Italy, Netherlands, Spain, Switzerland, Thailand, UK, USA (enzyme deficiency) (pyruvate kinase deficiency). |
| APR 2017          | Agios Fast Track, USA (enzyme deficiency) (pyruvate kinase deficiency).                                                                                               |
| MAR 2017          | Agios Orphan Drug, USA (enzyme deficiency) (pyruvate kinase deficiency).                                                                                              |
| 2015              | Agios trial started (Phase II), USA (enzyme                                                                                                                           |

|          |                                                |
|----------|------------------------------------------------|
| APR 2014 | deficiency) .                                  |
|          | Agios trial started (Phase I), USA (enzyme     |
|          | deficiency) (pyruvate kinase deficiency).      |
| 2013     | Agios Study started (preclinical), USA (enzyme |
|          | deficiency) (pyruvate kinase deficiency).      |

=>

# Abstract Submission

## 27. *Thalassemias*

EHA-2234

### DESIGN OF A PHASE 2, OPEN-LABEL, MULTICENTER STUDY OF MITAPIVAT (AG-348) IN ADULTS WITH NON-TRANSFUSION-DEPENDENT THALASSEMIA

Katrin Uhlig<sup>\*1</sup>, Elliott Vichinsky<sup>2</sup>, Kevin H. M. Kuo<sup>3</sup>, D. Mark Layton<sup>4</sup>, Lei Hua<sup>1</sup>, Chris Mix<sup>1</sup>

<sup>1</sup>Agios Pharmaceuticals, Inc., Cambridge, <sup>2</sup>UCSF Benioff Children's Hospital Oakland, Oakland, United States, <sup>3</sup>Toronto General Hospital, University Health Network, Toronto, Canada, <sup>4</sup>Hammersmith Hospital, Imperial College Healthcare NHS Trust, London, United Kingdom

**Does the study abide by applicable national and international regulations and guidelines, including but not limited to ethical committees, data protection and privacy regulations, informed consent and off-label use of drugs?:** Yes

**Background:** The thalassemias are a group of disorders in which mutation(s) in the  $\alpha$ - and/or  $\beta$ -globin locus lead to an imbalance between  $\alpha$ - and  $\beta$ -globin production. The disease is characterized by the precipitation of excess  $\alpha$ - or  $\beta$ -globin, ineffective erythropoiesis, and peripheral hemolysis, with resultant anemia. Energy supply in the form of ATP appears to be insufficient in thalassemic red blood cells (RBCs) to maintain RBC membrane fitness and clearance of globin precipitates. Mitapivat (AG-348) is an oral, small-molecule, allosteric activator of the RBC-specific form of pyruvate kinase (PK-R). In healthy adults, mitapivat activates wild-type PK-R and increases ATP levels in RBCs. In adults with a mutant PK-R and non-transfusion-dependent anemia due to pyruvate kinase deficiency (NCT02476916), oral mitapivat was well tolerated and induced rapid, durable hemoglobin (Hb) increases. In  $\beta$ -thalassemic mice, mitapivat increases ATP levels, reduces ineffective erythropoiesis, and improves anemia, RBC survival, and indices of iron overload. These data support the hypothesis that increased ATP synthesis mediated via the activation of PK-R by mitapivat may improve the survival of thalassemic RBCs in the bone marrow and/or peripheral circulation. A phase 2 study of mitapivat is ongoing to further inform this hypothesis.

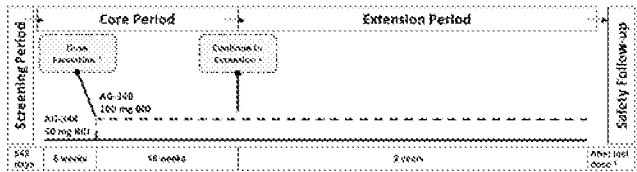
**Aims:** To report the design of the ongoing phase 2 study of mitapivat in adults with non-transfusion-dependent thalassemia (NTDT).

**Methods:** This phase 2, multicenter, open-label study (NCT03692052) is evaluating the efficacy, safety, pharmacokinetics, and pharmacodynamics of mitapivat in adults with NTDT. It consists of a 24-week core period followed by a 2-year extension period (Figure). Approximately 17 subjects with NTDT will be enrolled, including a minimum of 8 subjects with  $\beta$ -thalassemia. All eligible subjects will receive an initial mitapivat dose of 50 mg orally twice daily (BID). At the Week 6 visit the dose may be increased to 100 mg orally BID, depending on safety and Hb response. Key inclusion criteria are: 1)  $\beta$ -thalassemia (with or without  $\alpha$ -globin gene mutation), Hb E/ $\beta$ -thalassemia or Hb H disease; 2) baseline Hb  $\leq 9$  g/dL; 3) NTDT defined as  $\leq 5$  units of RBCs transfused in the preceding 24 weeks and no transfusions in the 8 weeks prior to the first day of study drug; 4) adequate organ function. The primary endpoint is the proportion of subjects who achieve an Hb response, defined as an increase in Hb of  $\geq 1.0$  g/dL from baseline at any time between Week 4 and Week 12 (inclusive). Key secondary endpoints include changes in Hb and markers of hemolysis and hematopoietic activity, and assessments of safety and pharmacokinetics. The study is currently enrolling.

**Results:** Not yet available.

#### Image/Pictures:

Study Schema



BID = twice daily; d = days.

<sup>1</sup> Subjects may undergo an intrasubject dose escalation at the Week 6 visit based on an evaluation of safety and hemoglobin concentration (as detailed in the body of the protocol).

<sup>2</sup> Subjects who complete the 24-week Core Period and achieve a hemoglobin response with an acceptable safety profile may continue study drug for an additional 2 years in the Extension Period.

<sup>3</sup> The safety follow-up visit will occur 28 days after the subject's last dose of study drug.

**Summary/Conclusion:** This is the first study of mitapivat in subjects with NTDT. The study will provide insights into the efficacy, safety, and pharmacokinetics of PK-R activation in NTDT. Mitapivat may represent a novel therapeutic approach in the management of anemia in thalassemic syndromes.

**Keywords:** Clinical trial, Thalassemia



101. RED CELLS AND ERYTHROPOIESIS, STRUCTURE AND FUNCTION, METABOLISM, AND SURVIVAL. EXCLUDING IRON | NOVEMBER 13, 2019

## Mitapivat (AG-348) in Adults with Pyruvate Kinase Deficiency Who Are Not Regularly Transfused: A Phase 3, Randomized, Multicenter, Double-Blind, Placebo-Controlled Study (ACTIVATE) in Progress

Eduard J. van Beers, MD PhD, Mallie P. Judge, BS, RN, Lei Hua, PhD, Chris Mix, MD



*Blood* (2019) 134 (Supplement 1): 4791.

<https://doi.org/10.1182/blood-2019-123100>

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**Background:** Pyruvate kinase (PK) deficiency is an under-recognized hereditary disease that causes lifelong hemolytic anemia. Mutations in the *PKLR* gene lead to reduced red cell PK (PK-R) enzyme activity, resulting in defective glycolysis and decreased lifespan of red blood cells. Mitapivat (AG-348) is a novel, first-in-class, oral, small-molecule allosteric activator of PK-R under clinical testing as the first targeted, disease-altering therapy for PK deficiency. The DRIVE PK study (NCT02476916; a phase 2, open-label, dose-ranging trial in adults with PK deficiency who are not regularly transfused) demonstrated that twice-daily (BID) dosing with mitapivat for >6 months was well tolerated and induced rapid, substantial, and durable responses (Grace et al. *ASH* 2017). Of 52 enrolled subjects, 26 (50%) had a maximum hemoglobin (Hb) increase of >1 g/dL in the 6-month treatment period. Among these 26 subjects, the mean maximum Hb increase was 3.4 g/dL, and 25 (96%) had ≥1 missense *PKLR* mutation. Based on these findings, mitapivat has entered phase 3 development in PK deficiency. The design of the ongoing phase 3 ACTIVATE study and its extension study are reported here, with an update on country/site activation.

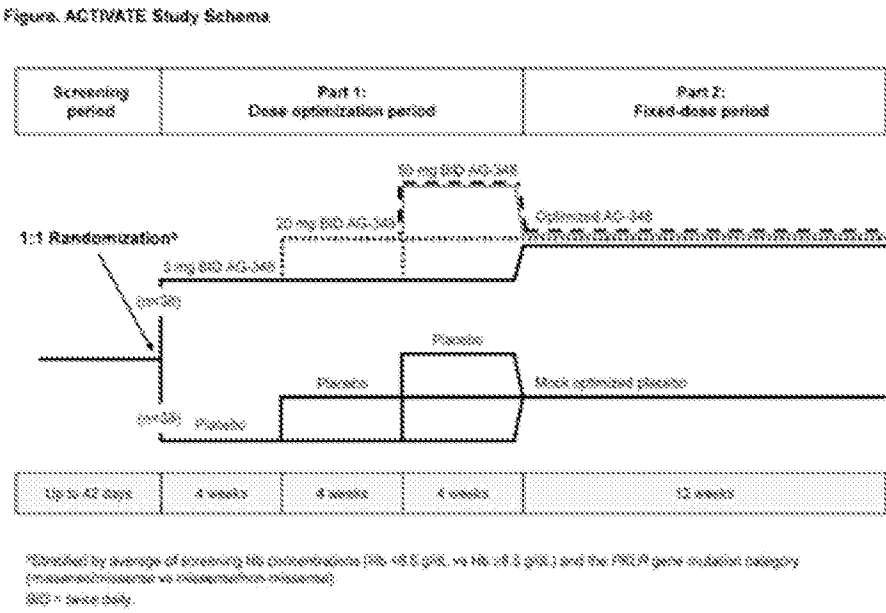
**Methods:** ACTIVATE is a phase 3, multicenter, randomized, double-blind, placebo-controlled study (NCT03548220) evaluating the efficacy and safety of mitapivat. Adults with PK deficiency who are not regularly transfused ( $\leq 4$  transfusion episodes in the previous year and no transfusions in the prior

3 months) will be randomized in a 1:1 ratio to mitapivat (administered orally, BID) or matched placebo. Additional criteria include baseline Hb  $\leq 10.0$  g/dL and adequate organ function. Subjects who are homozygous for the R479H mutation or have 2 non-missense mutations (without the presence of another missense mutation) in *PKLR* will be excluded.

The study consists of a screening period of up to 42 days, a 12-week dose optimization period, and a 12-week fixed-dose period (Figure). During the dose optimization period, mitapivat or matched placebo are titrated up to each subject's individually optimized dose, with an initial dose of 5 mg BID for all subjects and potentially 2 sequential dose increases (from 5 to 20 mg BID and from 20 to 50 mg BID), depending on safety and Hb change. The primary endpoint is the Hb response, defined as the proportion of subjects who achieve an increase of  $\geq 1.5$  g/dL in Hb sustained over at least 2 of the last 3 visits in the fixed-dose period. The average change in Hb during the fixed-dose period is considered a key secondary endpoint to further evaluate the magnitude of the treatment effect. Other secondary efficacy endpoints, such as markers of hemolysis and hematopoietic activity, will be evaluated to support the efficacy evaluation and confirm the mechanism of action, and safety is included as a secondary objective and endpoint. Two questionnaires, one specifically designed to measure the signs and symptoms of PK deficiency and the other designed to evaluate their impact on patients' lives, are also included for their validation and to further evaluate the efficacy of the treatment.

All subjects who complete the study have the opportunity to enroll in an open-label extension study (NCT03853798) in which all participants will receive mitapivat for up to 192 weeks. An independent data monitoring committee will review the study data periodically and provide safety oversight. The ACTIVATE study is currently ongoing.

§



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Disclosures

**van Beers:**Novartis: Consultancy, Research Funding; **Pfizer:** Research Funding; **RR Mechatronics:** Research Funding; **Agios Pharmaceuticals, Inc.:** Membership on an entity's Board of Directors or advisory committees, Research Funding. **Judge:**Agios: Employment, Equity Ownership. **Hua:**Agios Pharmaceuticals, Inc.: Employment, Equity Ownership. **Mix:**Agios: Employment, Equity Ownership. §

**Topics:** pyruvate kinase deficiency, blood transfusion, genetic disorder, hemoglobin, hemolytic anemia, pyruvate kinase, screening, surrogate endpoints, transfusion, hemolysis

Overview of your submission



EUROPEAN  
HEMATOLOGY  
ASSOCIATION



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- Your application will be reviewed by the organizers of the meeting.

Title

ACTIVATE: an ongoing phase 3 randomised, double-blind, placebo-controlled study with mitapivat (AG-348) in adults with pyruvate kinase deficiency who are not regularly transfused

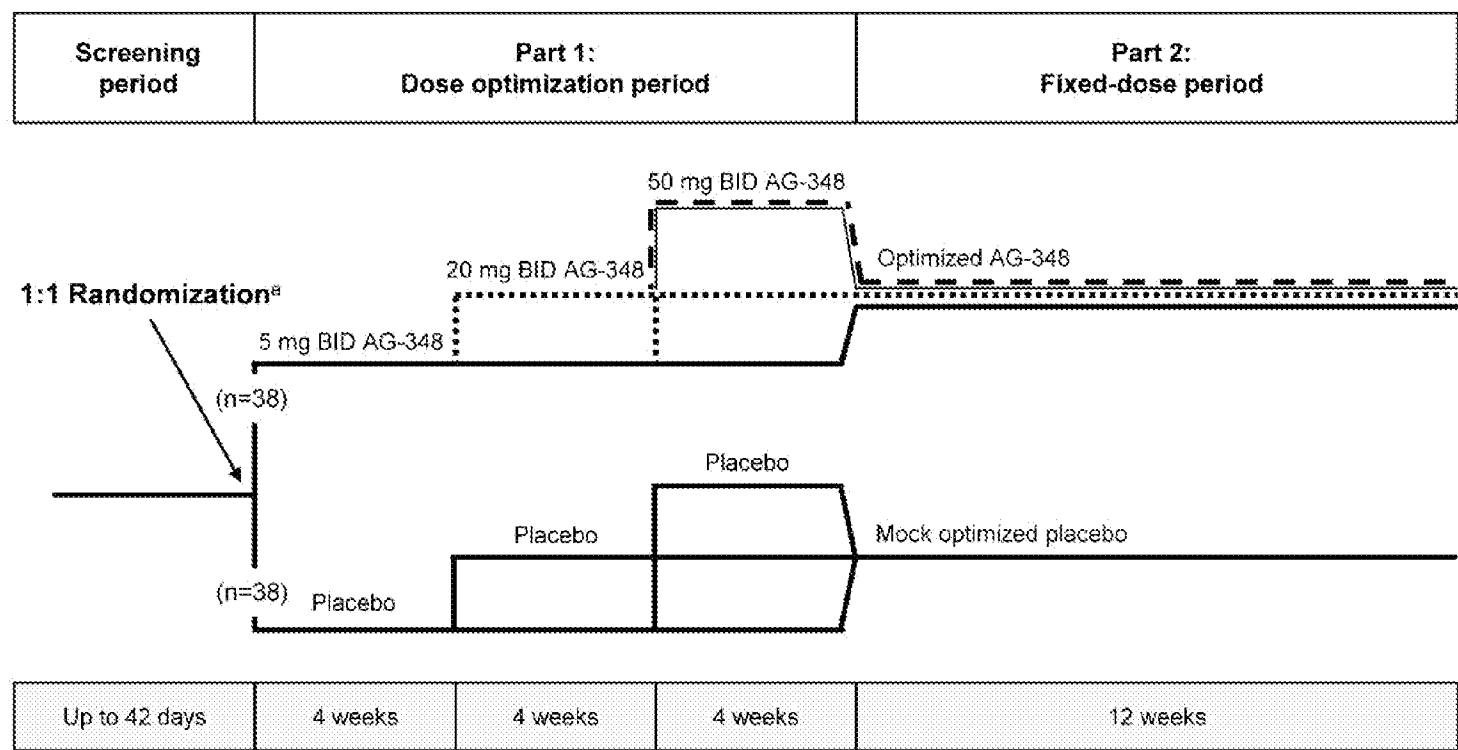
Case

**Background:** Pyruvate kinase (PK) deficiency is a red blood cell enzymopathy caused by mutations in the *PKLR* gene that lead to a deficit in red cell PK activity and chronic hemolytic anemia. Current disease management options are supportive only and have associated risks. Mitapivat (AG-348) is a novel, first-in-class, oral, small-molecule allosteric activator of PK-R in clinical development for treatment of PK deficiency. The phase 2 DRIVE PK study (NCT02476916; an open-label, dose-ranging trial in adults with PK deficiency who are not regularly transfused) demonstrated that twice-daily (BID) dosing with mitapivat was well tolerated and induced rapid, clinically significant ( $>1.0$  g/dL), increases in hemoglobin (Hb) with improvement in additional markers of hemolysis in 50% of patients (Grace et al. *N Engl J Med* 2019). Mitapivat currently is being studied in two phase 3 studies. Described here is the rationale and design of the ACTIVATE study and its extension study.

**Methods:** ACTIVATE is a global phase 3, multicenter, randomized, double-blind, placebo-controlled study (EudraCT 2017-003823-31 and NCT03548220) evaluating the efficacy and safety of mitapivat in adults with PK deficiency who do not receive regular transfusions ( $\leq 4$  transfusion episodes in the previous year and no transfusions in the prior 3 months). Subjects are randomized in a 1:1 ratio to mitapivat (administered orally, BID) or placebo. Subjects must have genetic confirmation of PK deficiency with at least one missense mutation. Patients who are homozygous for the R479H mutation or have 2 non-missense mutations (without the presence of another missense mutation) in *PKLR* will be excluded. A baseline Hb  $\leq 10.0$  g/dL is required for inclusion.

The study consists of a screening period (up to 42 days), a 12-week dose optimization period, and a 12-week fixed-dose period (Figure). During the optimization period, mitapivat or placebo are titrated up to each patient's individually optimized dose, with an initial dose of 5 mg BID and potentially 2 sequential dose increases (from 5 to 20 mg BID and from 20 to 50 mg BID), depending on tolerability and Hb response. The primary endpoint is the Hb response, defined as the proportion of patients who achieve an increase of  $\geq 1.5$  g/dL in Hb sustained over  $\geq 2$  of the last 3 visits in the fixed-dose period. The main secondary endpoint is average change in Hb levels during the fixed-dose period; additional secondary efficacy endpoints include markers of hemolysis and hematopoietic activity and safety. Two patient-reported outcome questionnaires, one specifically designed to measure the signs and symptoms of PK deficiency and the other designed to evaluate their impact on the subjects' lives, will be completed. All patients who complete the study will have the opportunity to enroll in an open-label extension study (EudraCT 2018-003459-39 and NCT03853798), in which all participants will receive mitapivat for up to 192 weeks. An independent data monitoring committee will review the study data periodically and provide safety oversight. The ACTIVATE study is currently enrolling.

Figure. ACTIVATE Study Schema



<sup>a</sup>Stratified by average of screening Hb concentrations (Hb <8.5 g/dL vs Hb ≥8.5 g/dL) and the *PKLR* gene mutation category (missense/missense vs missense/non-missense).  
BID = twice daily.

References

This trial is registered at clinicaltrials.gov NCT03548220 and NCT03853798 for the open-label extension study, and at EudraCT 2017-003823-31 and 2018-003459-39, respectively.

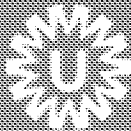
Topic

Red cell enzyme defects

Co-authors

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<sup>1</sup>Van Creveldkliniek, University Medical Center Utrecht, University of Utrecht Utrecht NETHERLANDS ;  
<sup>2</sup>Agiros Pharmaceuticals, Inc. Cambridge, MA UNITED STATES ;  
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Ex vivo treatment of red blood cells from 15 pyruvate kinase (PK)-deficient patients with AG-348, an allosteric activator of PK-R, increases enzymatic activity, protein stability and ATP levels



UMC Utrecht

Erinthe van Gerschoer<sup>1</sup>, Penelope A. Fowlsh<sup>2</sup>, Jeffrey Hixon<sup>2</sup>, Kaitlin Johnson<sup>2</sup>, Victor Chubukov<sup>2</sup>, Henry Dang<sup>2</sup>, Gerard Pasterkamp<sup>1</sup>, Stephanie van Straten<sup>1</sup>, Charles Kung<sup>2</sup>, and Richard van Vlegel<sup>1</sup> <sup>1</sup>Department of Clinical Chemistry and Hematology, University Medical Center Utrecht, Utrecht, The Netherlands; <sup>2</sup>Agios Pharmaceuticals Inc., Cambridge, MA, United States; <sup>3</sup>UCB Therapeutics, Cambridge, MA, United States

Background

Pyruvate kinase (PK) deficiency is a rare hereditary disorder affecting red blood cell (RBC) glycolysis caused by mutations in the *PKLR* gene. PK-deficient RBCs are characterized by changes in metabolism associated with defective glycolysis, including a build-up of the upstream metabolite 2,3-disphosphoglycerate and deficiency in the PK product ATP. It is hypothesized that insufficient energy production affects red cell homeostasis, promoting premature removal of PK-deficient RBCs from the circulation. Affected patients display chronic hemolytic anemia of variable severity.

Treatment of PK-deficient patients is generally supportive, focusing on the anemia and iron overload state, and there are no approved drugs that directly target mutated PK. AG-348 is an allosteric activator of the RBC isoform of PK (PK-R) and in clinical development for the treatment of PK deficiency.

Aim

To evaluate the ex vivo effect of AG-348 treatment on PK-R enzymatic function and RBC metabolism.

Study Design and Methods

This is an observational case-control study, approved by the Institutional Review Board.

Enrolled patients (N=15) were adults, transfusion-independent and compound heterozygous or homozygous for mutations in *PKLR*. Baseline metabolic profiling was performed by LC-MS/MS. Purified RBCs from patients and healthy control subjects were incubated with AG-348 (up to 10  $\mu$ M) for 24 hours at 37°C. After 6 and 24 hours PK-R activity and ATP levels were measured. For determination of PK-R thermal stability, RBC lysates were incubated for 2 hours with 2  $\mu$ M AG-348 (37°C) prior to testing. Baseline protein levels of PK-R were assessed by MSD-ELISA using antibodies against PK-R.

Table 1. Genotypes and Baseline characteristics of PK-deficient patients

| PKLR mutation | Amino acid change  | Hb (g/dL)                    | Retic (%) | PK (U/gHb) | PK (U/gHb) | PK-R/K  |
|---------------|--------------------|------------------------------|-----------|------------|------------|---------|
| AG050/F       | c.1121T>G (L486S)* | 137/140/140                  | 9.7       | 10         | 5.0        | 1.8     |
| AG018/M       | c.881G>A (L294S)*  | 11.2                         | 7         | 1.1        | 1.7        | 1.8     |
| AG012/M       | c.1178A>G (L392S)* | 139/135/140                  | 9.4       | 1.35       | 1.69       | 2.7     |
| AG044/F       | c.1462C>T (L490P)* | 140/139/140                  | 6.6       | 1.34       | 1.59       | 2.1     |
| AG012/F       | c.1121T>G (L486S)* | 139/139/140                  | 10.9      | 7          | 1.1        | 1.4     |
| AG008/F       | c.1507G>A (L502S)* | 139/139/140                  | 7.7       | 75         | 1.9        | 0.8     |
| AG057/M       | c.376A>G (L125K)*  | 139/139/140                  | 8.0       | 55         | 1.7        | 5.6     |
| AG020/F       | c.881G>A (L294S)*  | 13.3                         | 5         | 1.2        | 1.4        | 1.8     |
| AG020/M       | c.1315G>A (L438S)* | 10.5                         | 40        | 2.5        | 1.1        | 0.6     |
| AG023/M       | c.1154G>A (L382S)* | 13.4                         | 15        | 2.7        | 2.0        | 1.3     |
| AG006/M       | c.721G>T (L240P)*  | 13.4                         | 98        | 2.2        | 1.1        | 0.4     |
| AG046/M       | c.1421T>G (L474P)* | 12.9                         | 20        | 10.0       | 3.5        | 2.8     |
| AG010/M       | c.1269G>A (L423S)* | 9.7                          | 55        | 1.8        | 5.8        | 1.1     |
| AG055/F       | c.401T>A (L134S)*  | 13.6                         | 80        | 1.2        | 5.2        | 0.2     |
| AG050/M       | c.1529G>A (L509S)* | 12.5                         | 40        | 1.6        | 2.8        | 0.6     |
| Reference     |                    | N: 13.8-17.2<br>D: 11.9-15.7 | 1-2       | 5.2-16.9   | 0.8-1.4    | 50-16.6 |

Figure 1. Correlation between PK activity and PK-R protein levels in PK-deficient patients (red) and controls (blue). (inset) PK-deficient red cells with higher PK-R protein levels are more likely to have increased ATP and/or PK-R activity after (>1.5 fold, asterisk) after AG-348 treatment.

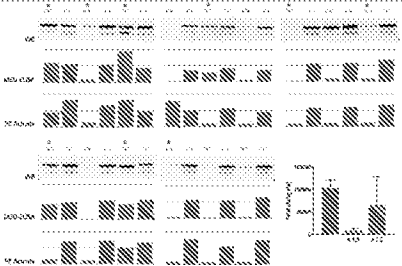


Figure 2. PK-deficient patients show glycolytic intermediates levels that are consistent with a block of glycolysis at the PK step.

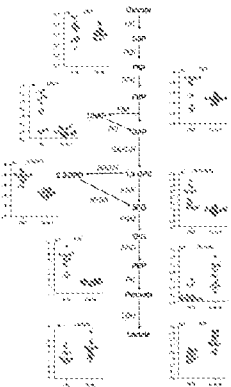


Figure 3. Upon incubation with AG-348 (10  $\mu$ M) for 6 (pink) and 24 hours (red), red cells from patients and controls show increased red cell PK activity.

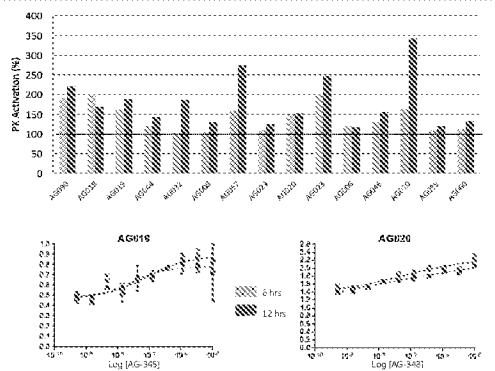


Figure 4. Upon incubation with AG-348 (10  $\mu$ M) for 6 (pink) and 24 hours (red), red cells from patients and controls show increased levels of ATP.

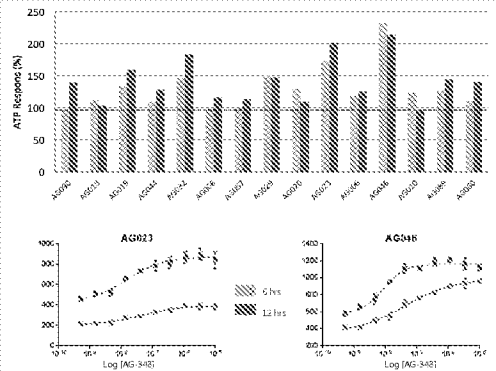
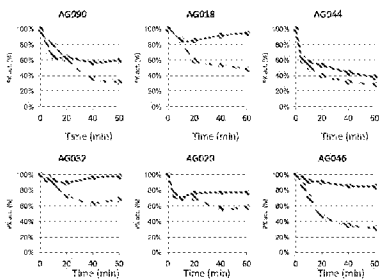


Figure 5. PK thermal stability is restored upon ex vivo treatment of red cell lysates with AG-348 (2  $\mu$ M). Dotted line untreated lysate, solid line treated with AG-348.



Results

Baseline patient characteristics show strongly reduced PK-R activity in all patient cells, in particular taking into account the degree of reticulocytosis (Table 1). Baseline protein level analyses suggests that a certain level of PK-R protein is required for cells to respond to AG-348 treatment *ex vivo* (i.e. 1.5-fold increase in PK activity or ATP levels), as treatment effects were minimal in patient cells with very low or undetectable levels of PK-R (Figures 1). Distinct metabolic changes were consistent with a block of glycolysis at the PK-R step (Figure 2). Treatment of PK-deficient RBCs with AG-348 resulted in increased enzymatic activity in all patient cells after 24 hours (Figure 3, mean increase 1.8-fold, range, 1.2-3.4). Similar increases were observed in control cells (mean fold increase 2.3, range, 1.2-7.1). ATP levels in PK-deficient cells increased upon AG-348 treatment (Figure 4, mean increase 1.5-fold, range: 1.0-2.2) similar to control cells (mean increase 1.6 fold, range, 1.4-1.8). Generally, PK-R thermal stability was strongly reduced in PK-deficient patient cells, illustrated by a mean loss of activity of 72% (19% for control cells) after incubation at 53 °C for 60 minutes. *Ex vivo* treatment with AG-348 prior to heat incubation resulted in residual activity 1.4 to >10-fold higher than residual activity of vehicle-treated samples (Figure 5).

Conclusion/summary

These data support the hypothesis that drug intervention with AG-348 effectively upregulates PK-R enzymatic activity and increases stability in PK-deficient RBCs over a broad range of *PKLR* genotypes. The concomitant increase in ATP levels suggests that glycolytic pathway activity may be restored. AG-348 treatment, which is being tested in an ongoing Phase II study in PK-deficient patients (NCT02476916), may represent an attractive way to correct the underlying pathologies of PK deficiency.

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UMC Utrecht

# Abstract Submission

## 27. Enzymopathies, membranopathies and other anemias

EHA-2288

### EX VIVO TREATMENT OF RED BLOOD CELLS FROM 15 PYRUVATE KINASE (PK)-DEFICIENT PATIENTS WITH AG-348, AN ALLOSTERIC ACTIVATOR OF PK-R, INCREASES ENZYMATIC ACTIVITY, PROTEIN STABILITY AND ATP LEVELS.

Brigitte van Oirschot<sup>1</sup>, Penelope A. Kosinksi<sup>2</sup>, Jeffrey Hixon<sup>3</sup>, Kendall Johnson<sup>2</sup>, Victor Chubukov<sup>2</sup>, Lenny Dang<sup>2</sup>, Gerard Pasterkamp<sup>1</sup>, Stephanie van Straaten<sup>1, 4</sup>, Charles Kung<sup>2</sup>, Richard van Wijk<sup>\* 1</sup>

<sup>1</sup>Clinical Chemistry and Haematology, University Medical Center Utrecht, Utrecht, Netherlands, <sup>2</sup>Agios Pharmaceuticals, Inc., <sup>3</sup>KSQ Therapeutics, Cambridge, MA, United States, <sup>4</sup>Van Creveldkliniek, University Medical Center Utrecht, Utrecht, Netherlands

Please indicate all of which apply to the presenting author: PhD

**Background:** Pyruvate kinase (PK) deficiency is a rare hereditary disorder affecting red blood cell (RBC) glycolysis. It is caused by mutations in the *PKLR* gene. PK-deficient RBCs are characterized by changes in metabolism associated with defective glycolysis, including a build-up of the upstream metabolite 2,3-disphosphoglycerate, and deficiency in the PK product ATP. It is hypothesized that insufficient energy production affects red cell homeostasis, promoting premature removal of PK-deficient RBCs from the circulation. Affected patients display chronic hemolytic anemia of variable severity. Treatment of PK-deficient patients is generally supportive, focusing on the anemia and iron overload state, and there are no approved drugs that directly target mutated PK. AG-348 is an allosteric activator of the RBC isoform of PK (PK-R) and in clinical development for the treatment of PK deficiency

**Aims:** To evaluate the *ex vivo* effect of AG-348 treatment on PK-R enzymatic function, RBC metabolism and deformability

**Methods:** Observational case-control study, approved by the Institutional Review Board. All patients gave informed consent. Enrolled patients (N=15) were adults, transfusion-independent and compound heterozygous or homozygous for mutations in *PKLR*. Baseline metabolic profiling was performed by LC-MS/MS. Purified RBCs from patients and healthy control subjects were incubated with AG-348 (up to 10 µM) for 24 hours at 37°C. After 6 and 24 hours PK-R activity, ATP levels and RBC deformability (by Lorrca) were measured. For determination of PK-R thermal stability, RBC lysates were incubated for 2 hours with 2 µM AG-348 (37°C) prior to testing. Baseline protein levels of PK-R were assessed using antibodies against PK-R

**Results:** Baseline patient characteristics show strongly reduced PK-R activity in all patient cells, in particular taking into account the degree of reticulocytosis (Table 1). Distinct metabolic changes were consistent with a block of glycolysis at the PK-R step. Treatment of PK-deficient RBCs with AG-348 resulted in increased enzymatic activity in all patient cells after 24 hours (mean increase 1.8-fold, range, 1.2-3.4). Similar increases were observed in control cells (mean fold increase 2.3, range, 1.2-7.1). ATP levels in PK-deficient cells increased upon AG-348 treatment (mean increase 1.5-fold, range: 1.0-2.2) similar to control cells (mean increase 1.6 fold, range, 1.4-1.8). Generally, PK-R thermal stability was strongly reduced in PK-deficient patient cells, illustrated by a mean loss of activity of 72% (19% for control cells) after incubation at 53 °C for 60 minutes. *Ex vivo* treatment with AG-348 prior to incubation resulted in residual activity 1.4 to >10-fold higher than residual activity of vehicle-treated samples. Baseline protein level analyses suggests that a certain level of PK-R protein is required for cells to respond to AG-348 treatment *ex-vivo*, as treatment effects were minimal in patient cells with very low or undetectable levels of PK-R. In approximately half of the patients, *ex vivo* treatment with AG-348 was associated with an increase in RBC deformability, although there doesn't appear to be a clear correlation with enzymatic or metabolic response

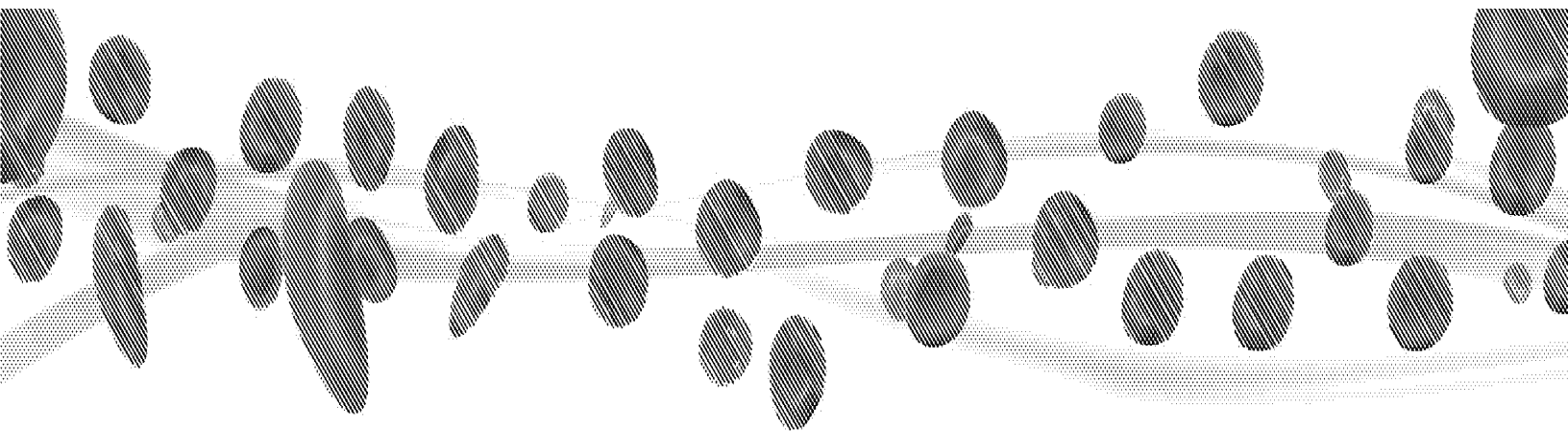
Image/Pictures:

Table 1. Baseline characteristics and genotypes of PK-deficient patients

| Patient/<br>Gender | <i>PKLR</i> mutation  | Amino acid change | Hb<br>(g/dL)                 | Retic<br>(%) | PK<br>(U/gHb) | HK<br>(U/gHb) | PK/HK    |
|--------------------|-----------------------|-------------------|------------------------------|--------------|---------------|---------------|----------|
| AG090/F            | c.1121T>C/c.1456C>T   | L374P/R486W       | 9.7                          | 10           | 9.0           | 5.0           | 1.8      |
| AG018/M            | c.331G>A/c.1456C>T    | G111R/R486W       | 11.2                         | 7            | 3.1           | 1.7           | 1.8      |
| AG019/M            | c.1178A>G/c.1456C>T   | N393S/R486W       | 9.4                          | >35          | 16.9          | 6.2           | 2.7      |
| AG044/F            | c.1462C>T/c.1529G>A   | R488*/R510Q       | 6.6                          | >34          | 15.9          | 7.4           | 2.1      |
| AG032/F            | c.1121T>C/c.1706G>A   | L374P/R569N       | 10.9                         | 7            | 2.1           | 1.5           | 1.4      |
| AG008/F            | c.587+1G>A/c.1436G>A  | Splicing/R479H    | 7.7                          | 75           | 1.9           | 6.0           | 0.3      |
| AG057/M            | c.326+2A>C/c.1529G>A  | Splicing/R510Q    | 8.0                          | 55           | 1.7           | 5.6           | 0.3      |
| AG029/F            | c.331G>A/c.1456C>T    | G111R/R486W       | 11.9                         | 5            | 2.2           | 1.4           | 1.6      |
| AG020/M            | c.331G>A/c.1492C>T    | G111R/R498C       | 10.3                         | 40           | 2.5           | 4.1           | 0.6      |
| AG023/M            | c.1134G>A/c.1529G>A   | R385K/R510Q       | 13.4                         | 15           | 2.7           | 2.0           | 1.3      |
| AG006/M            | c.721G>T/c.1529G>A    | E241*/R510Q       | 8.2                          | 85           | 2.2           | 6.1           | 0.4      |
| AG045/M            | c.142_159del/c.494G>T | T48_T53del/G165V  | 12.0                         | 20           | 10.0          | 3.5           | 2.8      |
| AG010/M            | c.1269G>A/c.1654G>A   | Splicing/Y552M    | 9.7                          | 55           | 1.8           | 5.8           | 0.3      |
| AG089/F            | c.401T>A/c.1529G>A    | V134D/R510Q       | 9.6                          | 60           | 1.2           | 5.2           | 0.2      |
| AG060/M            | c.1529G>A/c.1529G>A   | R510Q/R510Q       | 12.8                         | 40           | 1.6           | 2.8           | 0.6      |
| Reference          |                       |                   | M: 13.8-17.2<br>F: 11.9-15.5 | 1-2          | 9.2-16.9      | 0.9-1.4       | 9.0-16.6 |

**Summary/Conclusion:** These data support the hypothesis that drug intervention with AG-348 effectively upregulates PK-R enzymatic activity and increases stability in PK-deficient RBCs over a broad range of *PKLR* genotypes. The concomitant increase in ATP levels suggests that glycolytic pathway activity may be restored. AG-348 treatment may represent an attractive way to correct the underlying pathologies of PK deficiency





Phase 1 Multiple Ascending Dose Study  
of the Safety, Tolerability and  
Pharmacokinetics/Pharmacodynamics of AG-348, a First-in-Class  
Allosteric Activator  
of Pyruvate Kinase R, in Healthy Subjects

Hua Yang<sup>1</sup>, Elizabeth Merica<sup>1</sup>, Yue Chen<sup>1</sup>, Marvin Cohen<sup>2</sup>, Ronald Goldwater<sup>3</sup>, Hyeryun Kim<sup>1</sup>, Penelope A Kosinski<sup>1</sup>, Charles Kung<sup>1</sup>, Meredith Goldwasser<sup>1</sup>, Bruce Silver<sup>4</sup>, Luke Utley<sup>1</sup>, Sam Agresta<sup>1</sup>, Ann Barbier<sup>1</sup>

<sup>1</sup>Agios Pharmaceuticals, Cambridge, MA, USA; <sup>2</sup>MBC Pharma Solutions, Newtown, PA, USA; <sup>3</sup>Parexel International, Baltimore, MD, USA; <sup>4</sup>Bruce A Silver Clinical Science and Development, Dunkirk



6<sup>th</sup> EUROPEAN SYMPOSIUM ON RARE ANAEMIAS  
1<sup>st</sup> Dutch-Belgian meeting for patients and health professionals

21<sup>st</sup> - 22<sup>nd</sup> November 2015  
Amsterdam - The Netherlands

| Disclosures  |                  |          |            |             |                 |                |       |
|--------------|------------------|----------|------------|-------------|-----------------|----------------|-------|
| Company name | Research support | Employee | Consultant | Stockholder | Speakers bureau | Advisory board | Other |
| Agios        |                  | X        |            |             |                 |                |       |
|              |                  |          |            |             |                 |                |       |
|              |                  |          |            |             |                 |                |       |
|              |                  |          |            |             |                 |                |       |
|              |                  |          |            |             |                 |                |       |
|              |                  |          |            |             |                 |                |       |
|              |                  |          |            |             |                 |                |       |

| This talk is applicable for:              |          |          |
|-------------------------------------------|----------|----------|
|                                           | Definite | Probable |
| Thalassemia's                             |          | X        |
| Sickle cell disease                       |          |          |
| Membrane disorders<br>(e.g. sferocytosis) |          |          |
| Enzyme defects (e.g. PKD, G6PD)           | X        |          |
| PNH                                       |          |          |
| Other forms of hemolytic disease          |          |          |



# Disclosures

- ✧ This study was funded by Agios Pharmaceuticals
- ✧ Hua Yang, Elizabeth Merica, Yue Chen, Hyeryun Kim, Penelope A Kosinski, Charles Kung, Meredith Goldwasser, Luke Utley, Sam Agresta, Ann Barbier: Agios – employees and stockholders
- ✧ Marvin Cohen, Bruce Silver: Agios – consultants
- ✧ Ronald Goldwater: Parexel International – employee
- ✧ Editorial assistance was provided by Christine Tomlins, PhD, Excel Scientific Solutions, Horsham, UK, and supported by Agios



# Key Points

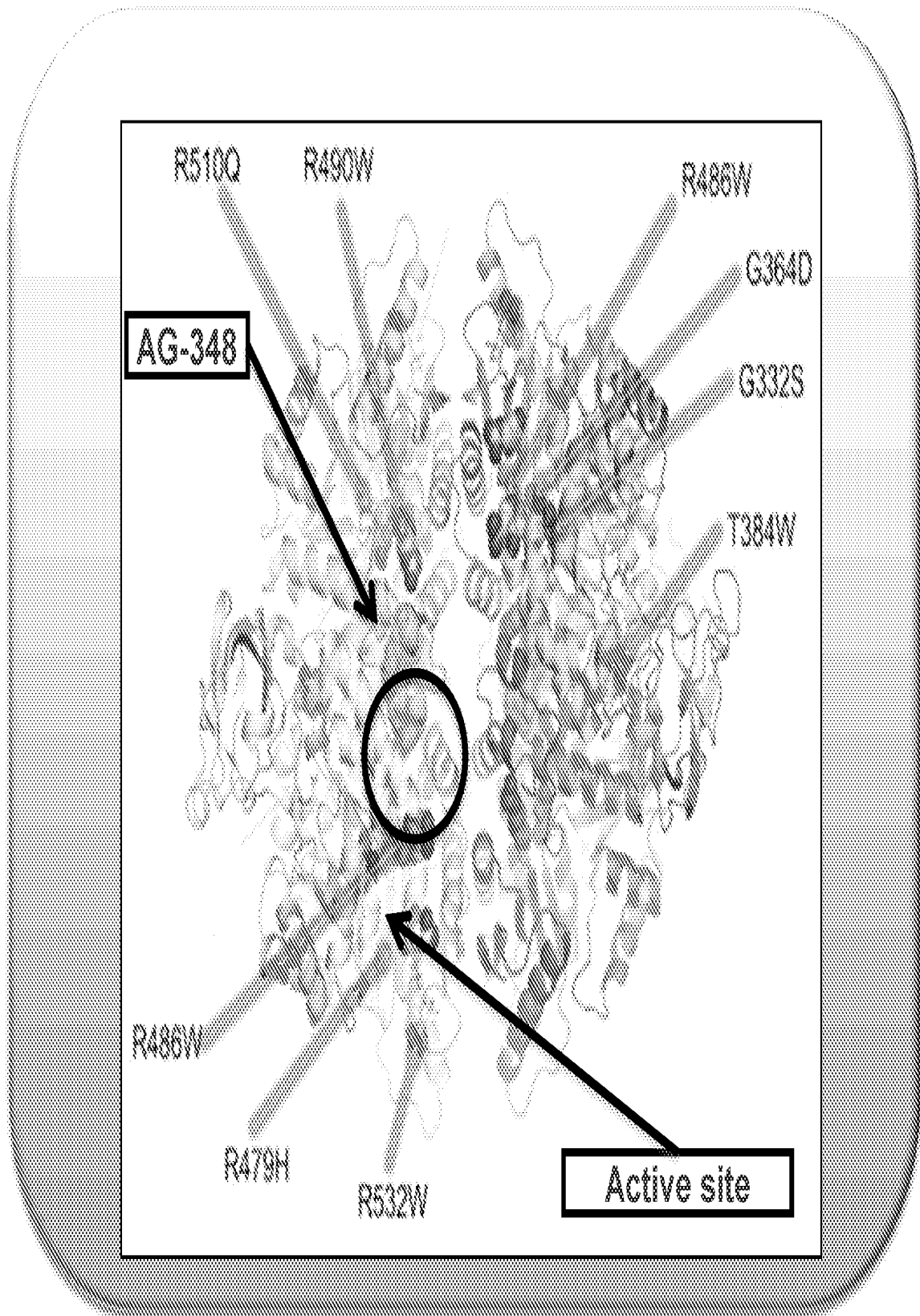
- Pyruvate Kinase (PK) deficiency is a serious, rare, genetic disease.
- AG-348 is a PK-R activator intended to correct the glycolytic pathway defect in patients with PK deficiency.
- Healthy volunteer study has established a safety profile at pharmacodynamically active doses that may be relevant to patients with PK deficiency.
- DRIVE PK, a global phase 2 study in patients, is now open and enrolling



# AG-348: Allosteric Activator of Wild-Type and Mutant PK-R

PK-R enzyme: active tetramer

AG-348 binds at the PK-R dimer-dimer interface

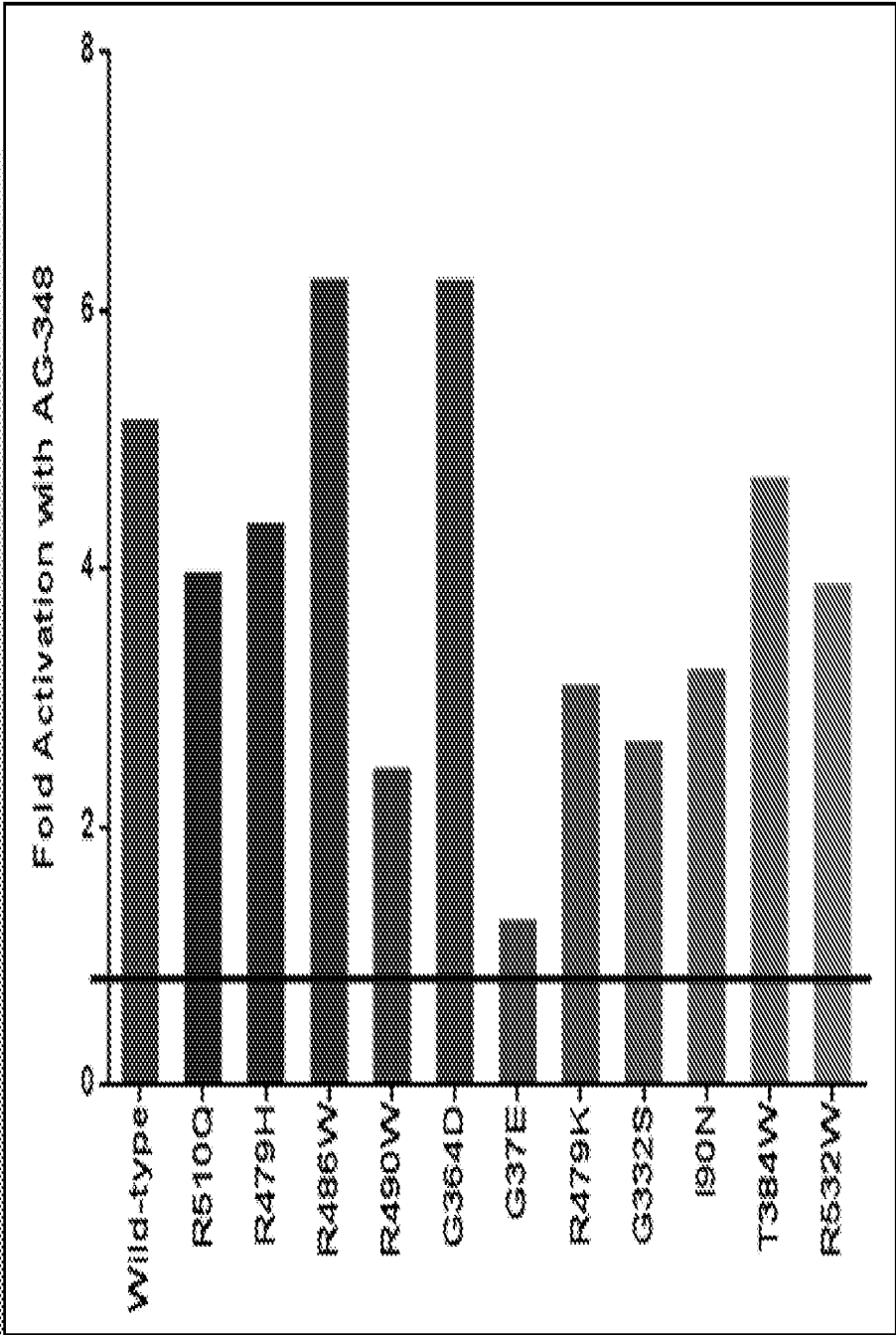


Kung C et al. 55<sup>th</sup> ASH Annual meeting 2013, Abstract 2180

6<sup>th</sup> European Symposium on Rare Anaemias - 1<sup>st</sup> Dutch-Belgian meeting for patients and health professionals

# AG-348 Activates PK-R Wild-Type and Mutant Subtypes

AG-348 activates PK-R wild type and nearly all mutant enzymes tested



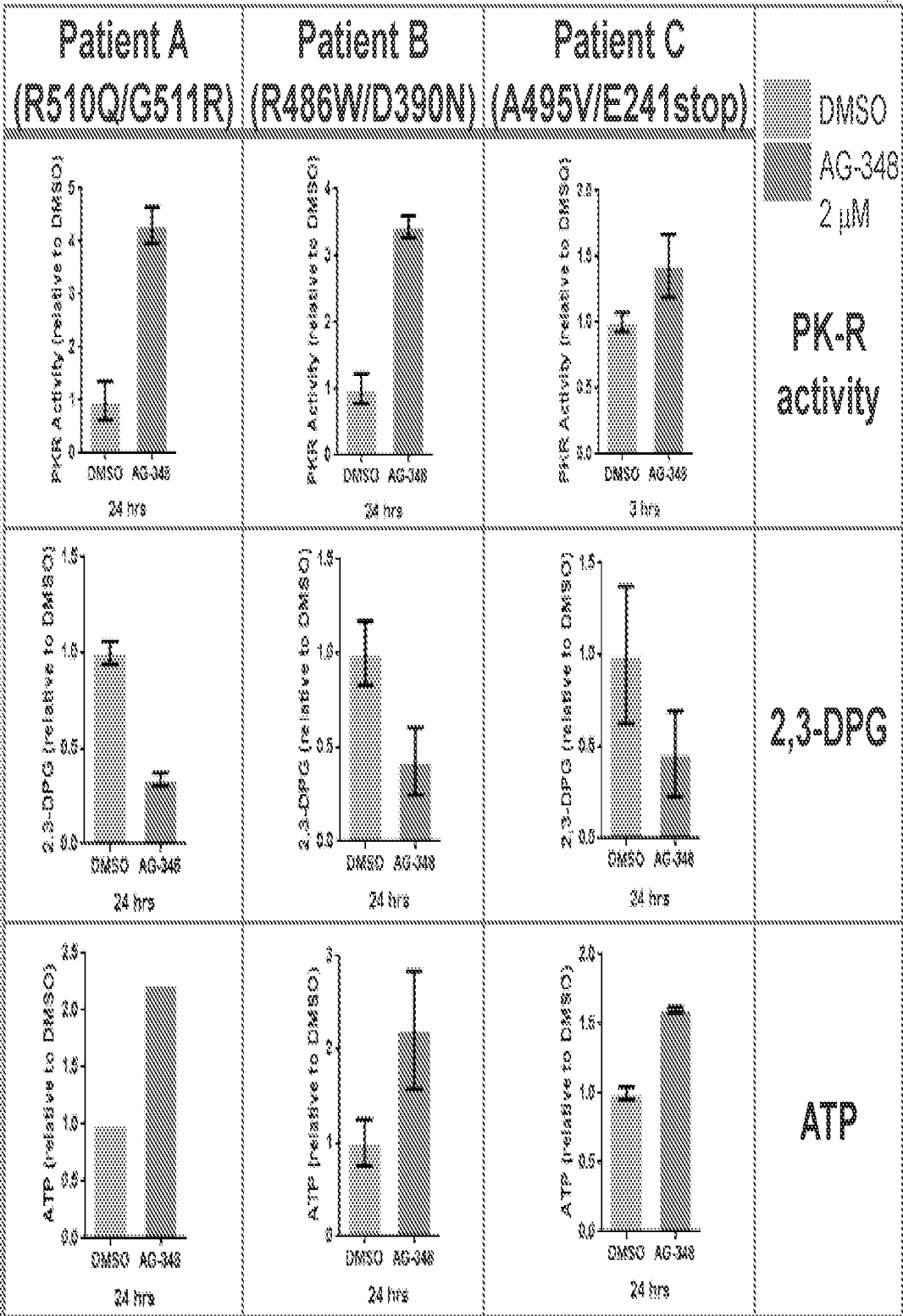
Kung C et al. 56<sup>th</sup> ASH Annual meeting 2014, Abstract 4010

6<sup>th</sup> European Symposium on Rare Anaemias - 1<sup>st</sup> Dutch-Belgian meeting for patients and health professionals



# AG-348 Restores the Glycolytic Pathway in Models

**PK Deficient  
Patient Red  
Blood Cell  
Samples**



Kung C et al. 56<sup>th</sup> ASH Annual meeting 2014, Abstract 4010

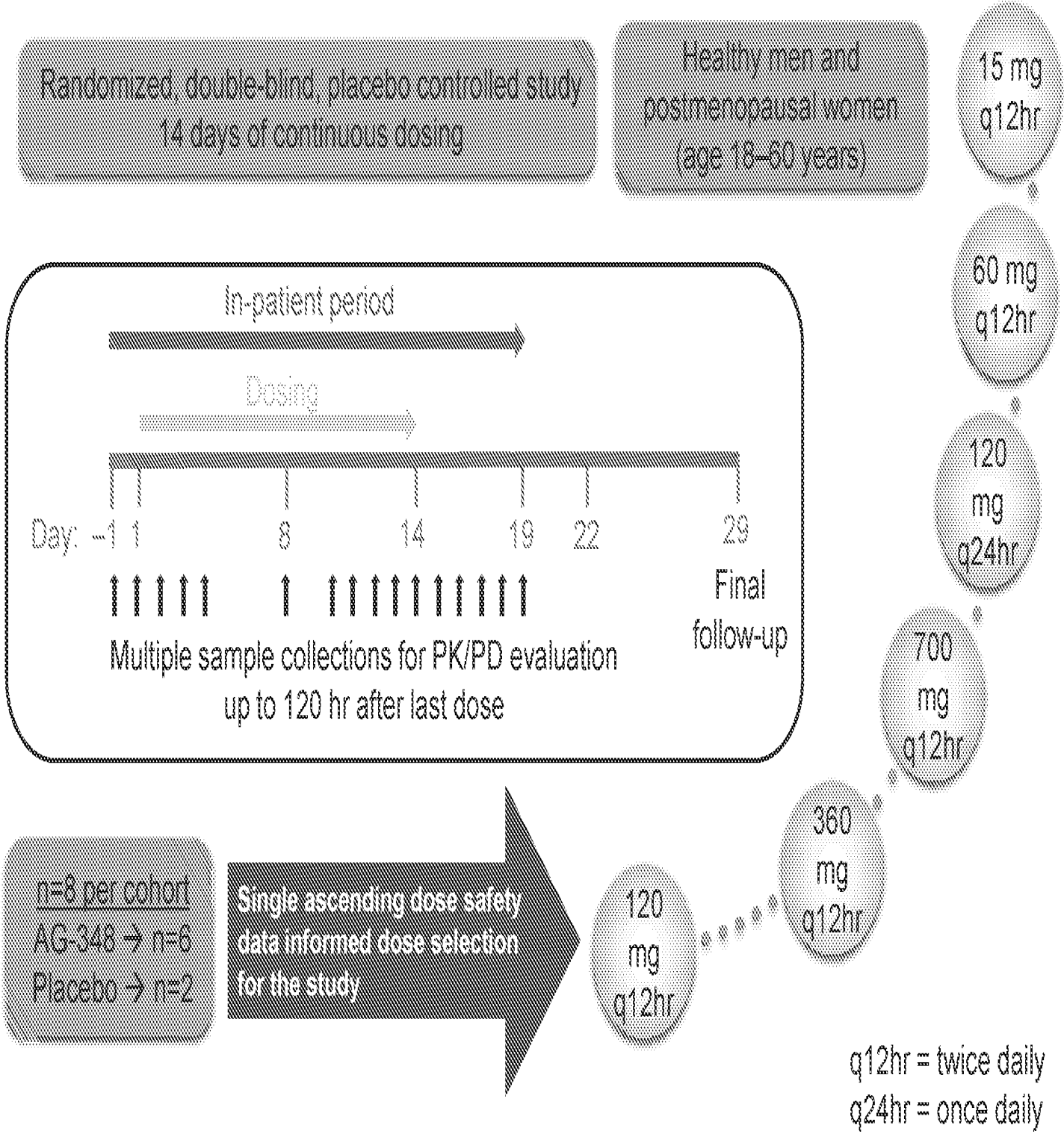
6<sup>th</sup> European Symposium on Rare Anaemias - 1<sup>st</sup> Dutch-Belgian meeting for patients and health professionals



# Multiple Ascending Dose Study Completed



**Objective: Establish the safety, tolerability and PD effects of AG-348**



PK/PD = pharmacokinetic/pharmacodynamic

CT02149966

# Demographics and Disposition



| Characteristic                                   | Placebo<br>n=12 | AG-348<br>n=36 |
|--------------------------------------------------|-----------------|----------------|
| Men, n (%)                                       | 9 (75)          | 33 (92)        |
| Women, n (%)                                     | 3 (25)          | 3 (8)          |
| Age in years, mean (SD)                          | 37.8 (11.4)     | 42.8 (12.2)    |
| Race, n (%)                                      |                 |                |
| Asian                                            | —               | 1 (3)          |
| Black                                            | 6 (50)          | 18 (50)        |
| White                                            | 6 (50)          | 17 (47)        |
| Body mass index in kg/m <sup>2</sup> , mean (SD) | 27.4 (2.3)      | 26.5 (2.0)     |

- 12 placebo treated and 32 of 36 AG-348 treated patients completed study:
  - Two withdrew due to AEs: Drug eruption (60 mg q12hr), grade 3 liver function test abnormalities at the highest dose explored (700 mg q12hr)
  - Two withdrew consent due to nausea and vomiting at the highest dose explored (700 mg q12hr)

AE = adverse event; SD = standard deviation



# AG-348 was well tolerated up to 360 mg oral q12 hr

- Drug-related events were grade 1 and 2
- One grade 3 event at 700 mg q12h (increased LFTs – DLT)

| AE, n (%)                                        | Placebo<br>n=12 | 15–360 mg q12hr<br>n=30 | 700 mg q12hr<br>n=6 |
|--------------------------------------------------|-----------------|-------------------------|---------------------|
| Any AE                                           | 4 (33)          | 10 (33)                 | 6 (100)             |
| Most common treatment-related AEs (≥2 subjects): |                 |                         |                     |
| Nausea                                           | —               | —                       | 5 (83)              |
| Headache                                         | 1 (8)           | 1 (3)                   | 4 (67)              |
| Vomiting                                         | 1 (8)           | —                       | 3 (50)              |
| Feeling hot                                      | 1 (8)           | —                       | 3 (50)              |
| Decreased appetite                               | —               | 2 (7)                   | 1 (17)              |
| Restlessness                                     | —               | —                       | 3 (50)              |
| Fatigue                                          | —               | —                       | 2 (33)              |
| Dizziness                                        | 1 (8)           | —                       | 2 (33)              |
| Hyperhidrosis                                    | 1 (8)           | —                       | 2 (33)              |
| Drug eruption                                    | —               | 2 (7)                   | —                   |
| Abdominal discomfort                             | 2 (17)          | —                       | —                   |

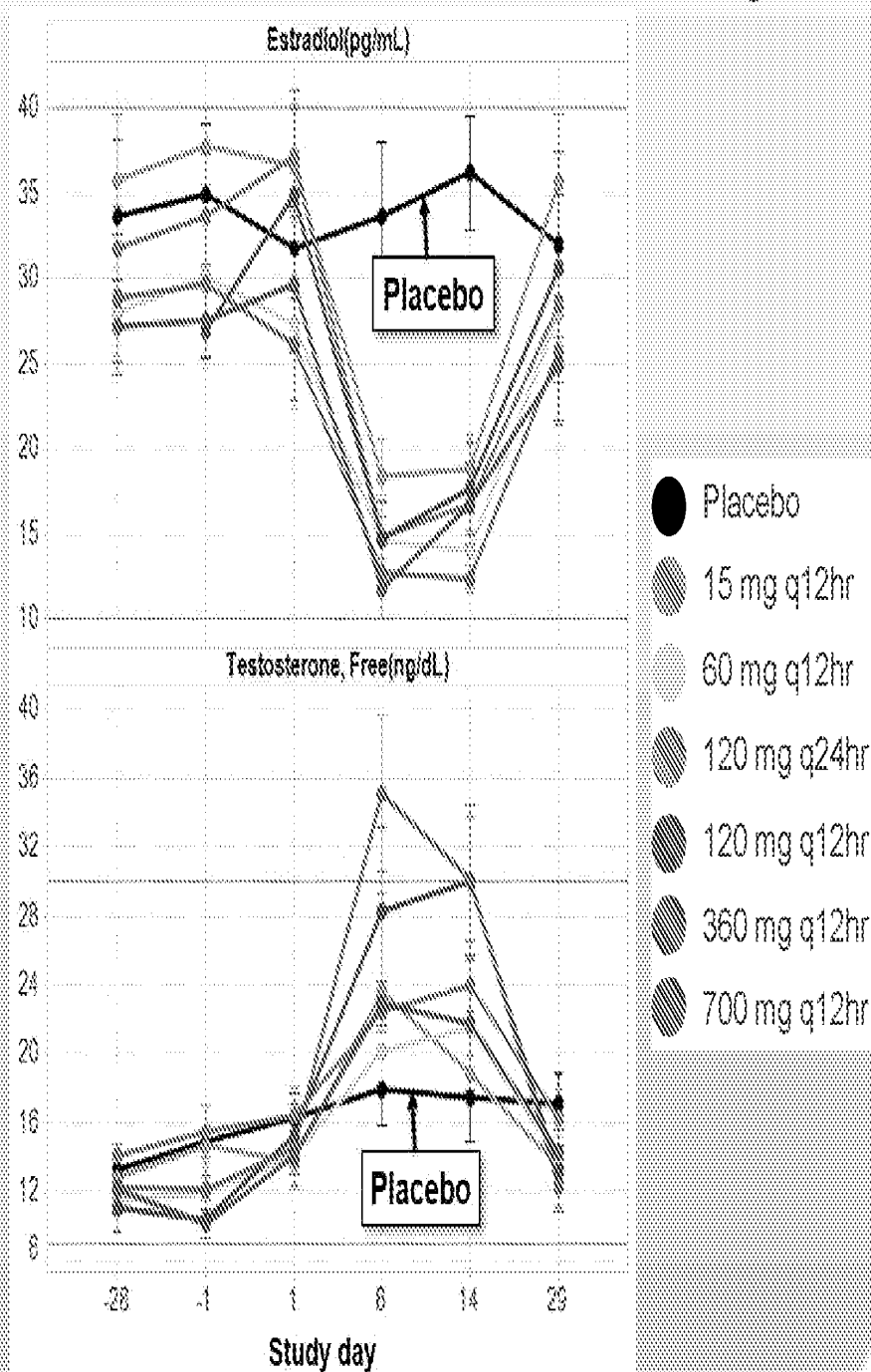
AEs were graded using National Cancer Institute Common Terminology Criteria, version 4.03



# Hormone Changes: Reversible Aromatase Inhibition

- Changes in serum androgens and estrogens were observed
- Most changes remained within normal reference ranges for age and sex
- Levels recovered to baseline
- Endocrine/physiologic significance to be evaluated in phase 2 study

Mean levels of serum estradiol and free testosterone in male subjects

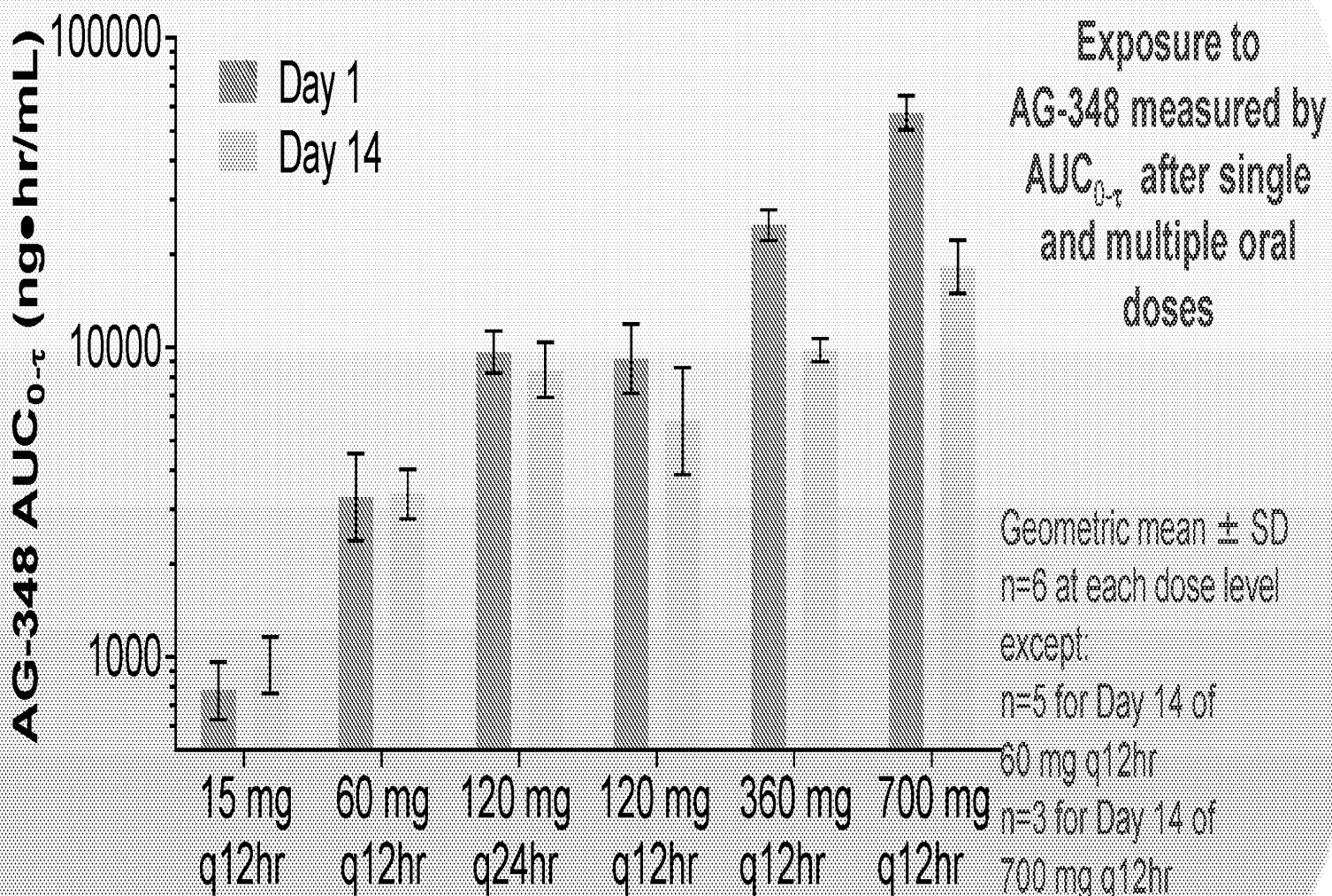


# Pharmacokinetic and Pharmacodynamic Results



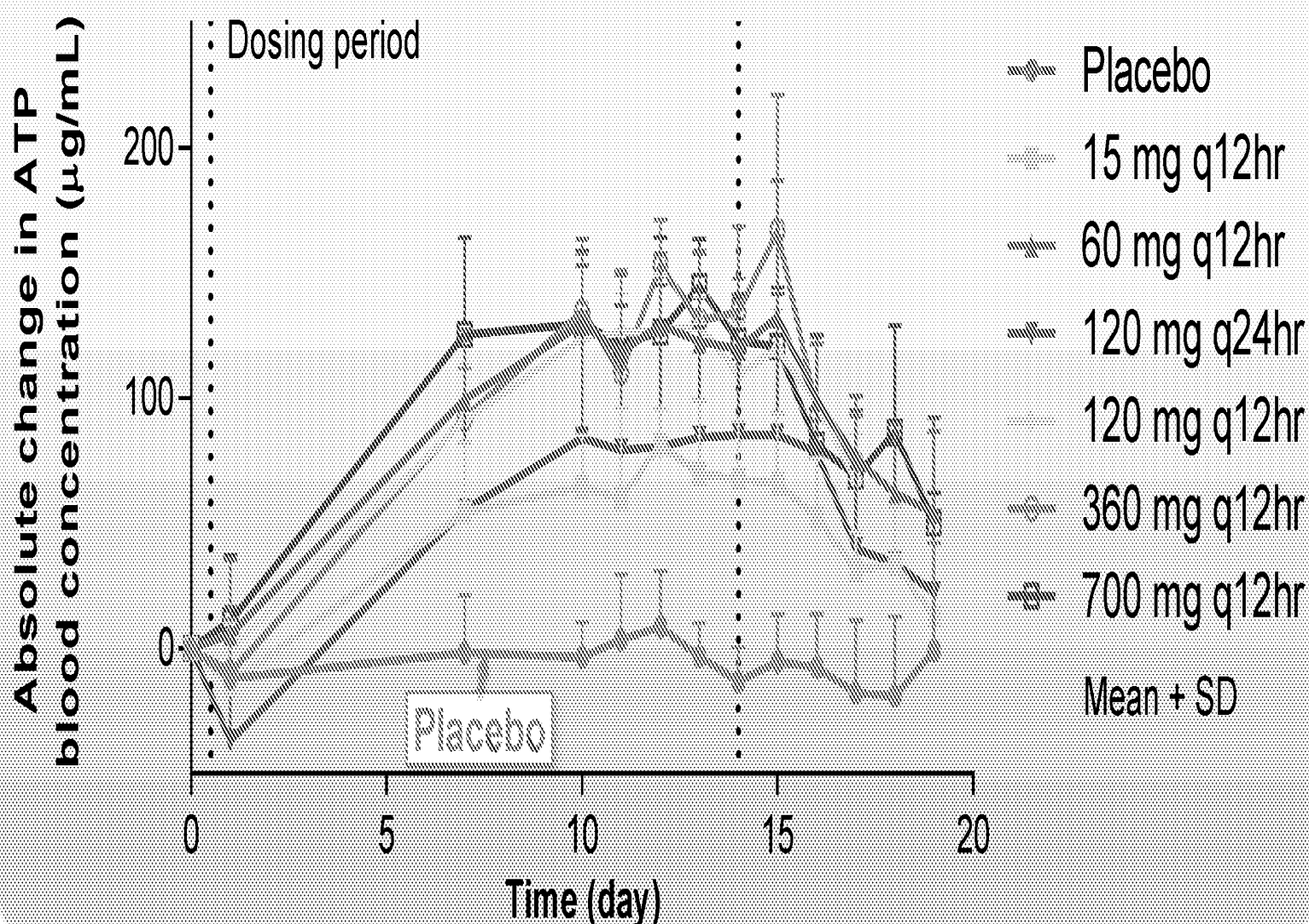
# Pharmacokinetics

- AG-348 displayed low to moderate pharmacokinetic variability
- Plasma exposure increased dose-proportionally
  - At higher doses, exposure at Day 14 was lower than on Day 1
  - AG-348 is a moderate inducer of CYP3A4, the main clearance pathway for AG-348



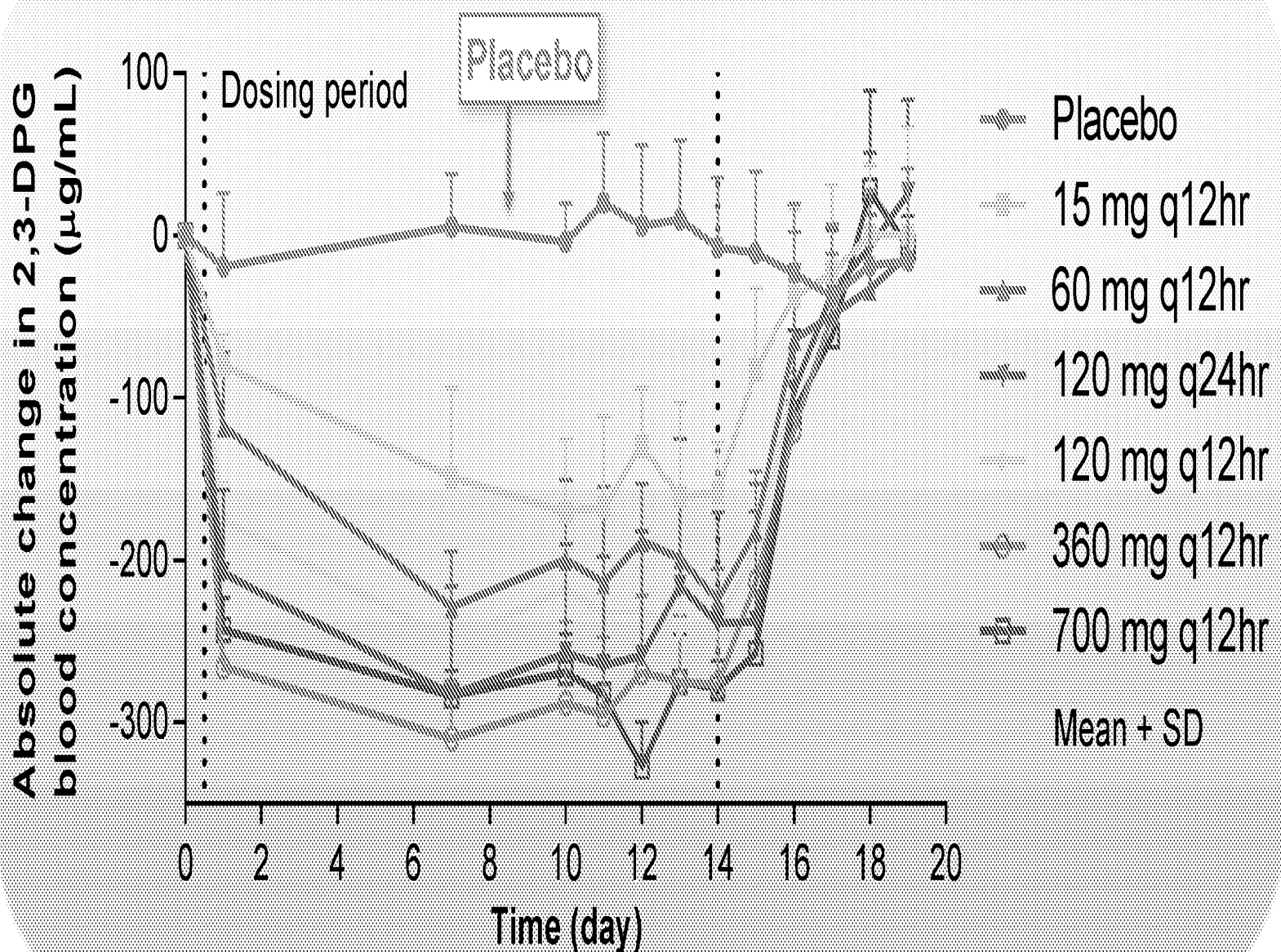
# Dose Dependent Increase in ATP

- A ~50% increase from Baseline was observed with  $\geq 60$  mg AG-348
- Levels remained elevated through 120 hours after the final dose



## Dose Dependent Decrease in 2,3-DPG

- A ~50% decrease from Baseline was observed with  $\geq 120$  mg AG-348
- The concentration returned to Baseline 72 hours after the final dose



Day 0 = Baseline value. Day 14 is the final day of dosing



Transfusion-independent PK-deficient adults  
n=25 in each arm

Screening

Randomization

Stratified by PK-R genotype

Arm 1  
300 mg q12hr

Optional  
3<sup>rd</sup> Arm

Arm 2  
50 mg q12hr

6-month  
dosing  
period

## Primary endpoints:

- Safety and tolerability

## Secondary endpoints:

- Pharmacokinetics of AG-348
- PD response: ATP, 2,3-DPG
- Indicators of clinical activity: hemoglobin, hematocrit, reticulocyte count, and other hematologic parameters.

*Natural History Study collaboration with Boston Children's Hospital ongoing*

# Visit Schedule

| Screening   | Month 1 | Months 2 & 3 | Months 4, 5 & 6 |
|-------------|---------|--------------|-----------------|
| Screening 1 | Day 1   | Week 6       | Week 16         |
| Screening 2 | Week 1  | Week 9       | Week 20         |
|             | Week 2  | Week 12      | Week 24         |
|             | Week 3  |              |                 |

- Most visits are 1 – 2 hours on average.
- The first and last visits (Screening 1 and Week 24) may be an additional 1 – 2 hours for additional testing.



# What happens during the Screening Period?

- The doctor and nurse will explain the study requirements and provide an informed consent document
  - the doctor and nurse have obligations to make sure that patients are treated safely during the study
  - Patients have an obligation to determine if this is the right study for them
- Patients who sign the consent form will have certain tests performed to see if they are eligible for the study
  - The doctor and nurse will explain these results and tell the patient if they can join the study
- The patient makes the final decision about participating
  - Discuss this with their family
  - Ask any questions to feel more comfortable with their decision



# DRIVE PK : Current Status

- ◆ The trial has been open in USA since June 2015
- ◆ Half of the sites are open for enrollment, with more coming soon
- ◆ For further information, please check [www.clinicaltrials.gov](http://www.clinicaltrials.gov) and type in “pyruvate kinase deficiency” or “NCT02476916” in the search window
- ◆ Investigator for Belgium and The Netherlands : Dr. E. De Beers (Utrecht)



# Summary and Conclusions

- PK deficiency is a serious hematologic disorder
- AG-348 is a novel, first-in-class, PK-R activator that is intended to restore the glycolytic pathway defect in patients with PK deficiency
- Healthy volunteer study:
  - ..... Established a clear range of well tolerated doses over 14 days and demonstrated maximal PD effects
- PD data are consistent with preclinical and *ex-vivo* data demonstrating:
  - ..... Sustained concomitant increases in ATP
  - ..... Decreases in 2,3-DPG
- Data overall provide compelling evidence to initiate global phase 2 study in PK deficiency



We would like to thank the volunteers who agreed to participate in this study and the PK-Deficiency patients who are participating in the DRIVE PK study, as well as the Clinical Site Staff and Investigators



## NEW TREATMENTS OF PYRUVATE KINASE DEFICIENCY : A PHASE 1 MULTIPLE ASCENDING DOSE STUDY OF THE SAFETY, TOLERABILITY, AND PHARMACOKINETICS/PHARMACODYNAMICS OF AG-348, A FIRST-IN-CLASS ALLOSTERIC ACTIVATOR OF PYRUVATE KINASE-R, IN HEALTHY SUBJECTS

Hua Yang<sup>\*1</sup>, Elizabeth Merica<sup>1</sup>, Yue Chen<sup>1</sup>, Marvin Cohen<sup>2</sup>, Ronald Goldwater<sup>3</sup>, Hyeryun Kim<sup>1</sup>, Penelope Kosinski<sup>1</sup>, Charles Kung<sup>1</sup>, Meredith Goldwasser<sup>1</sup>, Bruce Silver<sup>4</sup>, Luke Utley<sup>1</sup>, Sam Agresta<sup>1</sup>, Ann Barbier<sup>1</sup>

<sup>1</sup>Agios Pharmaceuticals, Cambridge, <sup>2</sup>MBC Pharma Solutions, Newtown, <sup>3</sup>PAREXEL, Baltimore, <sup>4</sup>Bruce A. Silver Clinical Science and Development, Dunkirk, United States

**Background:** Pyruvate kinase (PK) deficiency is a rare genetic disorder of metabolism resulting in chronic hemolytic anemia, with comorbidities including iron overload and multi-organ dysfunction. Approximately 180 mutations in the *PKLR* gene are recognized to cause functional deficiency of the PK-R isoform. Defective glycolysis in red blood cells leads to increases in the metabolic precursor, 2,3-diphosphoglycerate (2,3-DPG), and deficiency of the product, adenosine triphosphate (ATP). AG-348 is an oral allosteric activator of both wild type and multiple mutant PK-Rs. We report here a multiple ascending dose (MAD) trial of AG-348.

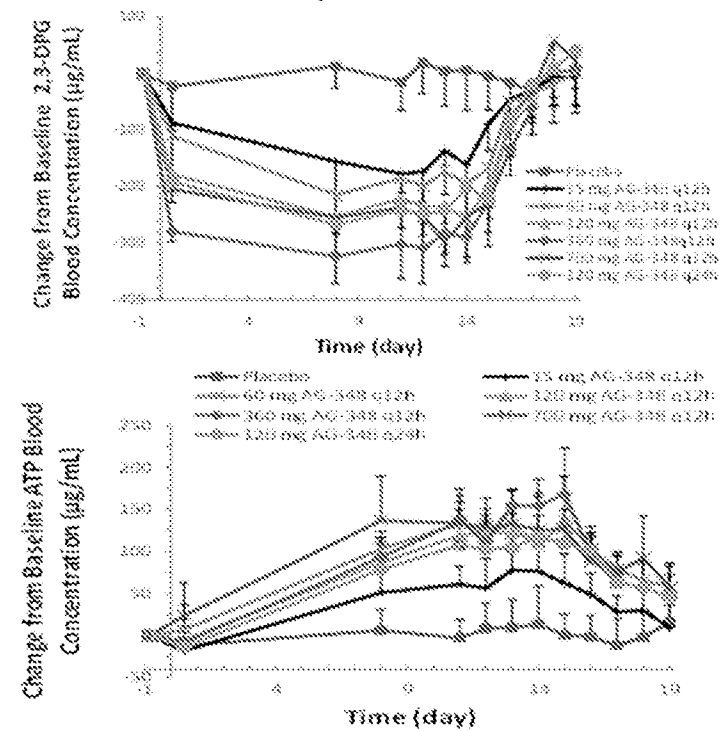
**Aims:** To assess safety, tolerability, and pharmacokinetics/pharmacodynamics (PK/PD) of AG-348 in healthy volunteers and identify a dosing schedule for future trials in patients with PK deficiency.

**Methods:** A phase 1, single-center, randomized, double-blind, placebo-controlled MAD study (NCT02149966) was conducted in healthy men and women (18–60 years) in 6 sequential cohorts (each cohort: n=6 AG-348, n=2 placebo [P]). Subjects gave informed consent and received oral AG-348 at 15–700 mg twice daily (q12h) or 120 mg once daily (q24h) for 14 days with follow-up to Day 29. Adverse events (AEs), laboratory parameters, ECGs, and vital signs were monitored. Plasma AG-348 concentrations and whole blood 2,3-DPG and ATP levels were measured in serial blood samples for PK/PD assessment. Hormone levels were monitored due to pre-clinical data suggesting potential modulation.

**Results:** 48 subjects (42M/6F), mean age 41.5 (range, 25–60) years, enrolled. Final, un-blinded safety data showed  $\geq 1$  AE in 16/36 (44%) AG-348-treated and 4/12 (33%) P subjects. Treatment-related AEs ( $\geq 1$ ) were noted in 11/36 (31%) AG-348-treated and 3/12 (25%) P subjects. All treatment-related AEs were mild or moderate in severity (only one grade 3 event) and often reversible despite continued dosing. The most frequent AG-348-related AEs were nausea and headache, 5/36 (14%) for each (P: nausea 0/12 [0%]; headache 1/12 [8%]). Gastrointestinal AEs occurred in AG-348-treated subjects only at the highest dose, 700 mg q12h. One grade 3 AE occurred (AG-348 700 mg q12h, elevated liver function tests [LFTs], which resolved after treatment discontinuation). There were 4 AG-348 discontinuations: due to AEs in 2 subjects (grade 2 drug eruption, 60 mg q12h; grade 3 elevated LFTs, 700 mg q12h), and 2 subjects withdrew consent (both had grade 1/2 nausea and grade 1/1 vomiting; both at 700 mg q12h). The highest well-tolerated dose was 360 mg q12h (doses between 360 and 700 mg were not explored). Plasma AG-348 exposure was dose dependent, with low to moderate variability in the PK parameters of AG-348 and its metabolite AGI-8702. There was a dose-dependent decrease in 2,3-DPG and increase in ATP with the effects plateauing at 360 mg q12h. Decrease in 2,3-DPG was robust after Dose 1, while the increase in ATP occurred gradually and was strongly evident at Day 8. Change from baseline in 2,3-DPG and ATP plateaued at  $\sim 300$   $\mu\text{g/mL}$  ( $\sim 50\%$  decrease) and  $\sim 175$   $\mu\text{g/mL}$  ( $\sim 50\%$  increase), respectively. After final Day 14 dose, 2,3-DPG returned to levels similar to baseline

between 72 and 120 hours. ATP levels remained elevated through 120 hours post dose (**Figure**). Details of all un-blinded data will be presented.

Change from baseline concentration–time profiles of 2,3-DPG and ATP in blood following multiple doses of AG-348



**Summary/Conclusion:** AG-348 showed a robust 2,3-DPG/ATP PD glycolytic profile at well-tolerated doses in healthy volunteers. AG-348 is currently being tested in a phase II trial in transfusion-independent adult patients with pyruvate kinase deficiency

# **Phase 1 Multiple Ascending Dose Study of the Safety, Tolerability and Pharmacokinetics/Pharmacodynamics of AG-348, a First-in-Class Allosteric Activator of Pyruvate Kinase R, in Healthy Subjects**

**Hua Yang<sup>1</sup>, Elizabeth Merica<sup>1</sup>, Yue Chen<sup>1</sup>, Marvin Cohen<sup>2</sup>,  
Ronald Goldwater<sup>3</sup>, Hyeryun Kim<sup>1</sup>, Penelope A Kosinski<sup>1</sup>,  
Charles Kung<sup>1</sup>, Meredith Goldwasser<sup>1</sup>, Bruce Silver<sup>4</sup>, Luke Utley<sup>1</sup>,  
Sam Agresta<sup>1</sup>**

*<sup>1</sup>Agios Pharmaceuticals, Cambridge, MA, USA; <sup>2</sup>MBC Pharma Solutions,  
Newtown, PA, USA; <sup>3</sup>Parexel International, Baltimore, MD, USA;*

*<sup>4</sup>Bruce A Silver Clinical Science and Development, Dunkirk, MD, USA*

*Presented at the 20th Congress of the European Hematology Association, 12 June 2015, Vienna, Austria*

# Disclosures

S138

- This study was funded by Agios Pharmaceuticals
- Hua Yang, Elizabeth Merica, Yue Chen, Hyeryun Kim, Penelope A Kosinski, Charles Kung, Meredith Goldwasser, Luke Utley, Sam Agresta: Agios – employees and stockholders
- Marvin Cohen, Bruce Silver: Agios – consultants
- Ronald Goldwater: Parexel International – employee
- Editorial assistance was provided by Christine Tomlins, PhD, Excel Scientific Solutions, Horsham, UK, and supported by Agios

## Key Points

- Pyruvate Kinase (PK) deficiency is a serious, rare, genetic disease.
- AG-348 is a PK-R activator intended to correct the glycolytic pathway defect in patients with PK deficiency.
- Healthy volunteer study has established a safety profile at pharmacodynamically active doses that may be relevant to patients with PK deficiency.
- DRIVE PK, a global phase 2 study in patients, is now open and enrolling

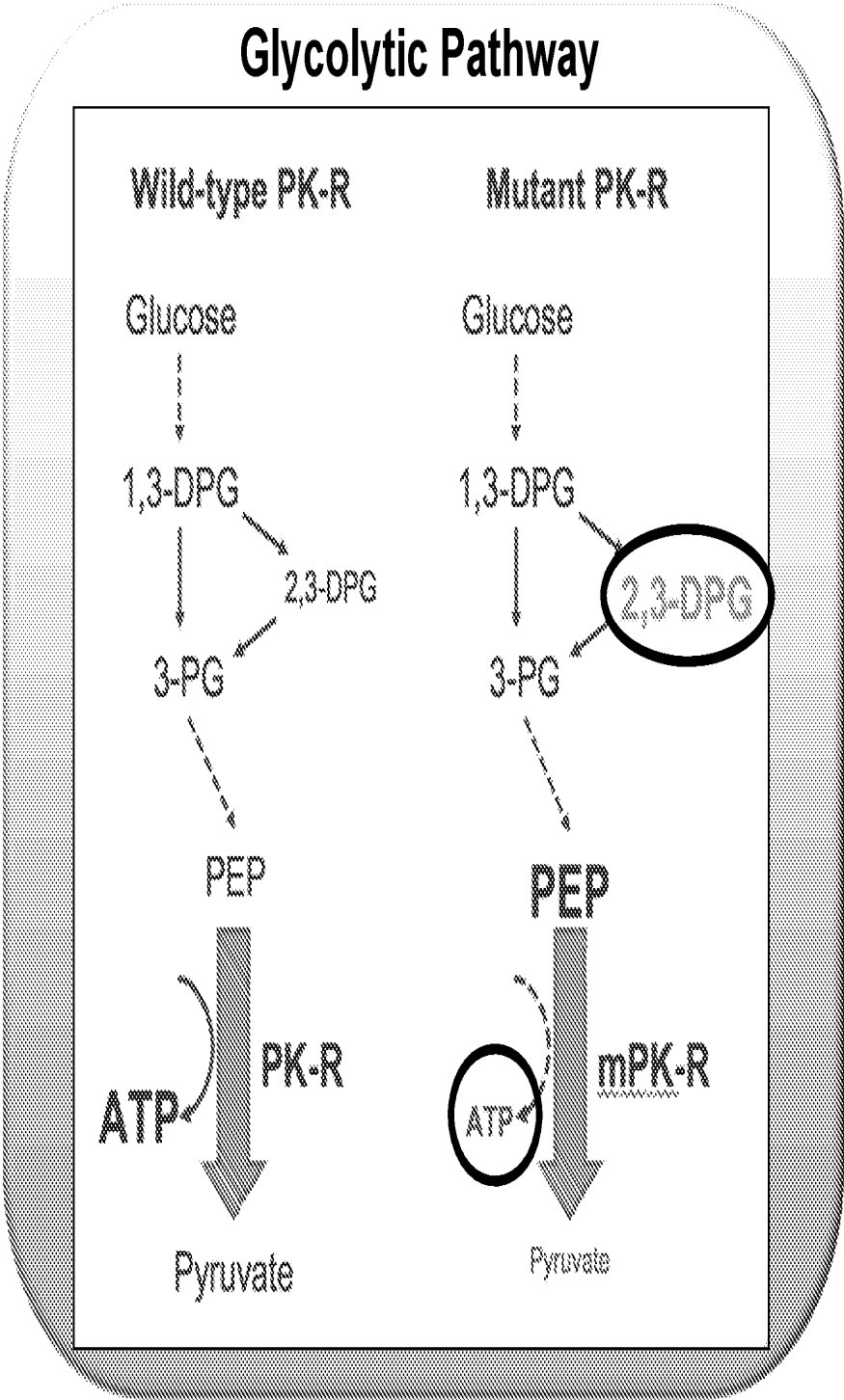
# PK Deficiency is a Severe Red Blood Cell Disease

## Disease overview

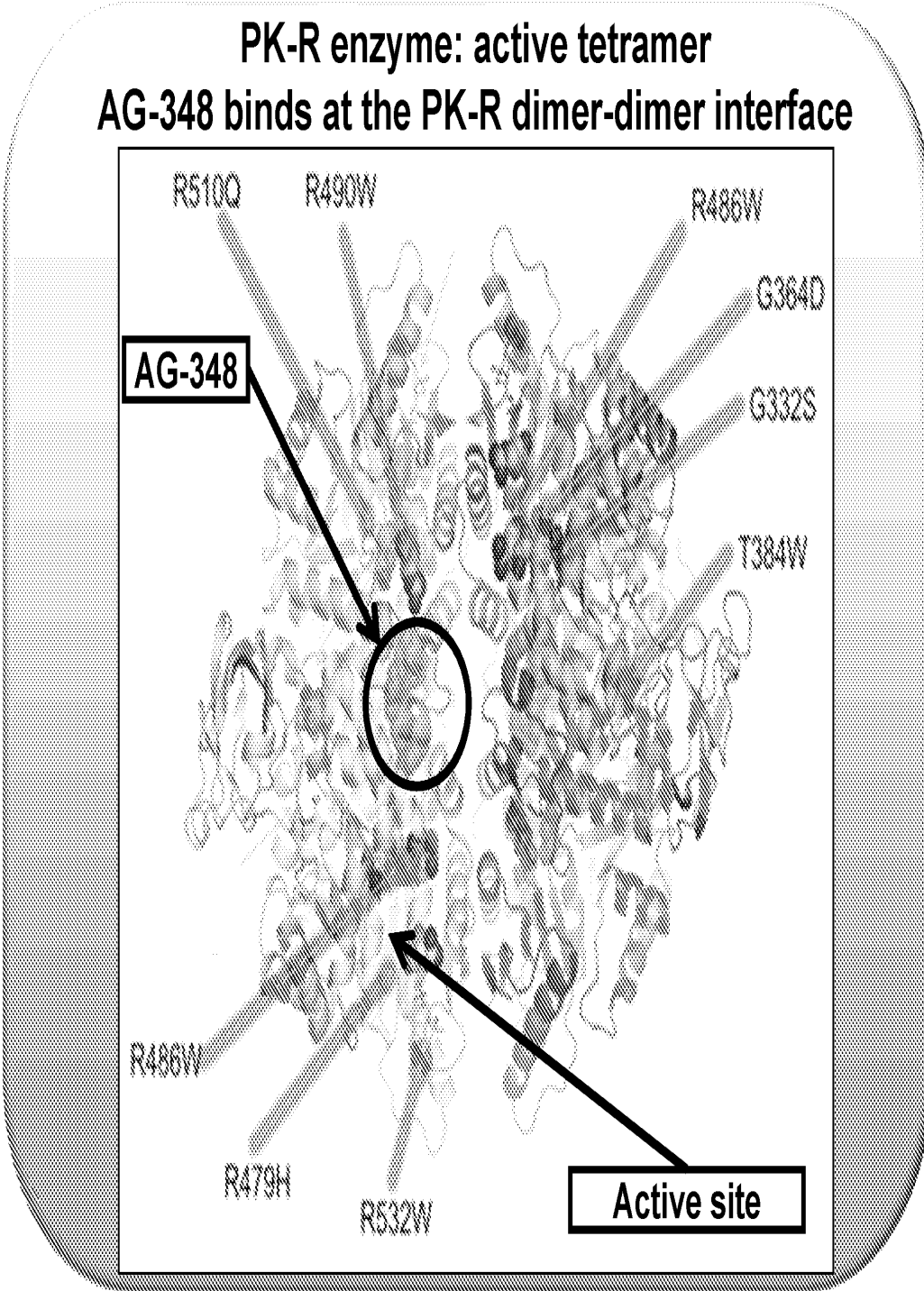
|                         |                                                                                                                                                                                |
|-------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Description             | <ul style="list-style-type: none"><li>• Presents at birth or in childhood with severe hemolytic anemia</li><li>• Estimated 2,400 patients diagnosed in the US and EU</li></ul> |
| Etiology                | <ul style="list-style-type: none"><li>• Caused by mutations in the <i>PK-LR</i> gene coding for erythrocyte pyruvate kinase (PK-R)</li></ul>                                   |
| Clinical Presentation   | <ul style="list-style-type: none"><li>• Lifelong hemolytic anemia</li><li>• Iron overload and jaundice</li><li>• Infection and aplastic anemia</li></ul>                       |
| Diagnosis/<br>Treatment | <ul style="list-style-type: none"><li>• PK-R enzyme activity and/or genetic testing</li><li>• Supportive treatment: transfusions, splenectomy, iron chelation</li></ul>        |

Sources: Zanella A et al. *Blood Rev* 2007;21:217-31; Blood and Bone Marrow Pathology; Wintrobe's Clinical Hematology; Physician Interviews; Market Research.

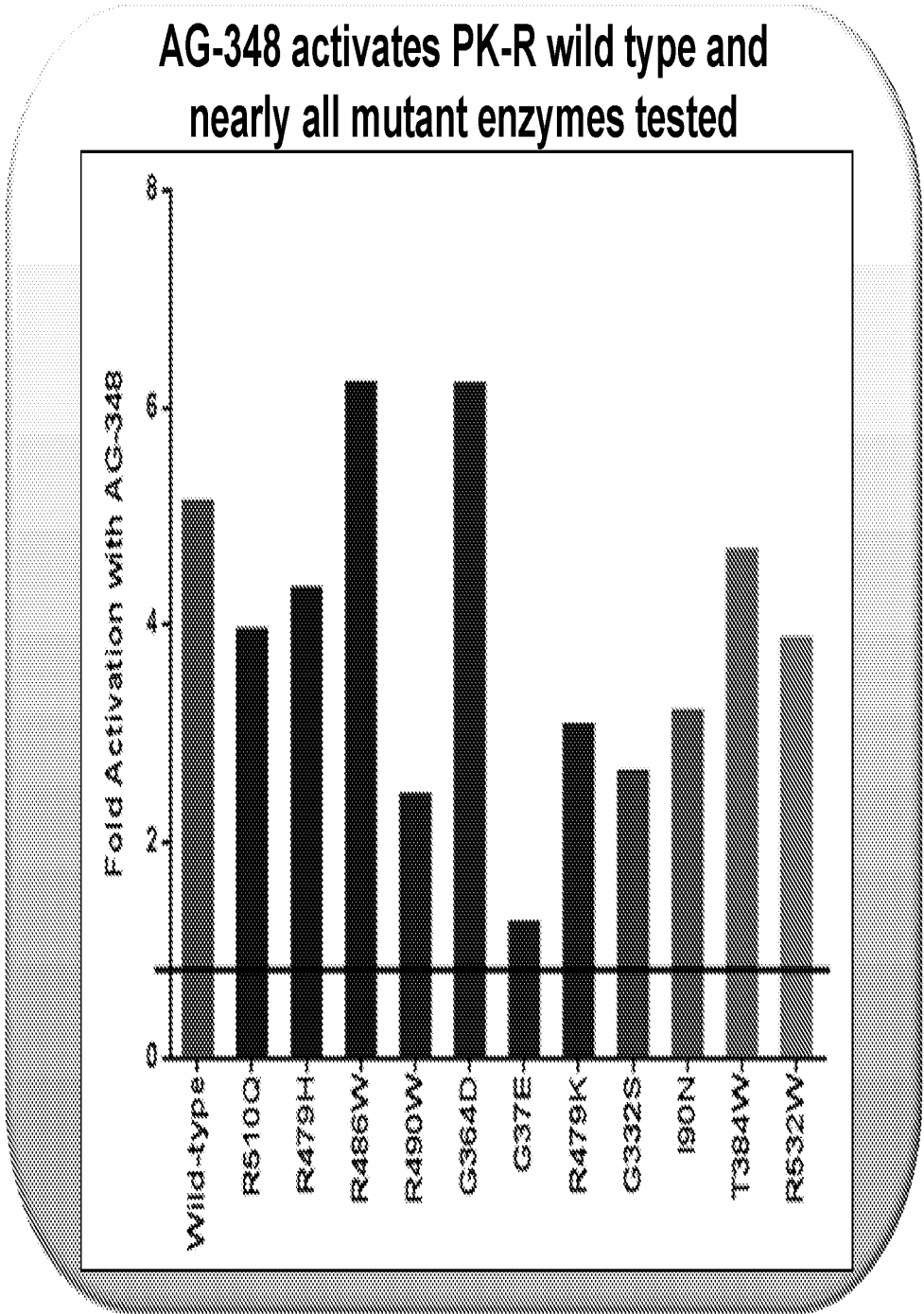
# Mutant PK-R: ATP Deficiency in Red Blood Cells



# AG-348: Allosteric Activator of Wild-Type and Mutant PK-R

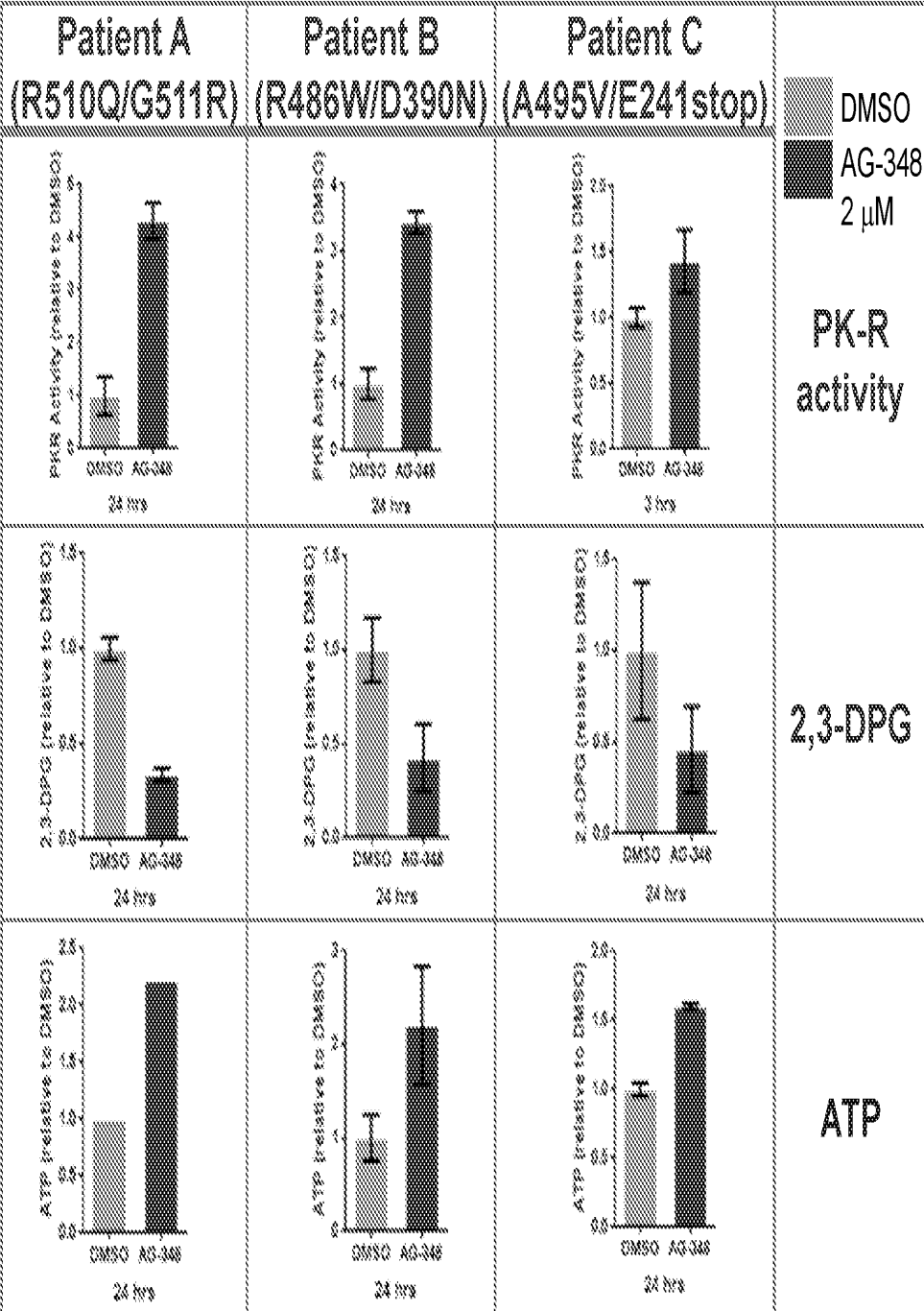


# AG-348 Activates PK-R Wild-Type and Mutant Subtypes



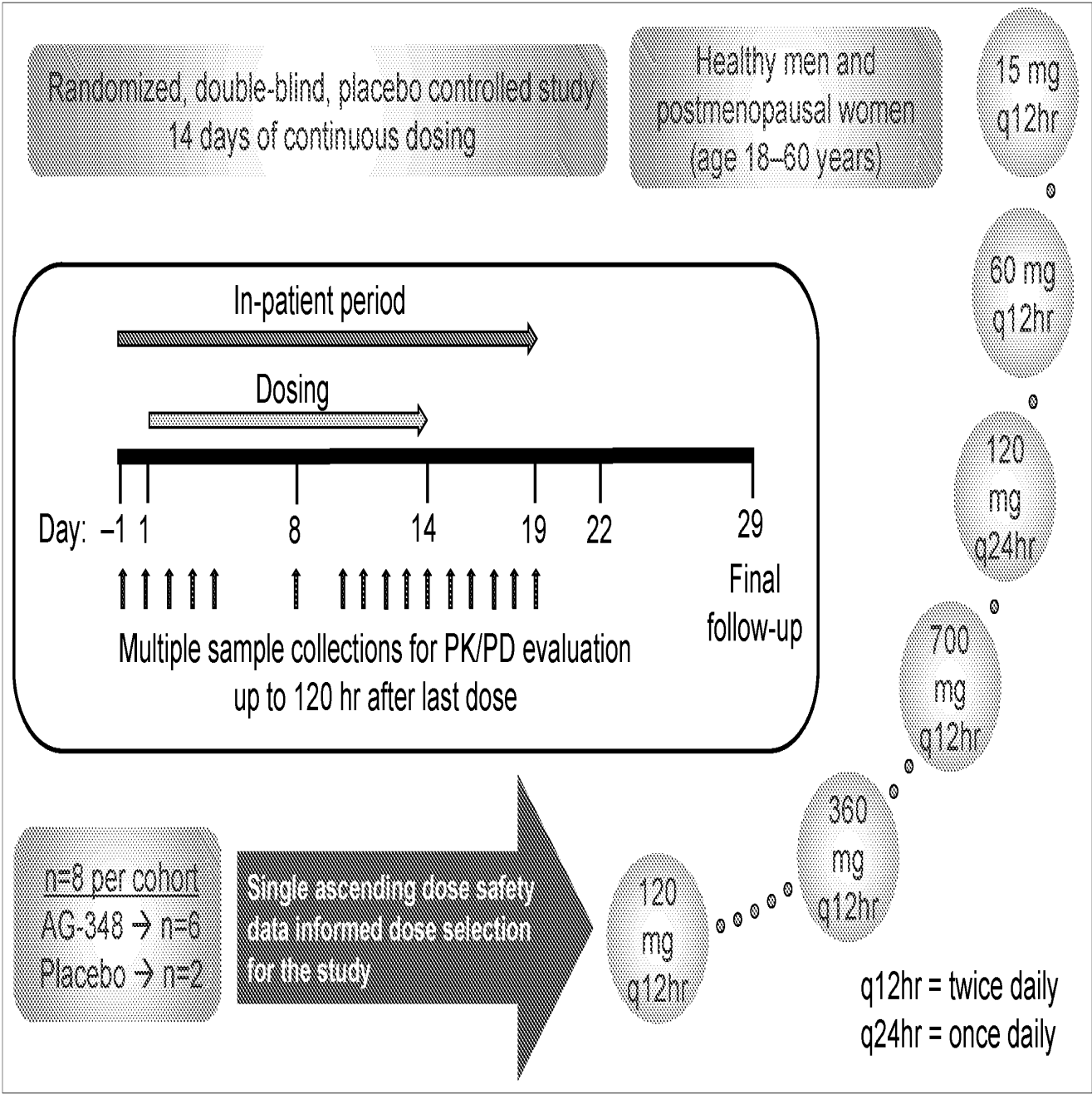
# AG-348 Restores the Glycolytic Pathway in Models

PK Deficient  
Patient Red  
Blood Cell  
Samples



# Multiple Ascending Dose Study Completed

**Objective: Establish the safety, tolerability and PD effects of AG-348**



# Demographics and Disposition

| Characteristic                                   | Placebo<br>n=12 | AG-348<br>n=36 |
|--------------------------------------------------|-----------------|----------------|
| Men, n (%)                                       | 9 (75)          | 33 (92)        |
| Women, n (%)                                     | 3 (25)          | 3 (8)          |
| Age in years, mean (SD)                          | 37.8 (11.4)     | 42.8 (12.2)    |
| Race, n (%)                                      |                 |                |
| Asian                                            | —               | 1 (3)          |
| Black                                            | 6 (50)          | 18 (50)        |
| White                                            | 6 (50)          | 17 (47)        |
| Body mass index in kg/m <sup>2</sup> , mean (SD) | 27.4 (2.3)      | 26.5 (2.0)     |

- 12 placebo treated and 32 of 36 AG-348 treated patients completed study:
  - Two withdrew due to AEs: Drug eruption (60 mg q12hr), grade 3 liver function test abnormalities at the highest dose explored (700 mg q12hr)
  - Two withdrew consent due to nausea and vomiting at the highest dose explored (700 mg q12hr)

AE = adverse event; SD = standard deviation

# AG-348 was well tolerated up to 360 mg oral q12 hr

- Drug-related events were grade 1 and 2
- One grade 3 event at 700 mg q12h (increased LFTs – DLT)

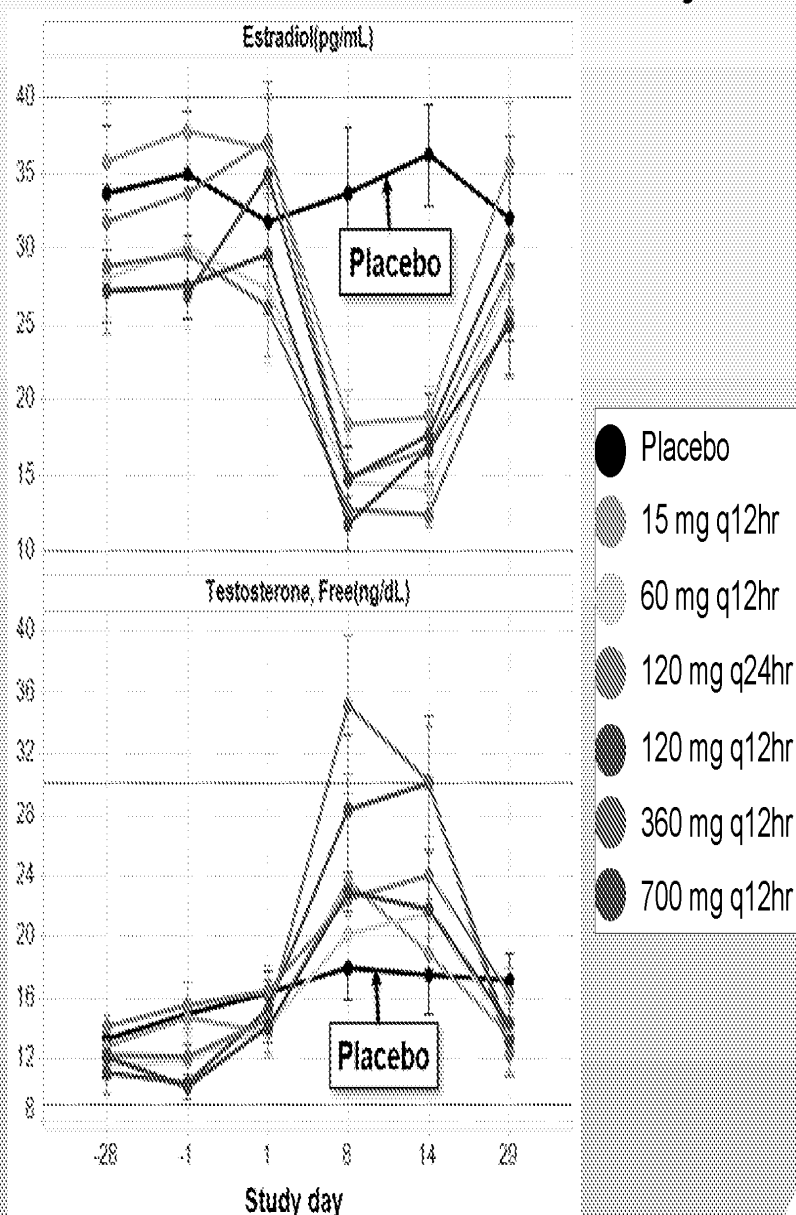
| AE n (%)                                         | Placebo<br>n=12 | 15–360 mg q12hr<br>n=30 | 700 mg q12hr<br>n=6 |
|--------------------------------------------------|-----------------|-------------------------|---------------------|
| Any AE                                           | 4 (33)          | 10 (33)                 | 6 (100)             |
| Most common treatment-related AEs (≥2 subjects): |                 |                         |                     |
| Nausea                                           | —               | —                       | 5 (83)              |
| Headache                                         | 1 (8)           | 1 (3)                   | 4 (67)              |
| Vomiting                                         | 1 (8)           | —                       | 3 (50)              |
| Feeling hot                                      | 1 (8)           | —                       | 3 (50)              |
| Decreased appetite                               | —               | 2 (7)                   | 1 (17)              |
| Restlessness                                     | —               | —                       | 3 (50)              |
| Fatigue                                          | —               | —                       | 2 (33)              |
| Dizziness                                        | 1 (8)           | —                       | 2 (33)              |
| Hyperhidrosis                                    | 1 (8)           | —                       | 2 (33)              |
| Drug eruption                                    | —               | 2 (7)                   | —                   |
| Abdominal discomfort                             | 2 (17)          | —                       | —                   |

AEs were graded using National Cancer Institute Common Terminology Criteria, version 4.03

# Hormone Changes: Reversible Aromatase Inhibition

- Changes in serum androgens and estrogens were observed
- Most changes remained within normal reference ranges for age and sex
- Levels recovered to baseline
- Endocrine/physiologic significance to be evaluated in phase 2 study

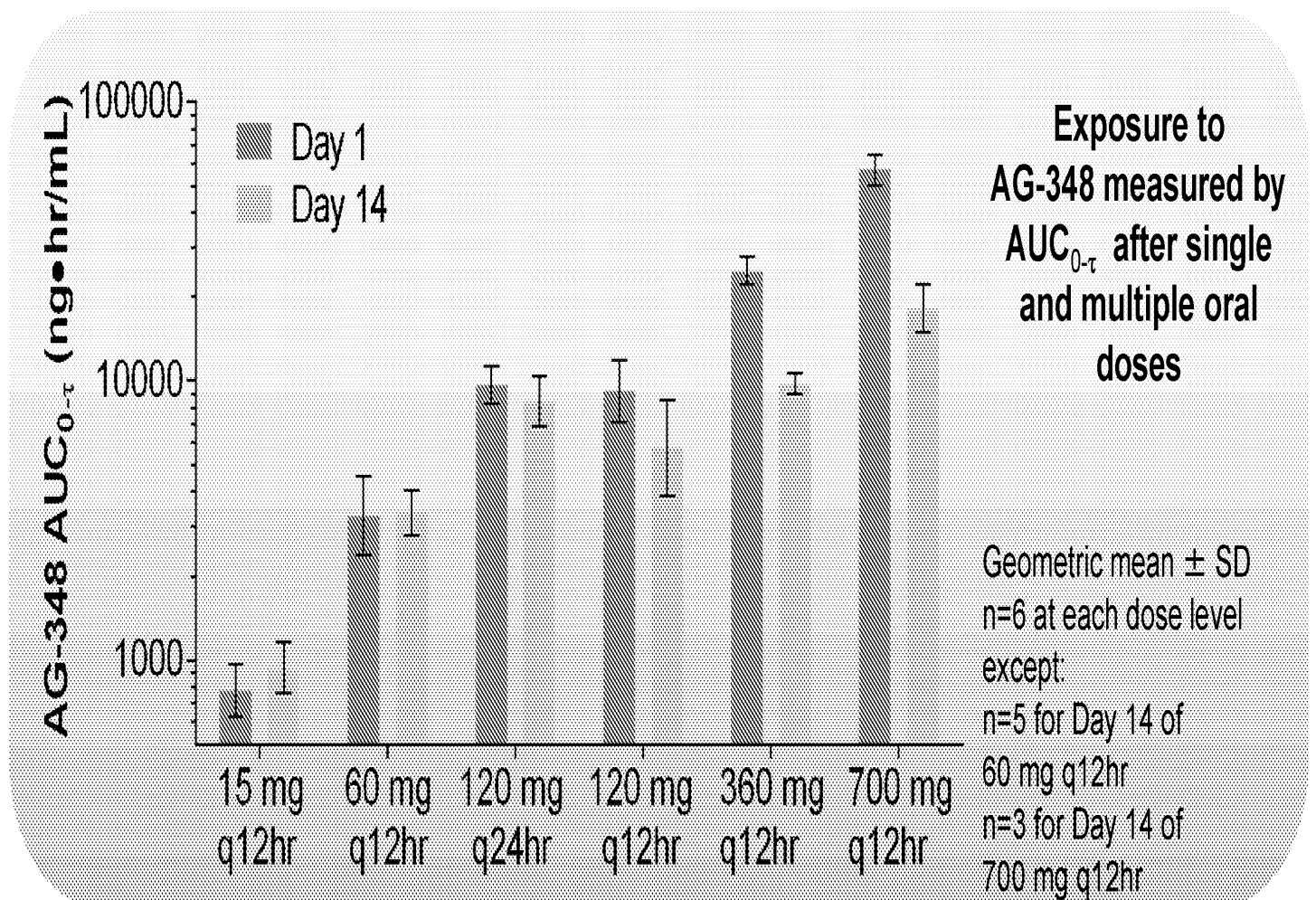
**Mean levels of serum estradiol and free testosterone in male subjects**



# **Pharmacokinetic and Pharmacodynamic Results**

# Pharmacokinetics

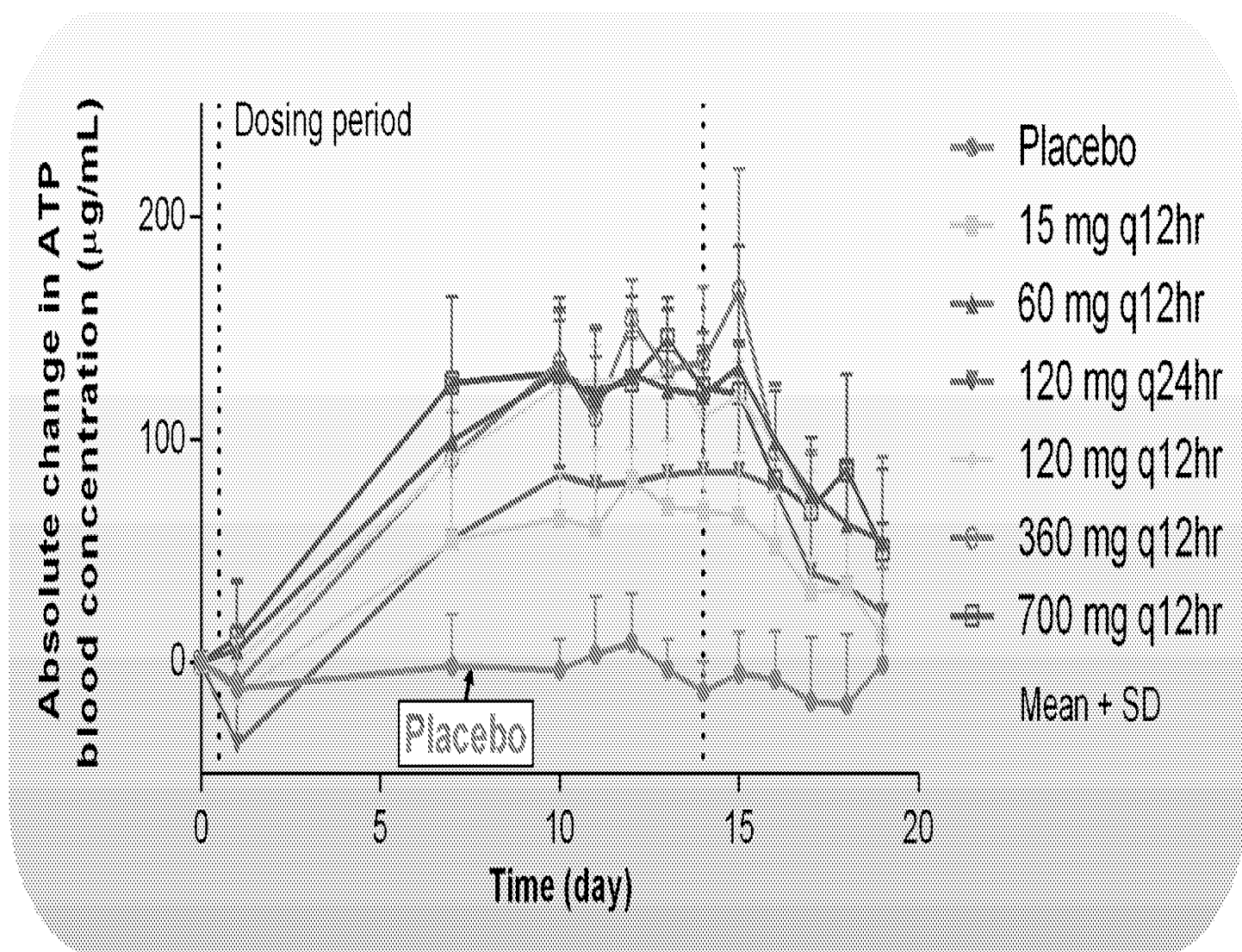
- AG-348 displayed low to moderate pharmacokinetic variability
- Plasma exposure increased dose-proportionally
  - At higher doses, exposure at Day 14 was lower than on Day 1
  - AG-348 is a moderate inducer of CYP3A4, the main clearance pathway for AG-348



AUC<sub>0-τ</sub> = area under the concentration–time curve from 0 to the end of the dosing interval  $\tau$

# Dose Dependent Increase in ATP

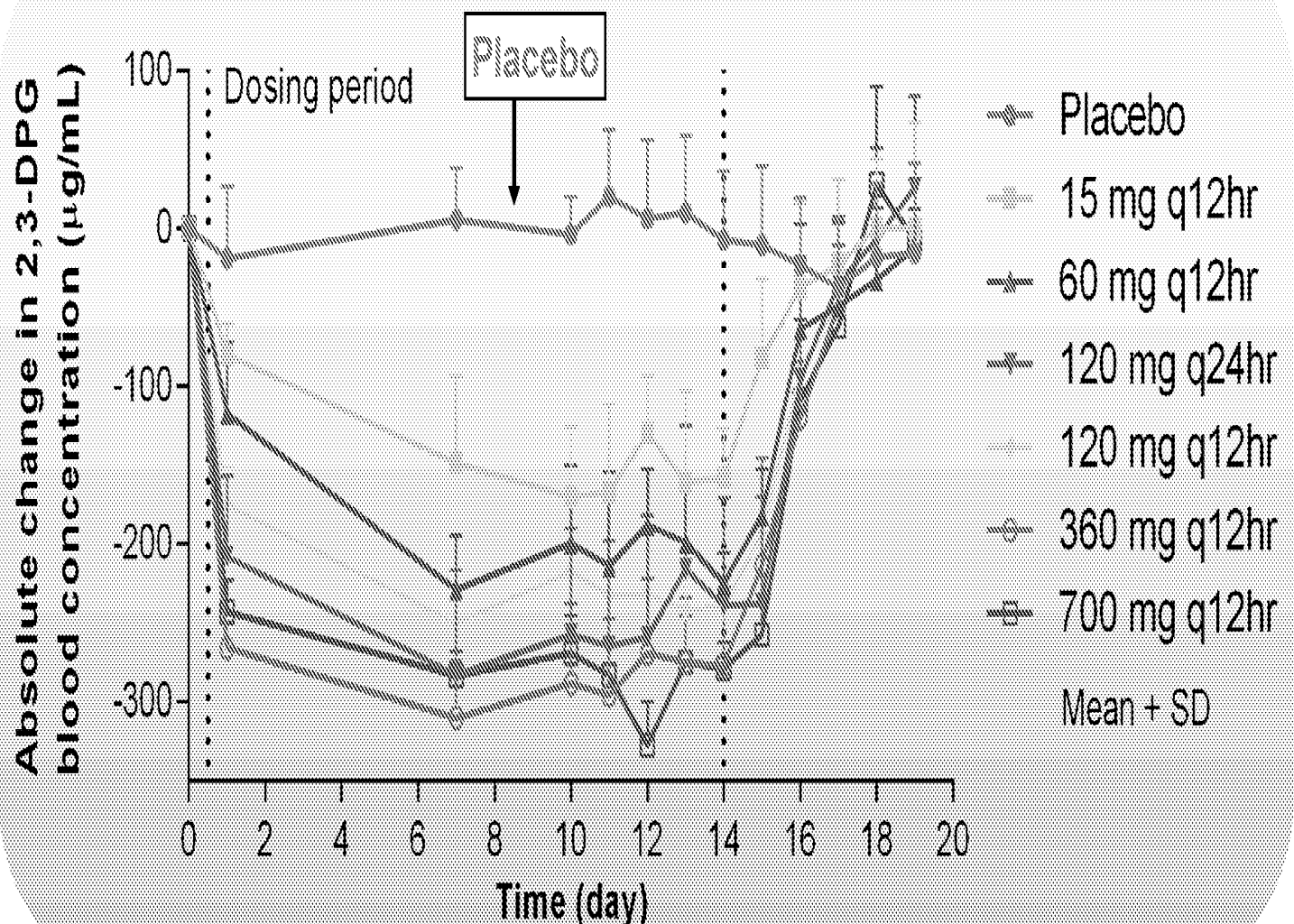
- A ~50% increase from Baseline was observed with  $\geq 60$  mg AG-348
- Levels remained elevated through 120 hours after the final dose



Day 0 = Baseline value. Day 14 is the final day of dosing

## Dose Dependent Decrease in 2,3-DPG

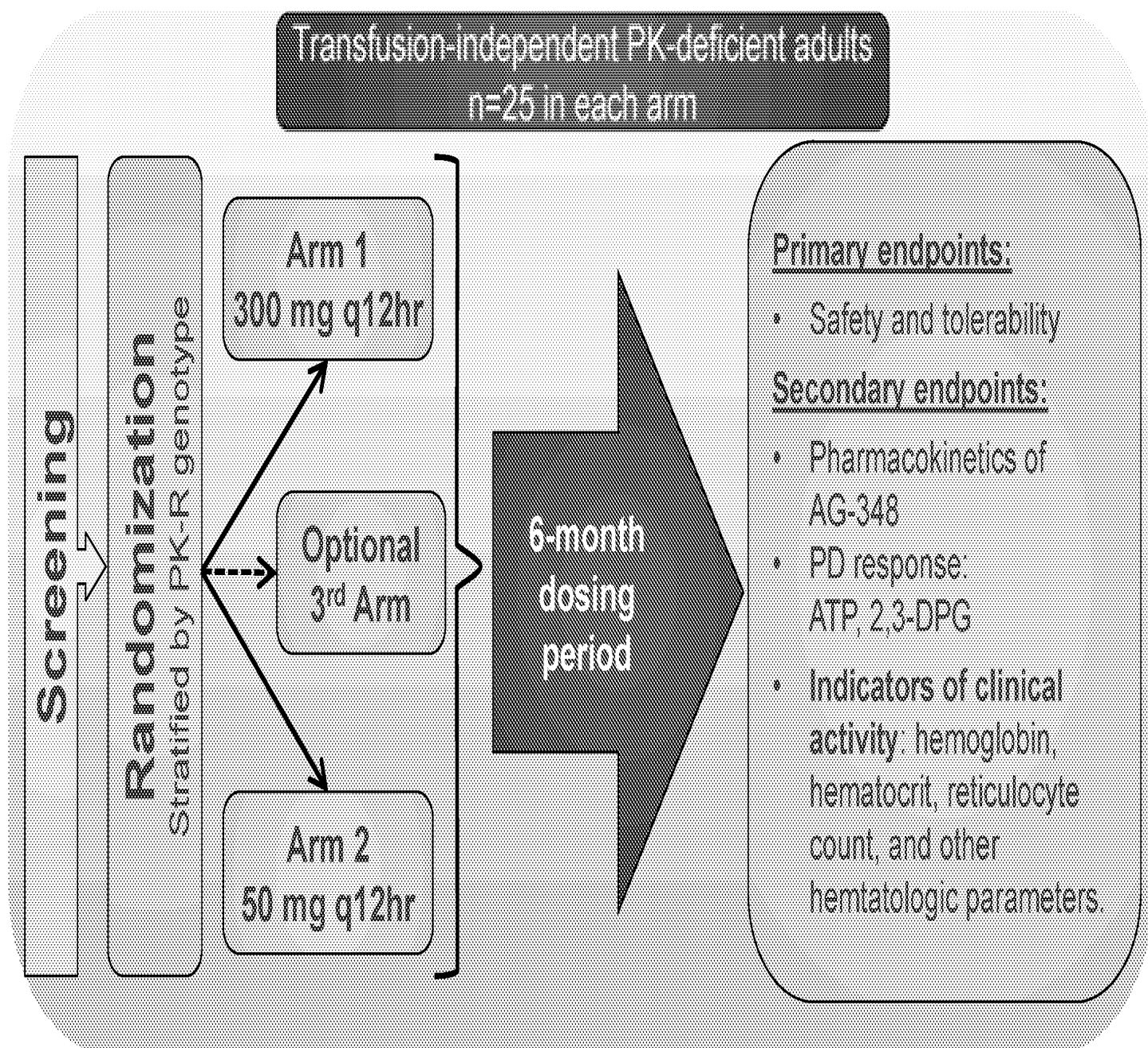
- A ~50% decrease from Baseline was observed with  $\geq 120$  mg AG-348
- The concentration returned to Baseline 72 hours after the final dose



Day 0 = Baseline value. Day 14 is the final day of dosing

# DRIVEPK

Open, global phase 2 study: 14 centers in the US, Canada, and EU



*Natural History Study collaboration with Boston Children's Hospital ongoing*

## Summary and Conclusions

- PK deficiency is a serious hematologic disorder
  - AG-348 is a novel, first-in-class, PK-R activator that is intended to restore the glycolytic pathway defect in patients with PK deficiency
  - Healthy volunteer study:
    - Established a clear range of well tolerated doses over 14 days and demonstrated maximal PD effects
  - PD data are consistent with preclinical and *ex-vivo* data demonstrating:
    - Sustained concomitant increases in ATP
    - Decreases in 2,3-DPG
  - Data overall provide compelling evidence to initiate global phase 2 study in PK deficiency
-

# Acknowledgments

We would like to thank the volunteers who agreed to participate in this study

# Abstract Submission

## 26. Red blood cells and iron - Clinical

EHA-3425

### PHASE 1 MULTIPLE ASCENDING DOSE STUDY OF THE SAFETY, TOLERABILITY, AND PHARMACOKINETICS/PHARMACODYNAMICS OF AG-348, A FIRST-IN-CLASS ALLOSTERIC ACTIVATOR OF PYRUVATE KINASE-R, IN HEALTHY SUBJECTS

Hua Yang<sup>\*1</sup>, Elizabeth Merica<sup>1</sup>, Yue Chen<sup>1</sup>, Marvin Cohen<sup>2</sup>, Ronald Goldwater<sup>3</sup>, Hyeryun Kim<sup>1</sup>, Penelope Kosinski<sup>1</sup>, Charles Kung<sup>1</sup>, Meredith Goldwasser<sup>1</sup>, Bruce Silver<sup>4</sup>, Luke Utley<sup>1</sup>, Sam Agresta<sup>1</sup>

<sup>1</sup>Agios Pharmaceuticals, Cambridge, <sup>2</sup>MBC Pharma Solutions, Newtown, <sup>3</sup>PAREXEL, Baltimore, <sup>4</sup>Bruce A. Silver Clinical Science and Development, Dunkirk, United States

**Please indicate your presentation preference:** No Preference

**Has the submitted material been published in a journal (printed or online)?:** No

**Has the submitted material been presented or submitted to another event?:** Yes

**New Information:** Complete safety, tolerability and PK/PD data from the MAD study will be presented at EHA 2015, in comparison to previous presentation at ASH 2014 which comprised the single ascending dose study and preliminary parts of the MAD study only.

**Background:** Pyruvate kinase (PK) deficiency is a rare genetic disorder of metabolism resulting in chronic hemolytic anemia, with comorbidities including iron overload and multi-organ dysfunction. Approximately 180 mutations in the *PKLR* gene are recognized to cause functional deficiency of the PK-R isoform. Defective glycolysis in red blood cells leads to increases in the metabolic precursor, 2,3-diphosphoglycerate (2,3-DPG), and deficiency of the product, adenosine triphosphate (ATP). AG-348 is an oral allosteric activator of both wild type and multiple mutant PK-Rs. We report here a multiple ascending dose (MAD) trial of AG-348.

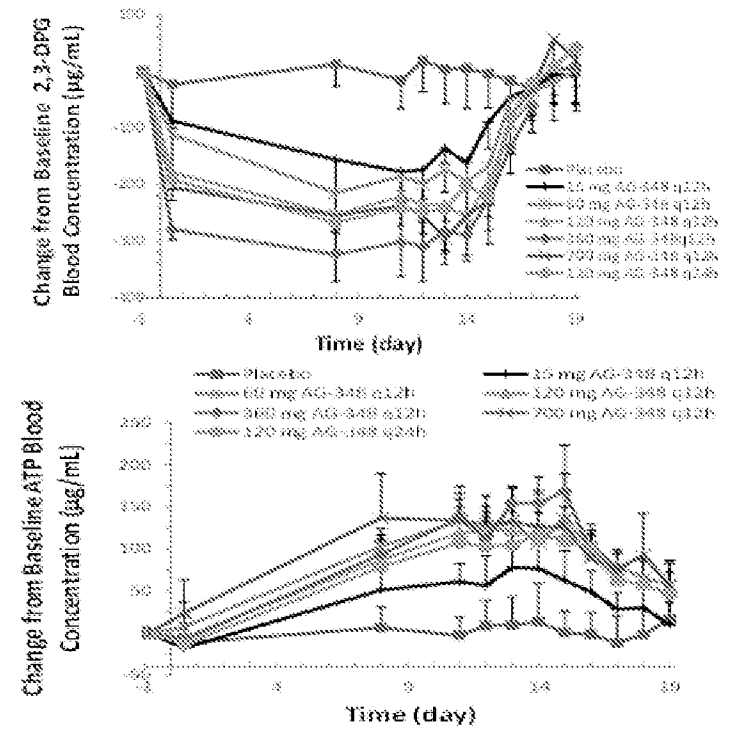
**Aims:** To assess safety, tolerability, and pharmacokinetics/pharmacodynamics (PK/PD) of AG-348 in healthy volunteers and identify a dosing schedule for future trials in patients with PK deficiency.

**Methods:** A phase 1, single-center, randomized, double-blind, placebo-controlled MAD study (NCT02149966) was conducted in healthy men and women (18–60 years) in 6 sequential cohorts (each cohort: n=6 AG-348, n=2 placebo [P]). Subjects gave informed consent and received oral AG-348 at 15–700 mg twice daily (q12h) or 120 mg once daily (q24h) for 14 days with follow-up to Day 29. Adverse events (AEs), laboratory parameters, ECGs, and vital signs were monitored. Plasma AG-348 concentrations and whole blood 2,3-DPG and ATP levels were measured in serial blood samples for PK/PD assessment. Hormone levels were monitored due to pre-clinical data suggesting potential modulation.

**Results:** 48 subjects (42M/6F), mean age 41.5 (range, 25–60) years, enrolled. Final, un-blinded safety data showed ≥1 AE in 16/36 (44%) AG-348-treated and 4/12 (33%) P subjects. Treatment-related AEs (≥1) were noted in 11/36 (31%) AG-348-treated and 3/12 (25%) P subjects. All treatment-related AEs were mild or moderate in severity (only one grade 3 event) and often reversible despite continued dosing. The most frequent AG-348-related AEs were nausea and headache, 5/36 (14%) for each (P: nausea 0/12 [0%]; headache 1/12 [8%]). Gastrointestinal AEs occurred in AG-348-treated subjects only at the highest dose, 700 mg q12h. One grade 3 AE occurred (AG-348 700 mg q12h, elevated liver function tests [LFTs], which resolved after treatment discontinuation). There were 4 AG-348 discontinuations: due to AEs in 2 subjects (grade 2 drug eruption, 60 mg q12h; grade 3 elevated LFTs, 700 mg q12h), and 2 subjects withdrew consent (both had grade 1/2 nausea and grade 1/1 vomiting; both at 700 mg q12h). The highest well-tolerated dose was 360 mg q12h (doses between 360 and 700 mg were not explored). Plasma AG-348 exposure was dose dependent, with low to moderate variability in the PK parameters of AG-348 and its metabolite AGI-8702. There was a dose-dependent decrease in 2,3-DPG and increase in ATP with the effects plateauing at 360 mg q12h. Decrease in 2,3-DPG was robust after Dose 1, while the increase in ATP occurred gradually and was strongly evident at Day 8. Change from baseline in 2,3-DPG and ATP plateaued at ~300 µg/mL (~50% decrease) and ~175 µg/mL (~50% increase), respectively. After final Day 14 dose, 2,3-DPG returned to levels similar to baseline between 72 and 120 hours. ATP levels remained elevated through 120 hours post dose (**Figure**). Details of all un-blinded data will be presented.

Image/Pictures:

Change from baseline concentration–time profiles of 2,3-DPG and ATP in blood following multiple doses of AG-348



**Summary/Conclusion:** AG-348 showed a robust 2,3-DPG/ATP PD glycolytic profile at well-tolerated doses in healthy volunteers. The results support proceeding with a planned phase 2 trial in patients with PK deficiency.

**Keywords:** Clinical trial, Pharmacokinetic, Phase I, Safety

**Title: Phase 1 Single- and Multiple-Ascending-Dose Randomized Studies of the Safety, Pharmacokinetics, and Pharmacodynamics of Mitapivat (AG-348), a First-in-Class Allosteric Activator of Pyruvate Kinase R, in Healthy Volunteers**

*Hua Yang<sup>\*‡</sup>, Elizabeth Merica<sup>1</sup>, Yue Chen<sup>1</sup>, Marvin Cohen<sup>2</sup>, Ronald Goldwater<sup>3</sup>, Penelope A.Kosinski<sup>1</sup>, Charles Kung<sup>1</sup>, Zheng (Jason) Yuan<sup>1</sup>, Lee Silverman<sup>1</sup>, Meredith Goldwasser<sup>1</sup>, Bruce A. Silver<sup>4</sup>, Sam Agresta<sup>1</sup>, and Ann J. Barbier<sup>1</sup>*

*\*[Vice President], <sup>1</sup>Agios Pharmaceuticals, Inc., Cambridge, MA, USA; <sup>2</sup>MBC Pharma Solutions, Newtown, PA, USA; <sup>3</sup>PAREXEL International, Baltimore, MD, USA; <sup>4</sup>Bruce A Silver Clinical Science and Development, Dunkirk, MD, USA.*

***Abstract***

Pyruvate kinase (PK) deficiency is a chronic hemolytic anemia caused by mutations in PK-R, a key glycolytic enzyme in erythrocytes. These 2 phase 1, randomized, placebo-controlled, double-blind, healthy volunteer studies assessed the safety, tolerability, and pharmacokinetics/pharmacodynamics of mitapivat (AG-348), a first-in-class allosteric PK-R activator. Twelve sequential cohorts were randomized 2:6 to receive oral placebo or mitapivat, respectively, as a single dose (30–2500 mg) in the single ascending dose (SAD) study (ClinicalTrials.gov: NCT02108106), or 15–700 mg every 12 hours (q12h), or 120 mg every 24 hours, for 14 days in the multiple ascending dose (MAD) study (ClinicalTrials.gov: NCT02149966). All 48 subjects completed the fasted SAD part; 44 of 48 completed the MAD (2 discontinued due to adverse events [AEs], 2 withdrew consent). The most common treatment-related AEs in mitapivat-treated subjects were headache (16.7% [SAD] and 13.9% [MAD]) and nausea (13.9%, both studies). AE frequency increased at mitapivat doses ≥700 mg (SAD) and at 700 mg q12h (MAD); 1 grade ≥3 AE occurred in the latter cohort. Pharmacokinetics were favorable: linear and with low variability. Dose-dependent changes in blood glycolytic intermediates consistent with glycolytic pathway activation were observed at all MAD doses, supporting the ongoing phase 2 and 3 trials investigating the potential of mitapivat for treating PK deficiency or other anemias. Yang et al. Phase 1 Single- and Multiple-Ascending-Dose Randomized Studies of the Safety, Pharmacokinetics, and Pharmacodynamics of AG-348, a First-in-Class Allosteric Activator of Pyruvate Kinase R, in Healthy Volunteers. *Clinical Pharmacology in Drug Development* 8(2):246. © 2018, The American College of Clinical Pharmacology. Reproduced with minor edits with permission from John Wiley & Sons Inc. and The American College of Clinical Pharmacology.

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# **Phase 1 Single- and Multiple-Ascending-Dose Randomized Studies of the Safety, Pharmacokinetics, and Pharmacodynamics of Mitapivat (AG-348), a First-in- Class Allosteric Activator of Pyruvate Kinase R, in Healthy Volunteers**

Hua Yang<sup>1</sup>, Elizabeth Merica<sup>1</sup>, Yue Chen<sup>1</sup>, Marvin Cohen<sup>2</sup>, Ronald Goldwater<sup>3</sup>,  
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International, Baltimore, MD, USA; <sup>4</sup>Bruce A Silver Clinical Science and Development, Dunkirk, MD, USA

Yang H et al. *Clin Pharmacol Drug Dev.* 2019;8:246-259.

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Presented at the 17<sup>th</sup> Annual Congress of International Drug Discovery Science and Technology, July 25–27, 2019, Kyoto, Japan

1

# Pyruvate Kinase Deficiency: Overview

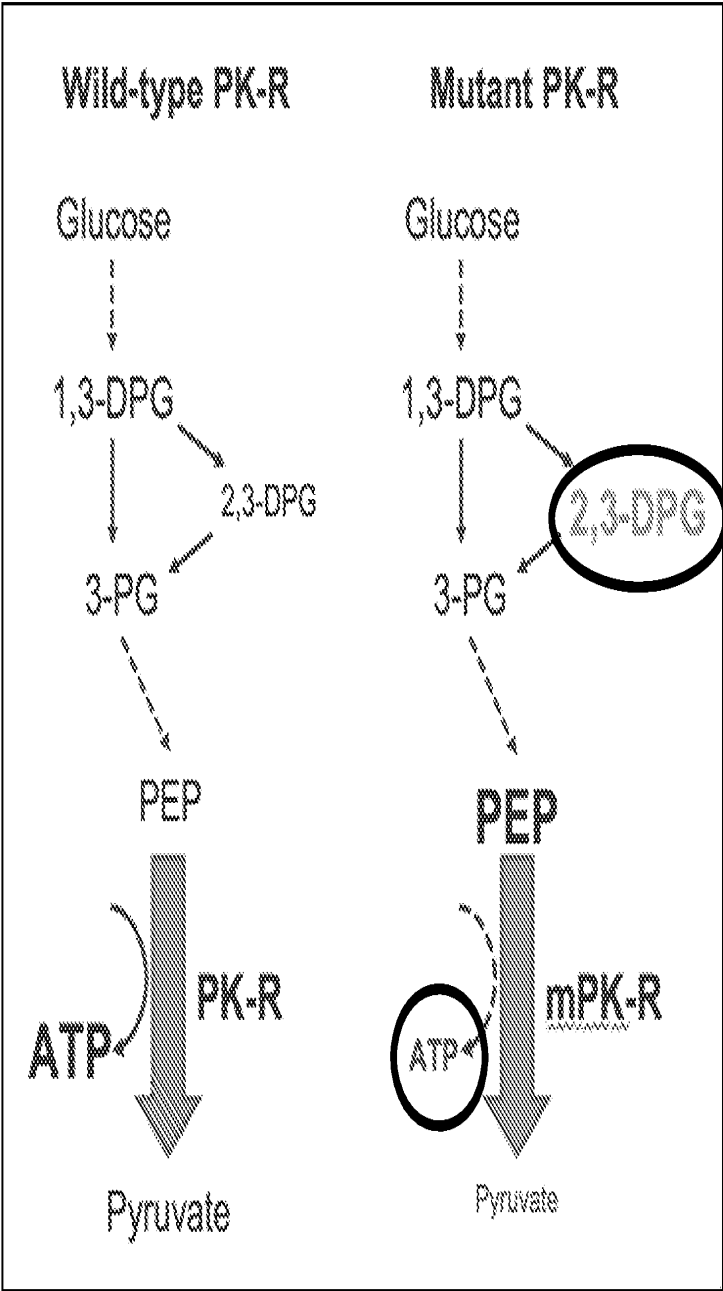
## Red Blood Cell Disease<sup>1-3</sup>

|                         |                                                                                                                                                                                                                                    |
|-------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Description             | <ul style="list-style-type: none"><li>• Under-recognized hereditary hemolytic anemia</li><li>• Heterogeneous disease with variable severity among all ages</li><li>• Most common form of nonspherocytic hemolytic anemia</li></ul> |
| Etiology                | <ul style="list-style-type: none"><li>• Caused by mutations in <i>PKLR</i> gene coding for erythrocyte pyruvate kinase R isoform (PK-R)</li><li>• Results in defective glycolysis and decreased red blood cell lifespan</li></ul>  |
| Disease burden          | <ul style="list-style-type: none"><li>• Lifelong hemolytic anemia</li><li>• Iron overload and jaundice</li><li>• Infection risk post splenectomy</li></ul>                                                                         |
| Diagnosis/<br>Treatment | <ul style="list-style-type: none"><li>• PK-R enzyme activity and genetic testing</li><li>• Supportive treatment: transfusions, splenectomy, iron chelation</li></ul>                                                               |

1. Grace RF et al. *Am J Hematol* 2015;90:825-30; 2. Percy MJ et al. *Blood Cells Mol Dis* 2007;39:189-94;  
3. Grace RF et al. *Blood* 2018;131:2183-92.

# Mutant PK-R: ATP Deficiency in Red Blood Cells

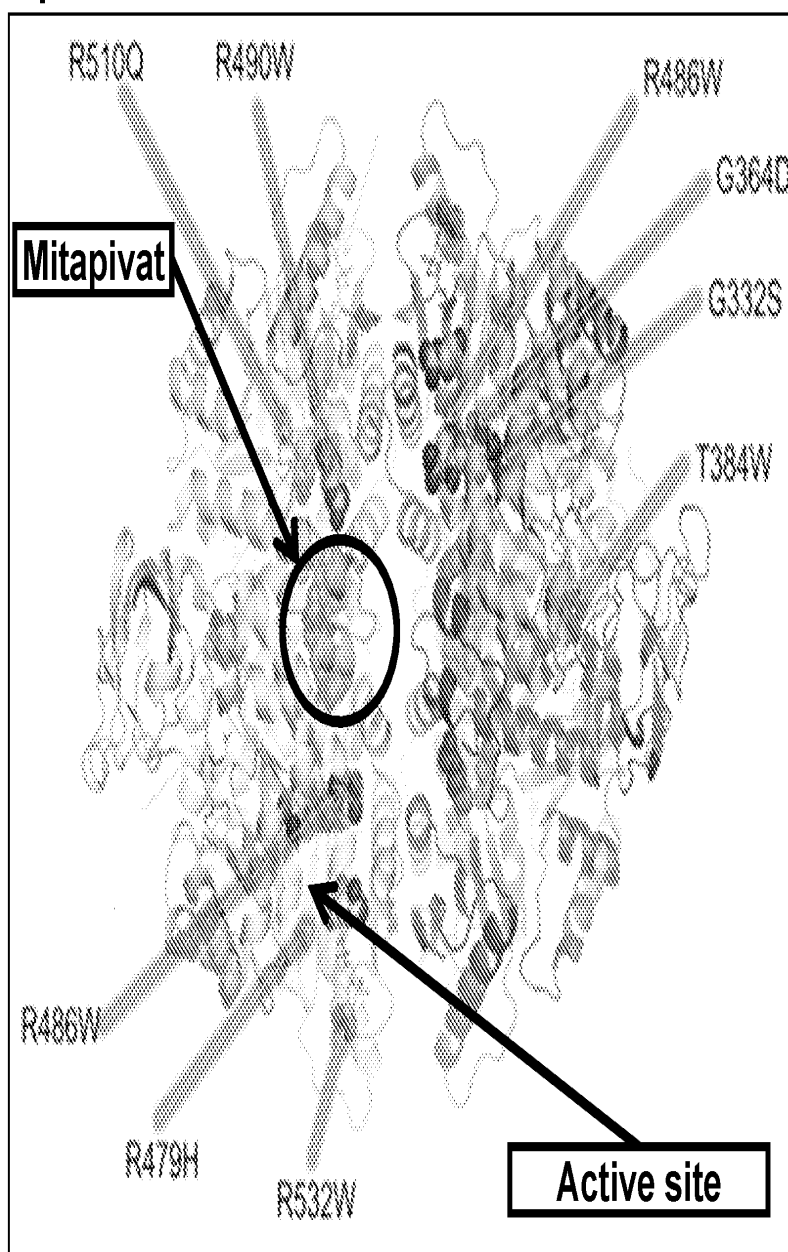
## Glycolytic Pathway



ATP, adenosine triphosphate; 1,3-DPG, 1,3-diphosphoglycerate; 2,3-DPG, 2,3-diphosphoglycerate; 3-PG, 3-phosphoglycerate; mPK-R, mutant pyruvate kinase-R isoform; PEP, Phosphoenolpyruvate; PK-R; pyruvate kinase-R isoform

# Mitapivat is a First-in-Class Allosteric Activator of Wild-Type and Mutant PK-R

PK-R enzyme: active tetramer  
Mitapivat binds at the PK-R dimer-dimer interface<sup>1</sup>

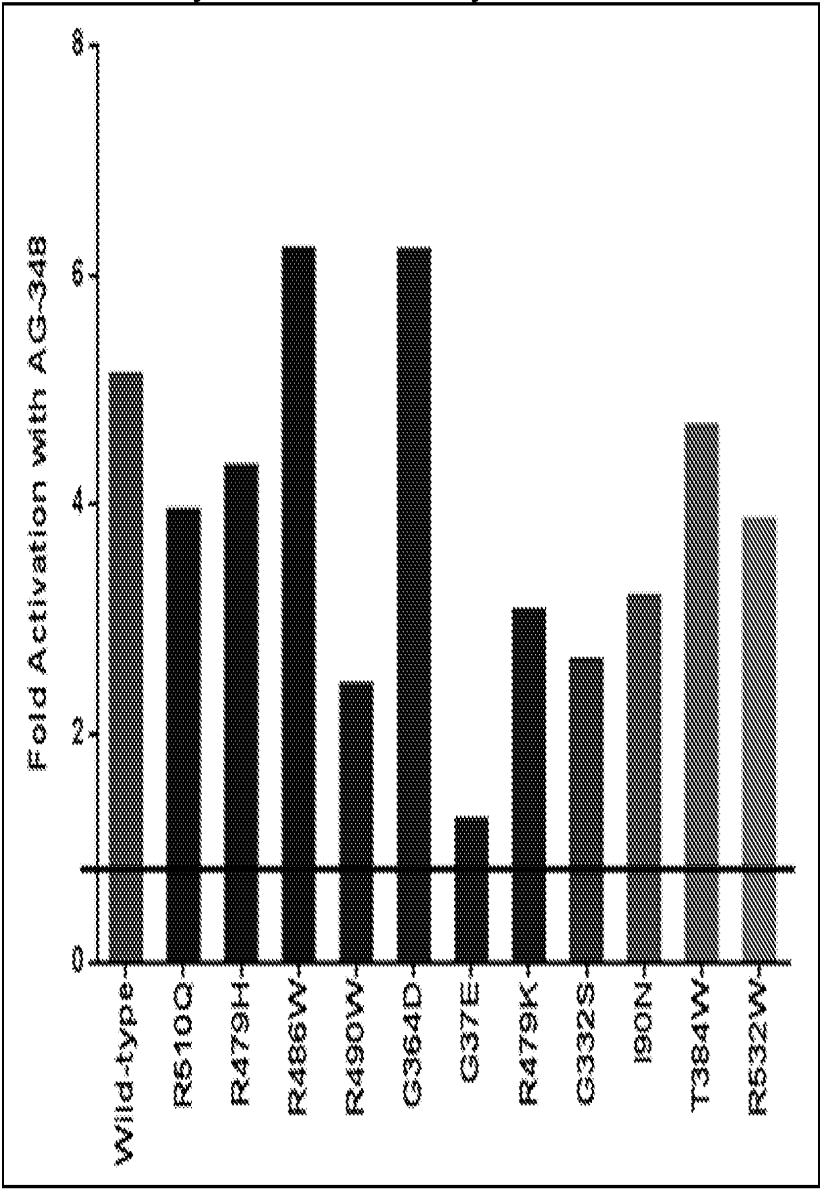


PK-R; pyruvate kinase-R isoform

1. Kung C et al. *Blood* 2017;130:1347-56.

# Mitapivat Activates PK-R Wild-Type and Mutant Subtypes

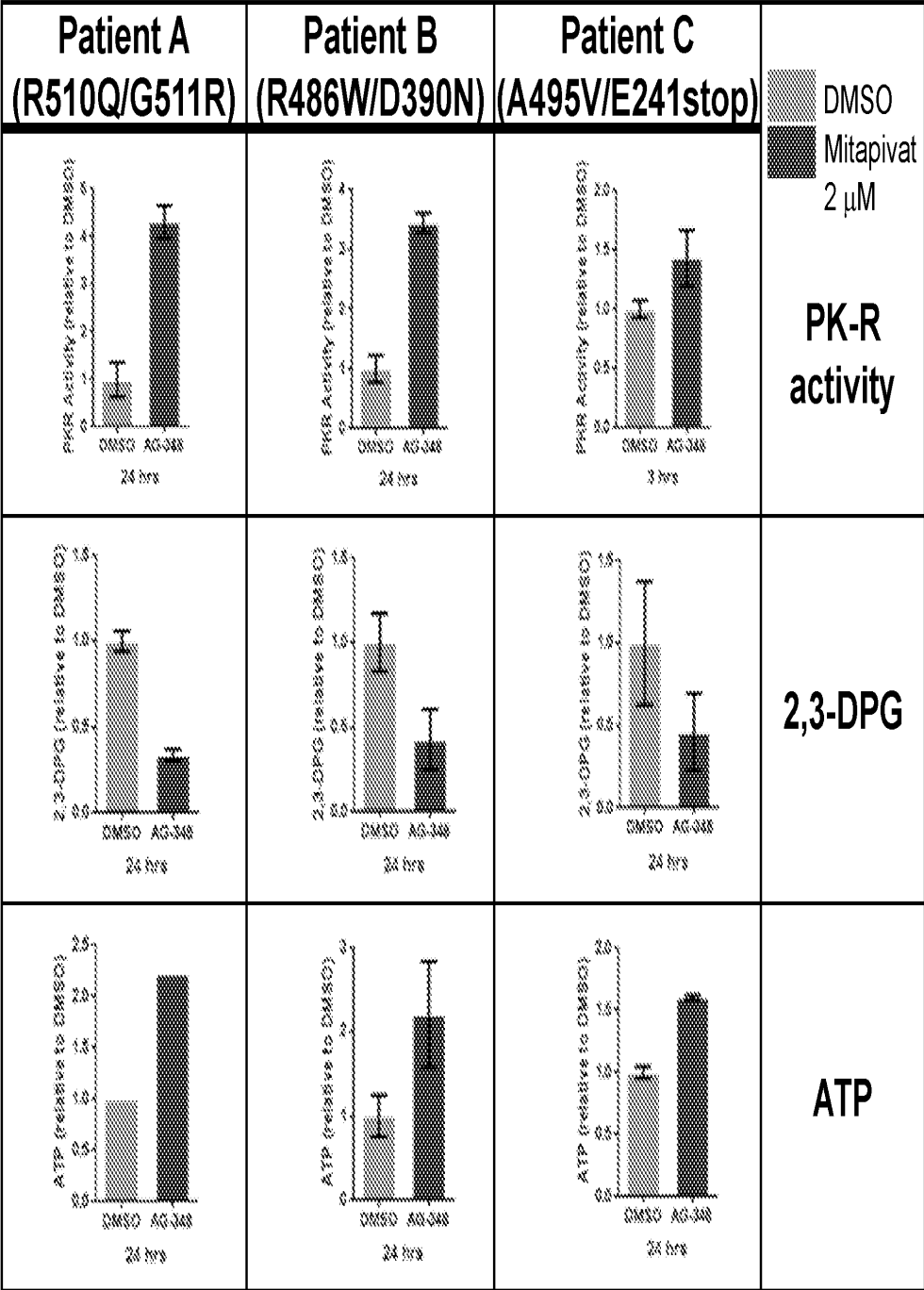
Mitapivat activates PK-R wild type and nearly all mutant enzymes tested<sup>1,2</sup>



1. Kung C et al. *Blood* 2017;130(11):1347-56; 2. Kung C et al. 56th ASH Annual meeting 2014, Poster 4010.

# Mitapivat Restores Glycolytic Pathway Activity

PK Deficient  
Patient Red  
Blood Cell  
Samples



2,3-DPG, 2,3-diphosphoglycerate; ATP, adenosine triphosphate; DMSO, dimethyl sulfoxide; PK, pyruvate kinase; PK-R; pyruvate kinase-R  
isoferr et al. 56<sup>th</sup> ASH Annual meeting 2014, Abstract 4010

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# Phase I Single- and Multiple-Ascending Dose Randomized Studies

Assessing the safety, tolerability, pharmacokinetics and pharmacodynamics of mitapivat (AG-348) in healthy subjects (aged 18–60 years) to inform dose selection and schedule for studies in patients with pyruvate kinase deficiency

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Single dose study: [Clinicaltrials.gov NCT02108106](https://clinicaltrials.gov/ct2/show/study/NCT02108106); Multiple dose study: [Clinicaltrials.gov NCT02149966](https://clinicaltrials.gov/ct2/show/study/NCT02149966).

## Study designs

---

- Cohorts: n=8 subjects per cohort (mitapivat n=6; placebo n=2)
- If safe and tolerable, sequentially escalate dose

### Single dose (n=48)

- Treatment: single oral doses of mitapivat on Day 1
  - 30 mg, 120 mg, 360 mg, 700 mg\*, 1400 mg, 2500 mg
- Multiple PK/PD sample collection from Days -1 to 6

### Multiple dose (n=48)

- Treatment: multiple oral mitapivat doses on Days 1 to 14
  - 15 mg, 60 mg, 120 mg, 360 mg, 700 mg q12h
  - 120 mg q24h
- Multiple PK/PD sample collection from Days -1 to 19

---

\*Food effects following high-fat meal were evaluated in the cohort of patients receiving mitapivat 700 mg dose/placebo after 3-week washout  
PD, pharmacodynamics; PK, pharmacokinetics; q12h, every 12 hours; q24h, every 24 hours.

# Demographics and Disposition

## Single dose

### Placebo (n=12)

Male: 11 (91.7%)  
Age: 36.8 (12.1) years  
Race: White 5 (41.7%), Black 6 (50%),  
Asian 1 (8.3%)  
BMI: 27.3 (3.3) kg/m<sup>2</sup>

### Mitapivat (n=36)

Male: 36 (100%)  
Age: 41.0 (10.6) years  
Race: White 10 (27.8%), Black 25 (69.4%),  
Native Hawaiian/Pacific Islander 1 (2.8%)  
BMI: 26.7 (3.4) kg/m<sup>2</sup>

## Multiple dose

### Placebo (n=12)

Male: 9 (75.0%)  
Age: 37.8 (11.4) years  
Race: White 6 (50%), Black 6 (50%)  
BMI: 27.4 (2.3) kg/m<sup>2</sup>

### Mitapivat (n=36)

Male: 33 (91.7%)  
Age: 42.8 (12.2) years  
Race: White 17 (47.2%), Black 18 (50%),  
Asian 1 (2.8%)  
BMI: 26.5 (2.9) kg/m<sup>2</sup>

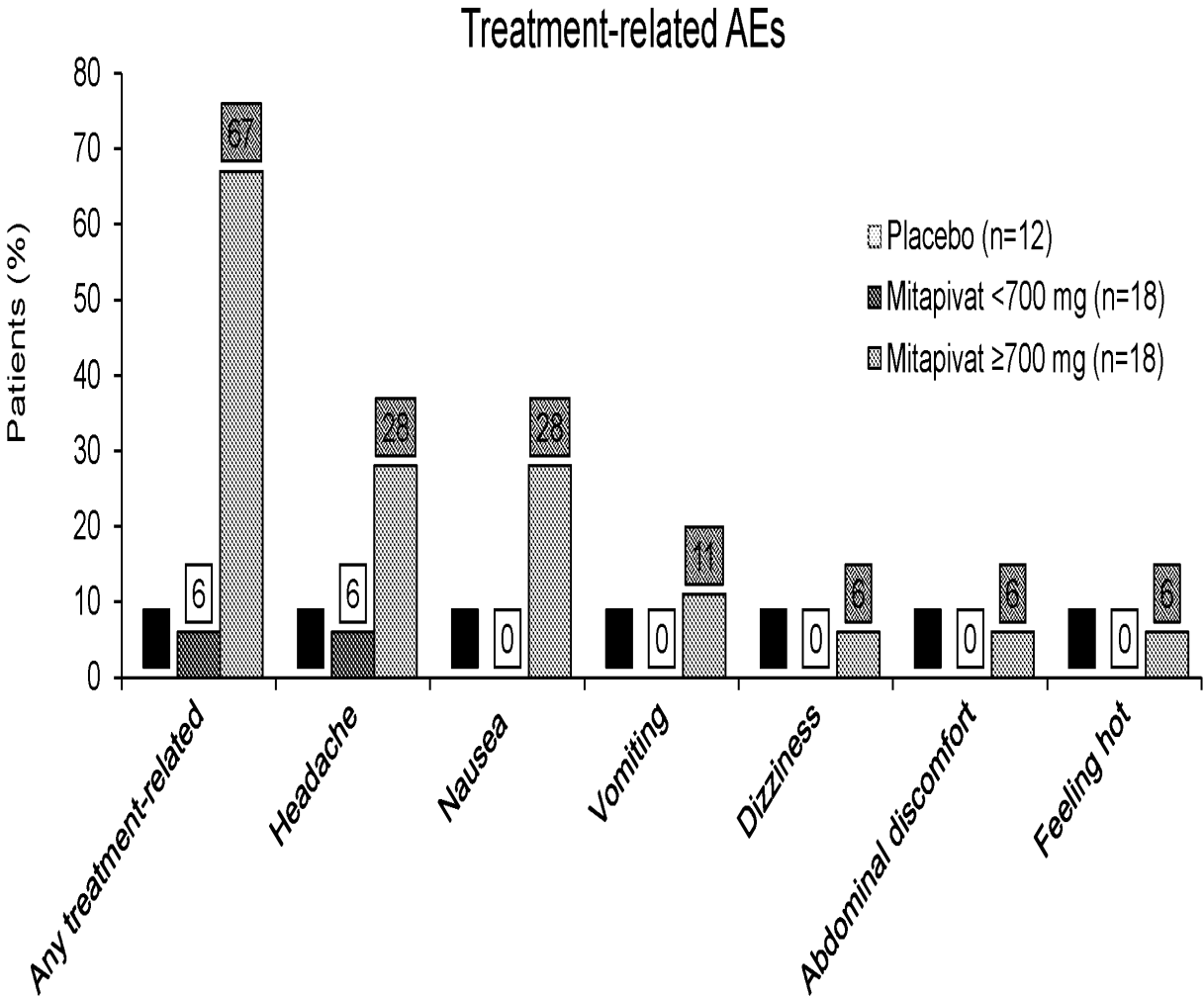
## Disposition

Single Dose: All 48 subjects completed the fasted part  
Multiple Dose: 44 of 48 completed (2 discontinued because of AEs, 2 withdrew consent)

Age and BMI data are mean (SD)  
AE, adverse event; BMI, body mass index; PD, pharmacodynamic; PK, pharmacokinetic.

# Single Dose Mitapivat: Adverse Event Summary

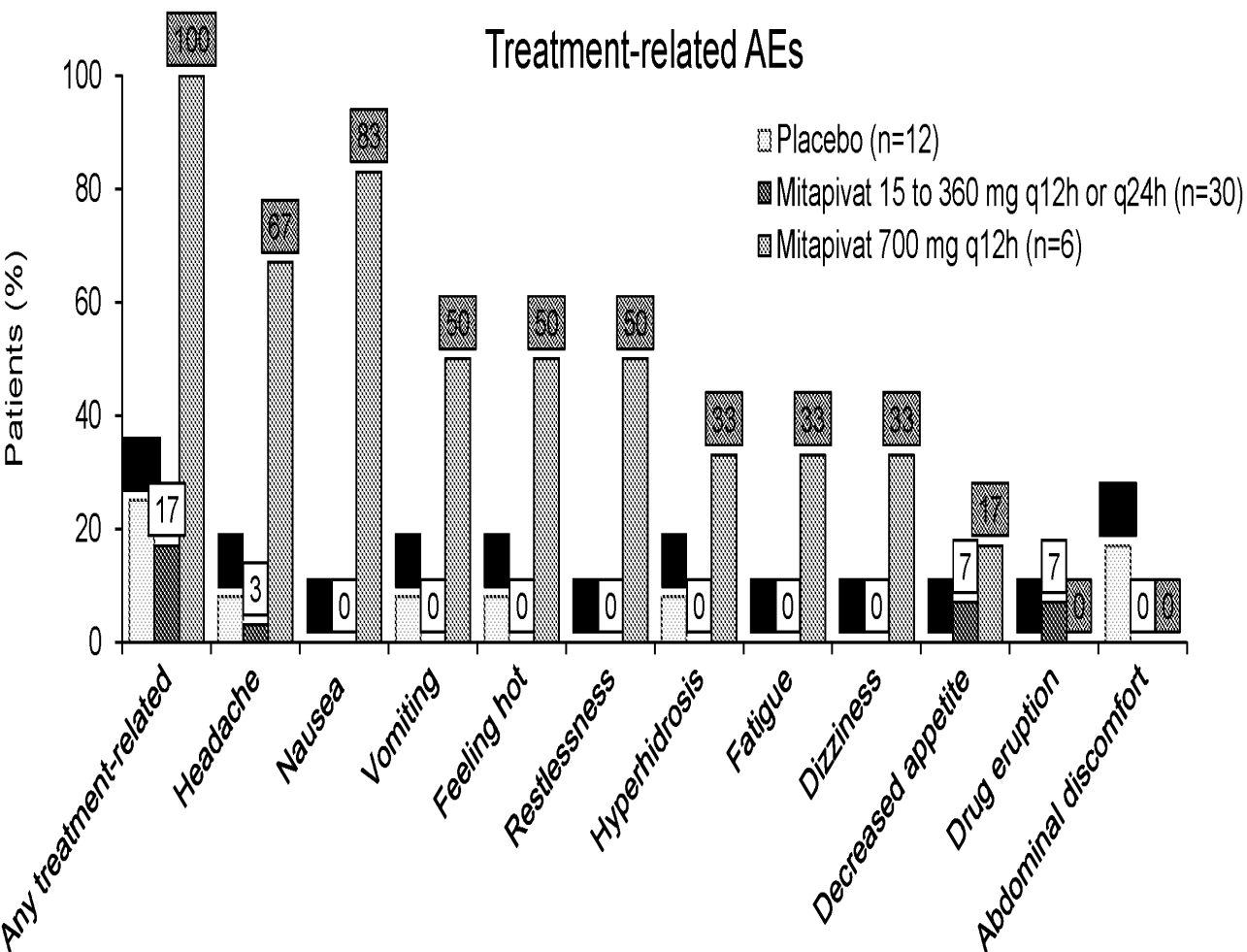
- Any TEAEs<sup>a</sup>: 2 (17%) subjects receiving placebo, 4 (22%) receiving <700 mg, 13 (72%) receiving ≥700 mg
- No SAEs, grade ≥3 TEAEs or DLTs



<sup>a</sup>Fasted period. During study period 2 (fed), TEAEs were reported in 3 of the 5 subjects who received 700 mg of mitapivat (single incidents of grade 1 abdominal discomfort [considered treatment-related], grade 1 upper abdominal pain, and grade 2 headache) AEs, adverse events; DLT, dose-limiting toxicity; TEAE, treatment-emergent adverse event.

# Multiple Doses of Mitapivat: Adverse Event Summary

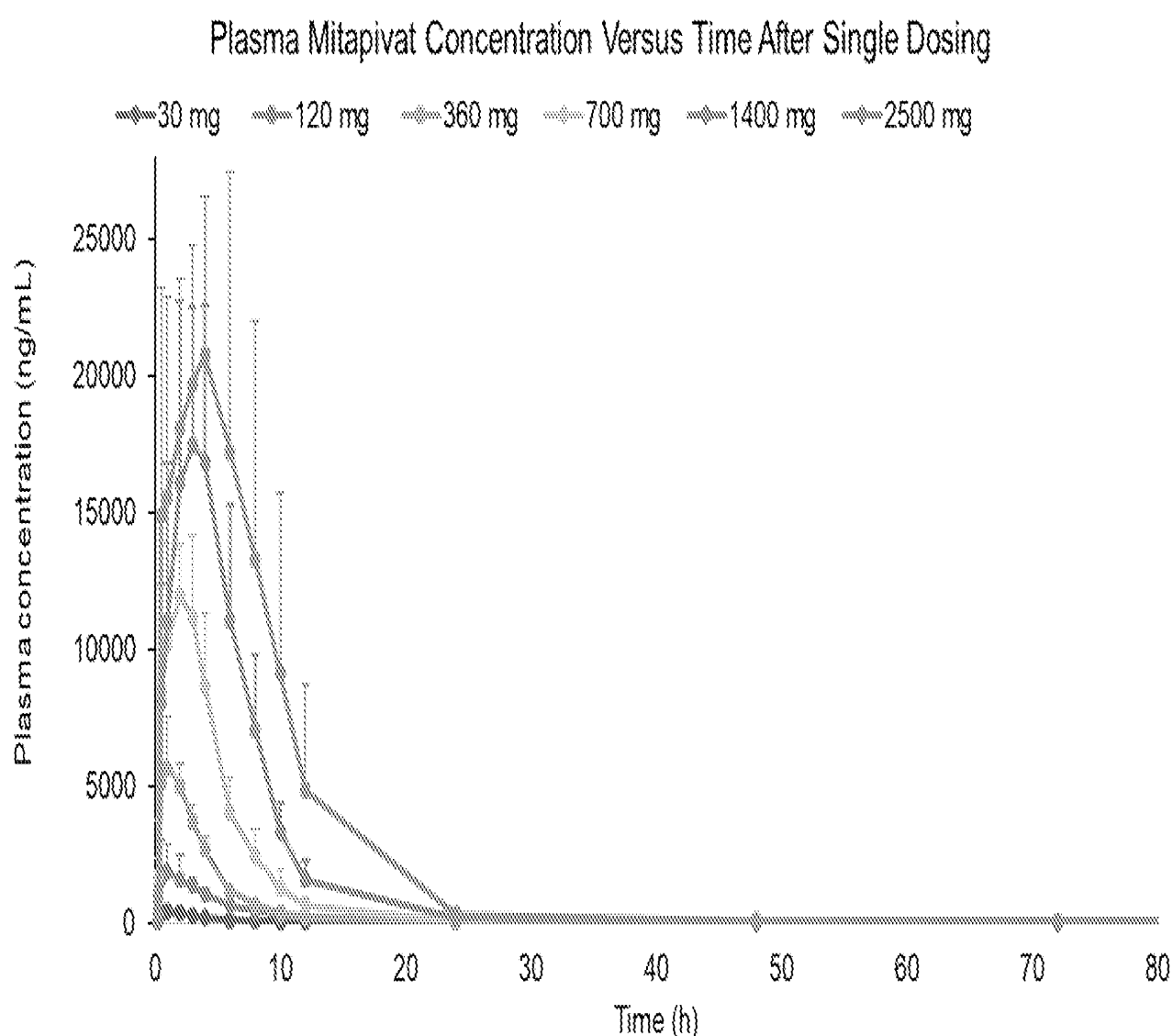
- Any TEAEs: 4 (33%) subjects receiving placebo, 10 (33%) receiving 15 to 360 mg (q12h/q24h), 6 (100%) receiving 700 mg q12h
- No SAEs
- Grade  $\geq 3$  TEAE or DLTs: 1 subject receiving 700 mg q12h experienced grade 3 abnormal liver function tests (a DLT)



AE, adverse event; DLT, dose-limiting toxicity; q12h, every 12 hours; q24h, every 24 hours; TEAE, treatment-emergent adverse event

# Single Dose Mitapivat: PK

- Readily absorbed; prolonged absorption at doses  $\geq 360$  mg
- After peak, decline was multiexponential – steep initial decline but remained above LLOQ (0.5 ng/mL) up to 72 h postdose



Data presented are mean (SD)

LLOQ, lower limit of quantification; PK, pharmacokinetic.

## Single Dose Mitapivat: PK

- Dose-normalized AUC generally remained constant at different dose levels suggesting linear PK across the dose levels studied
- Low PK variability
- Unchanged mitapivat in urine was 1.45% to 2.33% with renal clearance of 0.160 to 0.245 L/h; minor role in systemic elimination
- No delay in absorption and no apparent difference in the  $C_{\max}$ ,  $AUC_{0-t}$ , or  $AUC_{0-\infty}$  between fasting and fed regimens

# Plasma PK Parameters of Mitapivat after Single Doses

**t<sub>max</sub> (h), median (range)**

|              |                   |
|--------------|-------------------|
| 30 mg        | 0.77 (0.48, 2.00) |
| 120 mg       | 1.01 (0.50, 3.00) |
| 360 mg       | 0.77 (0.50, 2.00) |
| 700 mg       | 1.49 (0.58, 3.03) |
| 700 mg (fed) | 2.05 (2.02, 3.07) |
| 1400 mg      | 2.53 (1.72, 4.03) |
| 2500 mg      | 4.07 (0.48, 6.02) |

**t<sub>1/2</sub> (h), mean (SD)\***

|              |             |
|--------------|-------------|
| 30 mg        | 19.3 (3.6)  |
| 120 mg       | 17.8 (3.3)  |
| 360 mg       | 20.4 (3.6)  |
| 700 mg       | 79.3 (20.9) |
| 700 mg (fed) | 136 (44.1)  |
| 1400 mg      | 50.3 (14.9) |
| 2500 mg      | 73.9 (50.7) |

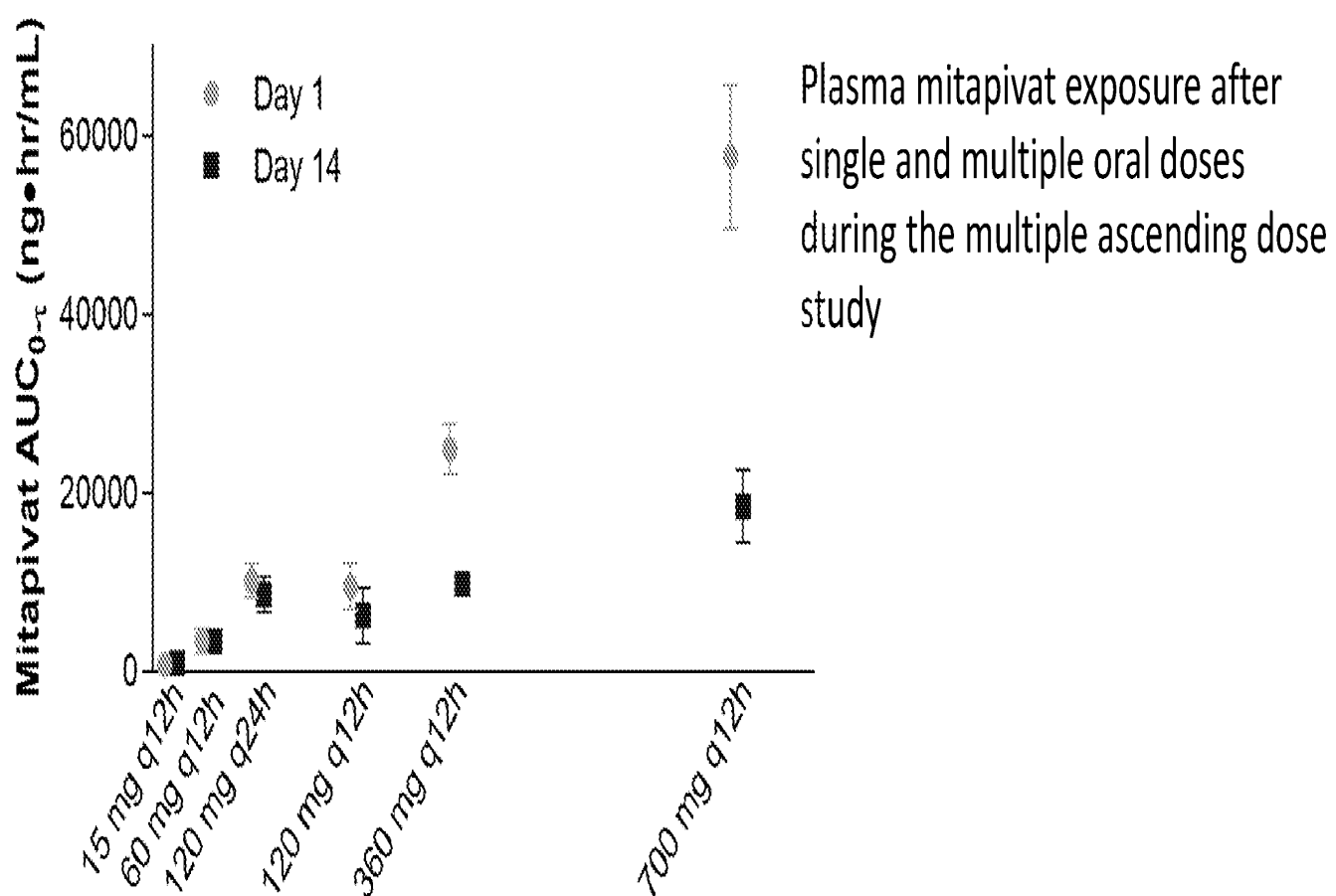
**CL/F (L/h), mean (SD)\***

|              |            |
|--------------|------------|
| 30 mg        | 14.9 (3.9) |
| 120 mg       | 11.2 (4.0) |
| 360 mg       | 13.9 (1.5) |
| 700 mg       | 10.6 (2.9) |
| 700 mg (fed) | 11.9 (2.6) |
| 1400 mg      | 10.9 (2.6) |
| 2500 mg      | 13.3 (4.4) |

N=6 for all doses except 700 mg fed n=5 for t<sub>max</sub> and n=3 for t<sub>1/2</sub> and CL/F which were not calculated for some subjects as concentration-time profile did not exhibit a terminal log-linear phase. \*Duration of sampling (from pre-dose through 72 hours for 30 to 360 mg doses and through 120 hours for 700 to 2500 mg doses had an impact on assessment of t<sub>1/2</sub> and CL/F  
CL/F, apparent clearance; PK, pharmacokinetic; t<sub>1/2</sub>, apparent terminal half-life; t<sub>max</sub>, time to maximal concentration

# Multiple Dose Mitapivat: PK

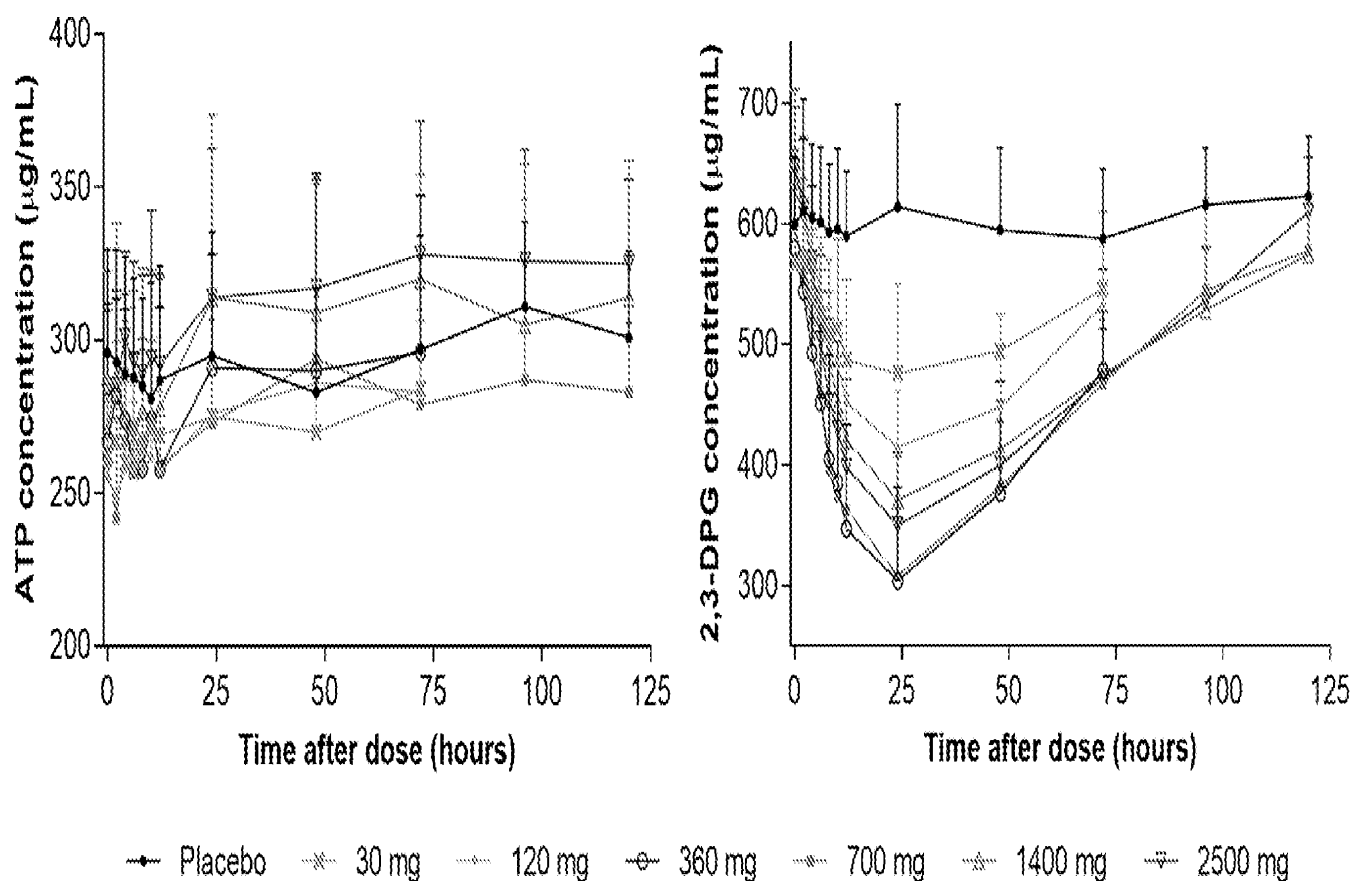
- For doses  $\geq 120$  mg q12h, exposure on day 14 was lower than day 1
- Mean plasma trough levels increased dose-proportionally, but for doses  $\geq 120$  mg q12h increase was less the dose-proportional
  - At 15 mg q12h dose, plasma trough levels reached plateau at day 2; steady state achieved at day 2
  - At 60 mg q12h dose, plasma trough levels reached plateau later than 15 mg dose; steady state achieved at day 7



Data presented as mean (SD). n=6 at each dose level except n=5 for 60 mg q12h and n=3 for 700 mg q12h at Day 14  
AUC<sub>0-∞</sub> area under the plasma concentration-versus-time curve from 0 extrapolated to infinity.

# Observed Concentrations of ATP and 2,3-DPG Following Single Dose of Mitapivat

- Minimal increases in ATP blood levels within 24 h of single dosing
- Decreased 2,3-DPG within 3 h over 30 to 2500 mg dose range
  - At doses  $\geq 360$  mg, maximum decreases reached at 24 h postdose
  - Mean 2,3-DPG levels back to baseline at  $\sim 120$  h postdose

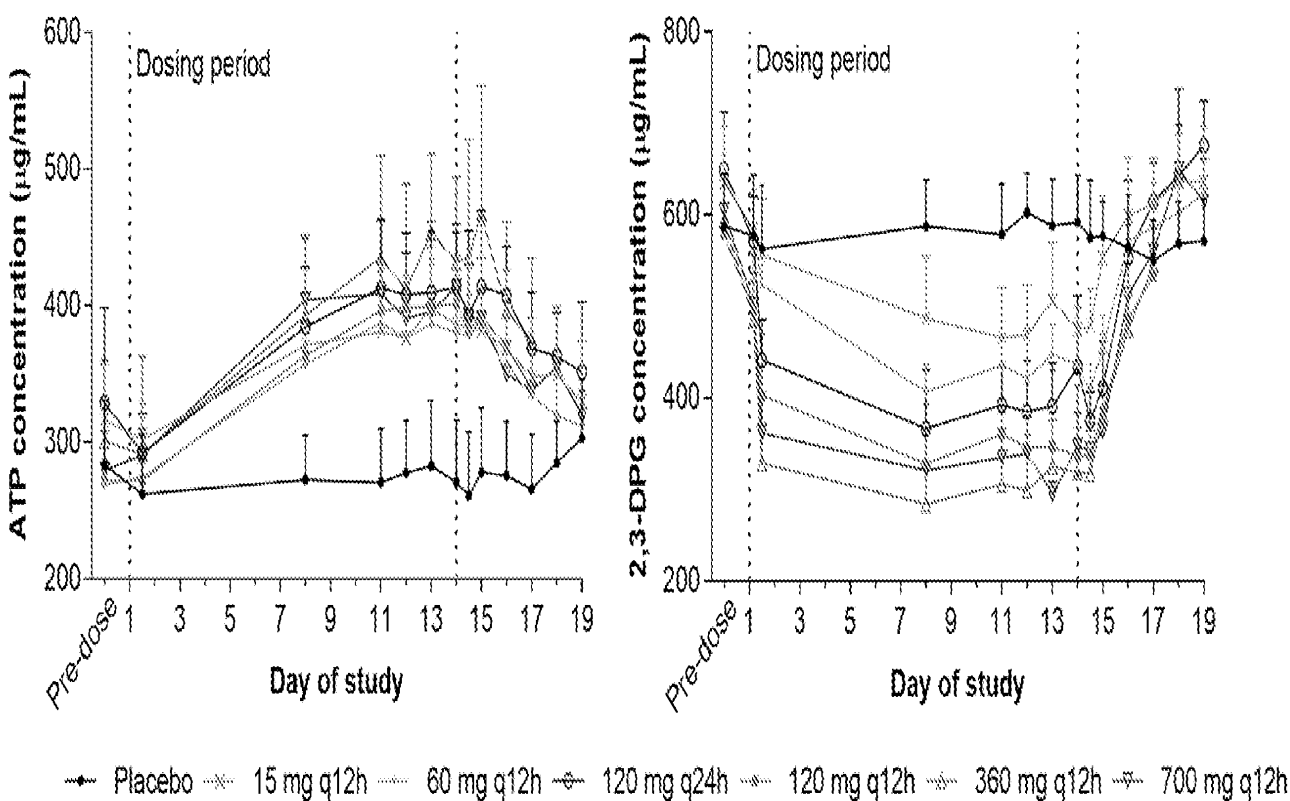


Data presented are mean (SD). n=6 for all doses except n=5 at 10 hours for 1400 mg, n=12 for placebo except at 96 and 120 hours where n=6.

2,3-DPG, 2,3-diphosphoglycerate; ATP, adenosine triphosphate.

# Observed Concentrations of ATP and 2,3-DPG Following Multiple Dosing of Mitapivat

- Maximum increase in ATP blood levels on day 14 was 60% from baseline; remained elevated 120 h after last dose
  - Increases not dose-proportional above 60 mg q12h dose; maximum effect achieved
- Maximum decrease in 2,3-DPG on day 14 was 47%
  - Mean 2,3-DPG level reached and plateaued after second dose on day 1; back to baseline at 72 h post final dose



Data presented are mean (SD). n=6 for all doses except n=5 from day 3 for 60 mg q12h, n=4 for days 3 to 11 and n=3 for day 12 onwards for 700mg q12h and n=12 for placebo except on days 1.5 to 14.5 where n= 10 and days 18 and 19 where n=7.  
2,3-DPG, 2,3-diphosphoglycerate; ATP, adenosine triphosphate.

# Conclusions

- Mitapivat was well tolerated at doses ranging from 15 to 360 mg administered every 12 hours (q12h) over 14 days
  - Most frequently reported treatment-related AEs were headache and nausea
  - Although maximum tolerated dose was not met, AEs increased with doses  $\geq 700$  mg
  - Dose-dependent changes in aromatase-dependent hormones were observed in males in the multiple dose study, consistent with reversible inhibition of human aromatase
  - Recommended dose for first clinical study was 360 mg q12h
- PK profile was favorable with low variability
- PD changes consistent with increased activity of the glycolytic pathway were observed for all doses
  - 90% of maximal response reached at 120 mg and 360 mg q12h doses
- PD data are consistent with preclinical and *ex vivo* data demonstrating:
  - Sustained increases in ATP
  - Decreases in 2,3-DPG
- The data overall provided compelling evidence to initiate a global phase 2 study in patients with pyruvate kinase deficiency

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# Acknowledgments

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We would like to thank the volunteers who agreed to participate in this study

## Disclosures

- Studies funded by Agios Pharmaceuticals, Inc.
- Hua Yang, Elizabeth Merica, Yue Chen, Penelope A. Kosinski, Charles Kung, Zheng (Jason) Yuan, Lee Silverman, Meredith Goldwasser, Sam Agresta, and Ann Barbier are employees of Agios Pharmaceuticals, Inc., or were at the time of the study. Current affiliations are: Elizabeth Merica, freelance; Zheng (Jason) Yuan, Vertex Pharmaceuticals; Sam Agresta, Infinity Pharmaceuticals; Ann Barbier, Translate Bio. Hua Yang, Elizabeth Merica, Yue Chen, Penelope A. Kosinski, Charles Kung, Lee Silverman, Meredith Goldwasser, and Sam Agresta hold stock or options in Agios Pharmaceuticals, Inc. Marvin Cohen and Bruce Silver are consultants to Agios Pharmaceuticals, Inc. Ronald Goldwater is an employee of PAREXEL International.
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Clinical Pharmacology in Drug Development / Volume 8, Issue 2

Original Manuscript

## Phase 1 Single- and Multiple-Ascending-Dose Randomized Studies of the Safety, Pharmacokinetics, and Pharmacodynamics of AG-348, a First-in-Class Allosteric Activator of Pyruvate Kinase R, in Healthy Volunteers

Hua Yang , Elizabeth Merica, Yue Chen, Marvin Cohen, Ronald Goldwater, Penelope A. Kosinski, Charles Kung, Zheng (Jason) Yuan, Lee Silverman, Meredith Goldwasser ... See all authors 

First published: 09 August 2018

<https://doi.org/10.1002/cpdd.604>

Citations: 7

Sam Agresta and Ann J. Barbier contributed equally to this article.

Authors who are fellows of the American College for Clinical Pharmacology: none.

### Abstract

Pyruvate kinase deficiency is a chronic hemolytic anemia caused by mutations in PK-R, a key glycolytic enzyme in erythrocytes. These 2 phase 1 randomized, placebo-controlled, double-blind healthy-volunteer studies assessed the safety, tolerability, and pharmacokinetics/pharmacodynamics of AG-348, a first-in-class allosteric PK-R activator. Twelve sequential cohorts were randomized 2:6 to receive oral placebo or AG-348, respectively, as a single dose (30-2500 mg) in the single-ascending-dose (SAD) study (ClinicalTrials.gov: NCT02108106) or 15-700 mg every 12 hours or 120 mg every 24 hours, for 14 days in the multiple-ascending-dose (MAD) study (ClinicalTrials.gov: NCT02149966). All 48 subjects completed the fasted SAD part; 44 of 48 completed the MAD (2 discontinued because of adverse events [AEs], 2 withdrew consent). The most common treatment-related AEs in AG-348-treated subjects were headache (16.7% [SAD] and 13.9% [MAD]) and nausea (13.9%, both studies). AE frequency increased at AG-348 doses  $\geq$  700 mg (SAD) and at 700 mg every 12 hours (MAD); 1 grade  $\geq$  3 AE occurred in the latter cohort. Pharmacokinetics were favorable with low variability. Dose-dependent changes in blood glycolytic

intermediates consistent with glycolytic pathway activation were observed at all MAD doses, supporting future trials investigating the potential of AG-348 for treating PK deficiency or other anemias.

Citing Literature

Number of times cited according to CrossRef: 7

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# PATENT COOPERATION TREATY

From the INTERNATIONAL SEARCHING AUTHORITY

# PCT

To:  
Davis, Steven G.  
McCARTER & ENGLISH, LLP  
265 Franklin Street  
Boston, MA 02110  
ETATS-UNIS D'AMERIQUE

NOTIFICATION OF TRANSMITTAL OF  
THE INTERNATIONAL SEARCH REPORT AND  
THE WRITTEN OPINION OF THE INTERNATIONAL  
SEARCHING AUTHORITY, OR THE DECLARATION

(PCT Rule 44.1)

|                                                                   |                                                                             |
|-------------------------------------------------------------------|-----------------------------------------------------------------------------|
| Date of mailing<br>(day/month/year) 20 February 2019 (20-02-2019) |                                                                             |
| Applicant's or agent's file reference<br>128748-00420             | <b>FOR FURTHER ACTION</b> See paragraphs 1 and 4 below                      |
| International application No.<br>PCT/US2018/062197                | International filing date<br>(day/month/year) 21 November 2018 (21-11-2018) |
| Applicant<br><br>AGIOS PHARMACEUTICALS, INC.                      |                                                                             |

- ☒ The applicant is hereby notified that the international search report and the written opinion of the International Searching Authority have been established and are transmitted herewith.  
**Filing of amendments and statement under Article 19:**  
 The applicant is entitled, if he so wishes, to amend the claims of the international Application (see Rule 46):  
**When?** The time limit for filing such amendments is normally two months from the date of transmittal of the international Search Report.  
**How?** Directly to the International Bureau of WIPO, 34 chemin des Colombelles  
1211 Geneva 20, Switzerland, Facsimile No.: (41-22) 338.82.70  
 For more detailed instructions, see *PCT Applicant's Guide*, International Phase, paragraphs 9.004 - 9.011.
- ☐ The applicant is hereby notified that no international search report will be established and that the declaration under Article 17(2)(a) to that effect and the written opinion of the International Searching Authority are transmitted herewith.
- ☐ With regard to any protest against payment of (an) additional fee(s) under Rule 40.2, the applicant is notified that:
  - ☐ the protest together with the decision thereon has been transmitted to the International Bureau together with any applicant's request to forward the texts of both the protest and the decision thereon to the designated Offices.
  - ☐ no decision has been made yet on the protest; the applicant will be notified as soon as a decision is made.
- Reminders**  
 The applicant may submit comments on an informal basis on the written opinion of the International Searching Authority to the International Bureau. These comments will be made available to the public after international publication. The International Bureau will send a copy of such comments to all designated Offices unless an international preliminary examination report has been or is to be established.  
 Shortly after the expiration of 18 months from the priority date, the international application will be published by the International Bureau. If the applicant wishes to avoid or postpone publication, a notice of withdrawal of the international application, or of the priority claim, must reach the International Bureau before the completion of the technical preparations for international publication (Rules 90bis 1 and 90bis 3).  
 Within 18 months from the priority date, but only in respect of some designated Offices, a demand for international preliminary examination must be filed if the applicant wishes to postpone the entry into the national phase until 30 months from the priority date (in some Offices even later); otherwise, the applicant must, within 20 months from the priority date, perform the prescribed acts for entry into the national phase before those designated Offices. In respect of other designated Offices, the time limit of 30 months (or later) will apply even if no demand is filed within 18 months. For details about the applicable time limits, Office by Office, see [www.wipo.int/pct/en/texts/time\\_limits.html](http://www.wipo.int/pct/en/texts/time_limits.html) and the *PCT Applicant's Guide*, National Chapters.  
 Within 22 months from the priority date, the applicant may request that a supplementary international search be carried out by a different International Searching Authority that offers this service (Rule 45bis 1). The procedure for requesting supplementary international search is described in the *PCT Applicant's Guide*, International Phase, paragraphs 8.006-8.032.

|                                                                                                                                                                                                                                                                                    |                                                                                          |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|
| Name and mailing address of the International Searching Authority<br> European Patent Office, P.B. 5818 Patentlaan 2<br>NL-2280 HV Rijswijk<br>Tel. (+31-70) 340-2040<br>Fax: (+31-70) 340-3016 | Authorized officer<br>PFITZNER, Gabriele<br>Tel. +49 (0)89 2399-8032<br>McCARTER ENGLISH |
|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------|

Form PCT/GA/220 (July 2017)

# PATENT COOPERATION TREATY

## PCT

### INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

|                                                       |                                                                                               |                                                                            |
|-------------------------------------------------------|-----------------------------------------------------------------------------------------------|----------------------------------------------------------------------------|
| Applicant's or agent's file reference<br>128748-00420 | <b>FOR FURTHER ACTION</b><br>see Form PCT/ISA/220 as well as, where applicable, item 5 below. |                                                                            |
| International application No.<br>PCT/US2018/062197    | International filing date (day/month/year)<br>21 November 2018 (21-11-2018)                   | (Earliest) Priority Date (day/month/year)<br>22 November 2017 (22-11-2017) |
| Applicant<br><br>AGIOS PHARMACEUTICALS, INC.          |                                                                                               |                                                                            |

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 7 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report
  - a. With regard to the language, the international search was carried out on the basis of:
 

☒ the international application in the language in which it was filed  
☐ a translation of the international application into \_\_\_\_\_, which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b))
  - b. ☐ This international search report has been established taking into account the rectification of an obvious mistake authorized by or notified to this Authority under Rule 91 (Rule 43.6bis(a)).
  - c. ☐ With regard to any nucleotide and/or amino acid sequence disclosed in the international application, see Box No. I.
2. ☐ Certain claims were found unsearchable (See Box No. II)
3. ☒ Unity of invention is lacking (see Box No. III)
4. With regard to the title,
 

☒ the text is approved as submitted by the applicant  
☐ the text has been established by this Authority to read as follows:
5. With regard to the abstract,
 

☒ the text is approved as submitted by the applicant  
☐ the text has been established, according to Rule 38.2, by this Authority as it appears in Box No. IV. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority
6. With regard to the drawings,
  - a. the figure of the drawings to be published with the abstract is Figure No. \_\_\_\_\_
 

☐ as suggested by the applicant  
☐ as selected by this Authority, because the applicant failed to suggest a figure  
☐ as selected by this Authority, because this figure better characterizes the invention
  - b. ☒ none of the figures is to be published with the abstract

Form PCT/ISA/210 (first sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.  
PCT/US2018/062197

Box No. II    Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

- 1. ☐ Claims Nos. :  
because they relate to subject matter not required to be searched by this Authority, namely:
- 2. ☐ Claims Nos. :  
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
- 3. ☐ Claims Nos. :  
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III    Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

- 1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
- 2. ☒ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
- 3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
- 4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest    ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.  
☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.  
☐ No protest accompanied the payment of additional search fees.

## INTERNATIONAL SEARCH REPORT

International application No  
PCT/US2018/062197

## A. CLASSIFICATION OF SUBJECT MATTER

INV. C07D215/36 A61P7/06 A61K31/4709  
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07D A61P A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, CHEM ABS Data, WPI Data, EMBASE

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                               | Relevant to claim No. |
|-----------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| X         | WO 2016/201227 A1 (AGIOS PHARMACEUTICALS INC [US]) 15 December 2016 (2016-12-15)<br>cited in the application<br>Claims 1, 17, 22, 38; page 35, example 1<br>with reference to US 8,785,450.<br>*****             | 1-42                  |
| X         | US 8 785 450 B2 (AGIOS PHARMACEUTICALS, INC.) 22 July 2014 (2014-07-22)<br>Claims 1, 24, 30, 51; column 172, lines<br>55-67 and column 175, lines 6-25: compound<br>VIII-8 = present compound 1.<br>*****<br>-/- | 1-42                  |

☒ Further documents are listed in the continuation of Box C.☒ See patent family annex.

## \* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&amp;" document member of the same patent family

Date of the actual completion of the international search

29 January 2019

Date of mailing of the international search report

20/02/2019

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040,  
Fax: (+31-70) 340-3016

Authorized officer

Weisbrod, Thomas

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                                                                                                                                                                                                     | Relevant to claim No. |
|-----------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| A,P       | DATABASE REGISTRY [Online]<br>CHEMICAL ABSTRACTS SERVICE, COLUMBUS,<br>OHIO, US;<br>5 December 2017 (2017-12-05),<br>XP002788333,<br>Database accession no. 2151847-10-6<br>abstract                                                                                                                                                                                                                                                                                                                                                   | 1-42                  |
| T         | DATABASE IMSRESEARCH [Online]<br>IQVIA, LONDON, UK;<br>13 December 2018 (2018-12-13),<br>XP002788334,<br>Database accession no. 2014:8<br>abstract                                                                                                                                                                                                                                                                                                                                                                                     | 1-42                  |
| L         | BERGE ET AL.: "PHARMACEUTICALS SALTS",<br>JOURNAL OF PHARMACEUTICAL SCIENCES,<br>vol. 66, no. 1,<br>1 January 1977 (1977-01-01), pages 1-19,<br>XP000562636,<br>ISSN: 0022-3549, DOI:<br>10.1002/JPS.2600660104<br>Page 1, paragraph bridging left and right<br>columns; page 2, table 1.                                                                                                                                                                                                                                              | 1-20,<br>23-42        |
| L         | STAHL ET AL.: "Pharmaceutical Salts:<br>Properties, Selections, and Use,<br>passages",<br>1 January 2002 (2002-01-01), HANDBOOK OF<br>PHARMACEUTICAL SALTS : PROPERTIES,<br>SELECTION, AND USE, ZÜRICH : VERL.<br>HELVETICA CHIMICA ACTA ; WEINHEIM [U.A.] :<br>WILEY-VCH, DE, PAGE(S) 212-214,254,305,<br>XP002550486,<br>ISBN: 978-3-906390-26-0<br>pages 212-214, 254, 305-306,<br>Page 212, section 8.1.2; page 306:<br>sulfuric acid. Cited as common general<br>knowledge.                                                       | 1-20,<br>23-42        |
| L         | BYRN ET AL.: "Pharmaceutical Solids: A<br>Strategic Approach to Regulatory<br>Considerations",<br>PHARMACEUTICAL RESEARCH,<br>vol. 12, no. 7,<br>1 January 1995 (1995-01-01), pages<br>945-954, XP055531015,<br>DOI: 10.1023/A:1016241927429<br>Abstract; page 945, left column paragraph<br>1 to right column, paragraph 1; page 946,<br>figure 1 and right column, paragraphs 2-3;<br>page 949, figure 6 and left column,<br>paragraph 2; page 952, figure 11 and left<br>column, paragraph 2. Cited as common<br>general knowledge. | 1-26                  |
|           | -/--                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   |                       |

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages                                                                                                                                                                                                                                                                           | Relevant to claim No. |
|----------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------|
| L        | CAIRA: "Crystalline Polymorphism of Organic Compounds", TOPICS IN CURRENT CHEMISTRY, vol. 198, 1 January 1998 (1998-01-01), pages 163-208, XP008166276, ISSN: 0340-1022<br>Paragraph bridging pages 165 and 166.<br>Cited as common general knowledge.                                                                                                       | 1-26                  |
| L        | HARWOOD; MOODY: "Experimental organic chemistry - Principles and practice", 1 January 1989 (1989-01-01), Blackwell Science, XP003025361, ISBN: 978-0-632-02016-4<br>pages 127-132,<br>Pages 127-132, chapter: "Crystallization".<br>Cited as common general knowledge.                                                                                       | 1-24,42               |
| L        | "Chapter 11, Tools for Purifying the Product: Column Chromatography, Crystallization and Reslurrying"<br>In: Anderson: "Practical Process Research & Development",<br>1 January 2000 (2000-01-01), Academic Press, San Diego, XP002565895, ISBN: 978-0-12-059475-7<br>pages 223-224,<br>Paragraph bridging pages 223-224. Cited as common general knowledge. | 1-24,42               |
| L        | HANCOCK; ZOGRAFI: "Characteristics and Significance of the Amorphous State in Pharmaceutical Systems", JOURNAL OF PHARMACEUTICAL SCIENCES, vol. 86, no. 1, 1 January 1997 (1997-01-01), pages 1-12, XP055274556, DOI: 10.1021/js9601896<br>Page 1, abstract; page 1, left column and figure 1. Cited as common general knowledge.                            | 25-40                 |

## INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2018/062197

| Patent document<br>cited in search report | Publication<br>date | Patent family<br>member(s) | Publication<br>date |
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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-42

relating to the subject-matter of claims 1-42

1.1. claims: 1-20, 25, 26, 41, 42(completely); 23, 24, 27-40(partially)

relating to a crystalline form or amorphous form of  
N-(4-(4-(cyclopropylmethyl)piperazine-1-carbonyl)phenyl)quinoline-8-sulfonamide x 1/2 H<sub>2</sub>SO<sub>4</sub> (= compound 1 x 1/2 H<sub>2</sub>SO<sub>4</sub>)  
as shown in claims 1 and 25.

1.2. claims: 21, 22(completely); 23, 24, 27-40(partially)

relating to a crystalline free base of compound 1 as shown in claim 21.

**Information on Search Strategy** - Pilot phase (see OJ 2015, A86)  
The type of information contained in this sheet may change during the pilot for improving  
the usefulness of this new service.

Application Number

PCT/US2018/062197

TITLE: CRYSTALLINE FORMS OF  
N-(4-(4-(CYCLOPROPYLMETHYL)PIPERAZINE-1-CARBONYL)PHENYL)QUINOLINE-8-SULFONAMIDE

APPLICANT: AGIOS PHARMACEUTICALS, INC.

IPC CLASSIFICATION: C07D215/36, A61P7/06, A61K31/4709

EXAMINER: Weisbrod, Thomas

CONSULTED DATABASES: CA, EPODOC, NPL, REAXYS, WPI, EMBASE

CLASSIFICATION SYMBOLS DEFINING EXTENT OF THE SEARCH:

IPC:

CPC:

FI/F-TERMS:

KEYWORDS OR OTHER ELEMENTS FEATURING THE INVENTION:

PATENT COOPERATION TREATY

From the  
INTERNATIONAL SEARCHING AUTHORITY

PCT

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |                                                          |                                                                                   |                                                                                                                                                                        |
|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------------------------------------------|-----------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| To:<br><br>see form PCT/ISA/220                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |                                                          | WRITTEN OPINION OF THE<br>INTERNATIONAL SEARCHING AUTHORITY<br>(PCT Rule 43bis.1) |                                                                                                                                                                        |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                |                                                          | Date of mailing<br>(day/month/year) see form PCT/ISA/210 (second sheet)           |                                                                                                                                                                        |
| Applicant's or agent's file reference<br>see form PCT/ISA/220                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                                                          | FOR FURTHER ACTION<br>See paragraph 2 below                                       |                                                                                                                                                                        |
| International application No.<br>PCT/US2018/062197                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             | International filing date (day/month/year)<br>21.11.2018 | Priority date (day/month/year)<br>22.11.2017                                      |                                                                                                                                                                        |
| International Patent Classification (IPC) or both national classification and IPC<br>INV. C07D215/36 A61P7/06 A61K31/4709                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                                                          |                                                                                   |                                                                                                                                                                        |
| Applicant<br>AGIOS PHARMACEUTICALS, INC.                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       |                                                          |                                                                                   |                                                                                                                                                                        |
| <p>1. This opinion contains indications relating to the following items:</p> <p><input checked="" type="checkbox"/> Box No. I Basis of the opinion</p> <p><input checked="" type="checkbox"/> Box No. II Priority</p> <p><input type="checkbox"/> Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability</p> <p><input checked="" type="checkbox"/> Box No. IV Lack of unity of invention</p> <p><input checked="" type="checkbox"/> Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement</p> <p><input checked="" type="checkbox"/> Box No. VI Certain documents cited</p> <p><input type="checkbox"/> Box No. VII Certain defects in the international application</p> <p><input checked="" type="checkbox"/> Box No. VIII Certain observations on the international application</p> <p>2. FURTHER ACTION</p> <p>If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1bis(b) that written opinions of this International Searching Authority will not be so considered.</p> <p>If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.</p> <p>For further options, see Form PCT/ISA/220.</p> |                                                          |                                                                                   |                                                                                                                                                                        |
| Name and mailing address of the ISA:<br><br>European Patent Office<br>D-80298 Munich<br>Tel. +49 89 2399 - 0<br>Fax: +49 89 2399 - 4465                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                          | Date of completion of<br>this opinion<br><br>see form<br>PCT/ISA/210              | Authorized Officer<br><br>Weisbrod, Thomas<br><br>Telephone No. +49 89 2399-0<br> |

WRITTEN OPINION OF THE  
INTERNATIONAL SEARCHING AUTHORITY

International application No.  
PCT/US2018/062197

Box No. I Basis of the opinion

1. With regard to the language, this opinion has been established on the basis of:
  - ☒ the international application in the language in which it was filed.
  - ☐ a translation of the international application into , which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1 (b)).
2. ☐ This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43bis.1(a))
3. ☐ With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of a sequence listing:
  - a. ☐ forming part of the international application as filed:
    - ☐ in the form of an Annex C/ST.25 text file.
    - ☐ on paper or in the form of an image file.
  - b. ☐ furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
  - c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
    - ☐ in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).
    - ☐ on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).
4. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

see separate sheet

Box No. II Priority

1. ☒ The validity of the priority claim has not been considered because the International Searching Authority does not have in its possession a copy of the earlier application whose priority has been claimed or, where required, a translation of that earlier application. This opinion has nevertheless been established on the assumption that the relevant date (Rules 43bis.1 and 64.1) is the claimed priority date.
2. ☐ This opinion has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid (Rules 43bis.1 and 64.1). Thus for the purposes of this opinion, the international filing date indicated above is considered to be the relevant date.
3. Additional observations, if necessary:

WRITTEN OPINION OF THE  
INTERNATIONAL SEARCHING AUTHORITY

International application No.  
PCT/US2018/062197

Box No. IV Lack of unity of invention

1. ☐ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has, within the applicable time limit:
- ☐ paid additional fees
  - ☐ paid additional fees under protest and, where applicable, the protest fee
  - ☐ paid additional fees under protest but the applicable protest fee was not paid
  - ☐ not paid additional fees
2. ☒ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.
3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is
- ☐ complied with
  - ☒ not complied with for the following reasons:  
see separate sheet
4. Consequently, this report has been established in respect of the following parts of the international application:
- ☒ all parts.
  - ☐ the parts relating to claims Nos.

Box No. V Reasoned statement under Rule 43bis.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

|                               |             |             |
|-------------------------------|-------------|-------------|
| Novelty (N)                   | Yes: Claims | <u>1-42</u> |
|                               | No: Claims  |             |
| Inventive step (IS)           | Yes: Claims |             |
|                               | No: Claims  | <u>1-42</u> |
| Industrial applicability (IA) | Yes: Claims | <u>1-42</u> |
|                               | No: Claims  |             |

2. Citations and explanations

see separate sheet

**WRITTEN OPINION OF THE  
INTERNATIONAL SEARCHING AUTHORITY**

International application No.  
PCT/US2018/062197

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**Box No. VI Certain documents cited**

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1. Certain published documents (Rules 43*bis*.1 and 70.10)

and / or

2. Non-written disclosures (Rules 43*bis*.1 and 70.9)

see form 210

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**Box No. VIII Certain observations on the international application**

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The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

**Re Item I**

**Basis of the report**

The claims are directed to

1. a crystalline form of N-(4-(4-(cyclopropylmethyl)piperazine-l-carbonyl)phenyl)-quinoline-8-sulfonamide x 1/2 H<sub>2</sub>SO<sub>4</sub> (claims 1-20, 23-24);
2. a crystalline form of N-(4-(4-(cyclopropylmethyl)piperazine-l-carbonyl)phenyl)-quinoline-8-sulfonamide free base (claims 21-22);
3. an amorphous form of N-(4-(4-(cyclopropylmethyl)piperazine-l-carbonyl)phenyl)-quinoline-8-sulfonamide x 1/2 H<sub>2</sub>SO<sub>4</sub> (claims 25-26); and
4. subject-matter referring to one or more of these forms (claims 27-42).

For the sake of simplicity and in accordance with the description the present application N-(4-(4-(cyclopropylmethyl)piperazine-l-carbonyl)phenyl)quinoline-8-sulfonamide is referred to as compound 1 in this report.

**Re Item IV**

**Lack of unity of invention**

1 Reference is made to the following documents.

- |    |                                                                                            |
|----|--------------------------------------------------------------------------------------------|
| D1 | WO 2016/201227 A1, 15 December 2016; cited in the application.                             |
| D2 | US 8 785 450 B2, 22 July 2014.                                                             |
| D3 | DATABASE REGISTRY, Database accession no. 2151847-10-6 (RN), 5 December 2017, XP002788333. |
| D4 | DATABASE IMSRESEARCH, Database accession no. 2014:8, 13 December 2018, XP002788334.        |
| D5 | BERGE ET AL. JOURNAL OF PHARMACEUTICAL SCIENCES 1977, 66, 1-19, XP000562636.               |

- D6 STAHL ET AL. HANDBOOK OF PHARMACEUTICAL SALTS: PROPERTIES, SELECTION, AND USE, 2002, pages 212-214, 254, 305-306, XP002550486.
- D7 BYRN ET AL. PHARMACEUTICAL RESEARCH 1995, 12, 945-954, XP055531015.
- D8 CAIRA TOPICS IN CURRENT CHEMISTRY 1998, 198, 163-208, XP008166276.
- D9 HARWOOD; MOODY Experimental organic chemistry - Principles and practice, 1989, pages 127-132, XP003025361.
- D10 Anderson Practical Process Research & Development, 2000, pages 223-224, XP002565895.
- D11 HANCOCK; ZOGRAFI JOURNAL OF PHARMACEUTICAL SCIENCES 1997, 86, 1-12, XP055274556.

Under the assumption that the priority is valid, the P-document D3 is not considered as prior art under Rule 64.1 PCT. D4 has been published after the filing date of the present application. It is no prior art under Rule 64.1 PCT.

## 2 Unity

The application lacks unity of invention since its subject-matter relates not to one but rather to 2 separate inventions not linked together by a common underlying inventive concept as required by Rules 13.1 and 13.2 PCT. The claims and inventions to which the separate inventions relate are grouped as follows (in the order chosen by the applicant).

1. Claims 1-20, 25-26, 41-42 (complete) and claims 23-24, 27-40 (all part) relating to a crystalline or amorphous form of compound 1 x 1/2 H<sub>2</sub>SO<sub>4</sub>.
2. Claims 21-22 (complete) and claims 23-24, 27-40 (all part) relating to a crystalline free base of compound 1.

The common matter linking together the identified inventions is represented by "solid state forms of compounds comprising the formula of compound 1". This common matter does not comprise a single general inventive concept, based on same or corresponding special technical features, because it does not define a contribution over the prior art. The reasons are as follows:

D1 relates to therapeutic methods involving the present compound 1 and pharmaceutically acceptable salts thereof (cf. claims 1, 17, 22, 38). Furthermore, D2 discloses a group of compounds and pharmaceutically acceptable salts thereof (D2, claim 1), including compound 1 (D2: claim 24, column 417; claim 30), pharmaceutically acceptable salts thereof (claims 1, 51; column 17, lines 53-59), and a corresponding pharmaceutical composition (claim 51). The synthesis of compound 1 is disclosed in column 175, lines 6-25 in combination with column 172, lines 55-67 of D2, and D2 is incorporated by reference into D1 in its entirety, particularly with respect to the synthesis of compound 1 (cf. D1, page 35, example 1). In the mentioned prior art documents it is not described in which physical form compound 1 had been obtained by the procedure of D1. Starting from D1 or D2, the problem underlying the inventions (1) and (2) may be seen in selecting and/or providing more specific embodiments of compound 1 for the same therapeutic application as in D1 and D2. The contributions claimed in the present application which are possibly made over the prior art are:

1. for the invention (1): the selection of compound 1 x 1/2 H<sub>2</sub>SO<sub>4</sub> in solid amorphous or solid crystalline form; and
2. for the invention (2): the provision of compound 1 in crystalline form.

These contributions, however, diverge into 2 different directions, because one contribution relates to the selection of a salt in solid form, whereas the second contribution relates to the provision of the free base in crystalline form. The solid state forms of the identified inventions do not qualify as unifying features, because in the pharmaceutical development of compound 1 or a pharmaceutically acceptable salt thereof, it is routine for the skilled artisan to provide and investigate solid state forms of the development candidate, that is crystalline forms (polymorphs), solvates, and amorphous forms (cf. common general knowledge: e.g. D7, page 945, left column, 1st sentence, page 946,

figure 1, page 949, figure 6, page 952, figure 11; D8, paragraph bridging pages 165-166). Hence, the identified inventions comprise neither the same, nor corresponding special technical features. Therefore, the technical relationship between the subject-matter of the identified inventions required by Rule 13.2 PCT is lacking and the identified inventions are not so linked as to form a single inventive concept as required by Rule 13.1 PCT. The application does thus not fulfil the requirement of unity of invention.

**Re Item V**

**Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

- 1 Claims 32-40 relate to a subject-matter considered by this Authority to be covered by the provisions of Rule 39.1(iv)/67.1(iv) PCT. The patentability can be dependent upon the formulation of the claims. The EPO, for example, does not recognise as patentable claims to the use of a compound in medical treatment, but may allow claims to a product, in particular substances or compositions for a first or further medical treatment.

- 2 Novelty

The aspects (1) and (2) as defined under item IV.2 are generally comprised in the teaching of D1 and D2. The aspect (1) is a novel selection of D1 and D2 with the hemisulfate ( $1/2 \text{ H}_2\text{SO}_4$ ) as salt former with compound 1 and the solid state aspects (crystalline respectively amorphous) of the claimed products as novel technical features. The aspect (2) is novel over D1 and D2 on account of the crystalline state of the compound 1 as defined in claim 21. D5-D11 are not relevant to the question of novelty of the application, because the subject-matter of the present claims is not disclosed therein.

- 3 Inventive step of the invention (1) as defined under item IV.2 above

Merely as a solution of the problem of selecting more specific embodiments for the same therapeutic application from D1 or D2, the subject-matter of the aspect (1) is obvious because pharmaceutically acceptable salts and their utility is already taught in D1 and D2. It is noted that salts of sulfuric acid are

commonly used for active pharmaceutical ingredients (cf. common general knowledge: D5, table 1; D6, page 212, 8.1.2, and page 306). Furthermore, salt forming agents are often chosen empirically and the preferred form is selected on practical basis including ease of crystallisation (D5, page 1, right column, sentences 1 and 2). In addition, polymorph and solid state form screening covering solvates, hydrates and the amorphous form belong to the routine task of the skilled artisan (cf. common general knowledge: e.g. D7, page 945, left column, 1st sentence, page 946, figure 1, page 949, figure 6, page 952, figure 11; D8, paragraph bridging pages 165-166). In the absence of any substantiated unexpected effect of the compounds as defined in claims 1 and 25 in comparison with compound 1 free base prepared according to D1, no inventive step will be acknowledged for the claims of the aspect (1).

4 Inventive step of the invention (2) as defined under item IV.2 above

In view of D1 or D2, the problem underlying the aspect (2) lies in the provision of a more specific form of compound 1 for the same therapeutic application. For practical reason the skilled person would always crystallise a substance, because crystalline substances are easy to handle and crystallisation generally provides the best purification and may be essential for preparing API of the desired purity and polymorphic state (cf. common knowledge: e.g. D9, page 127, paragraph 3; D10, paragraph bridging pages 223-224). Furthermore, polymorphism is common place among APIs and polymorph screening is routine in the pharmaceutical industry (cf. D8, page 165, last paragraph). Hence, in the absence of any substantiated unexpected effect of the compound of claim 21 in comparison with compound 1 free base prepared according to D1, no inventive step will be acknowledged for the claims of the aspect (2).

Re Item VI

**Certain documents cited**

The P-document D3 shows compound 1 hemisulfate sesquihydrate. As database entry it is no enabling disclosure and cannot challenge the novelty of the application.

Re Item VIII

**Certain observations on the international application**

The claims do not comply with Article 6 PCT for the following reasons.

- 1      The subject-matter of claims 2-4, 6-11, and 18-19 is not covered by independent claim 1, because solvates are not defined by claim 1. This inconsistency renders the claims unclear.
- 2      The vague terms "substantially" and "about" render the claims 24, 26, and 30 unclear.
- 3      Claims 31 and 41 lack clarity, because Form A is not defined therein.
- 4      Claim 37 does not comply with Article 6 PCT, because the therapeutic application is functionally defined by a mechanism of action which does not allow any practical application in the form of a defined, real treatment of a pathological condition. This objection may be overcome by introducing in the claims a list of pathological conditions cited in the application.

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1/1 - (C) FILE REGISTRY

XP-002788333

RN - 2151847-10-6 REGISTRY

ED - Entered STN: 05 Dec 2017

CN - 8-Quinolinesulfonamide, N-[4-[[4-(cyclopropylmethyl)-1-piperazinyl]carbonyl]phenyl]-, sulfate, hydrate (2:1:3) (CA INDEX NAME)

OTHER NAMES:

CN - AG 348 sulfate hydrate

CN - Mitapivat sulfate

CN - N-(4-[4-(Cyclopropylmethyl)piperazine-1-carbonyl]phenyl)quinoline-8-sulfonamidesulfate hydrate (2:1:3)

MF - C24 H26 N4 O3 S . 1/2 H2 O4 S . 3/2 H2 O

SR - CAS Client Services

LC - STN Files: EMBASE, IMSRESEARCH

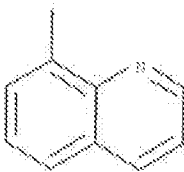
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CRN 1260075-17-9

CMF C24 H26 N4 O3 S

PAGE 1-2

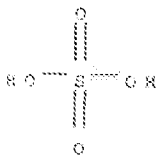




CM 2

CBN 7664-93-9

CMF R2 O4 S



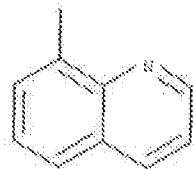
XP-002788334

1/1 - (C) FILE IMSRESEARCH

ACCESSION NUMBER: 2014:8 IMSRESEARCH  
SOURCE: R&D Focus, (13 Dec 2018)  
DOCUMENT NUMBER: 2505849  
GENERIC NAME: mitapivat; mitapivat sulfate  
REFERENCE: INN; USAN  
LABORATORY NAME: AG 348  
CHEMICAL NAME: N-(4-[4-(cyclopropylmethyl)piperazine-1-carbonyl]phenyl)quinoline-8-sulfonamide  
CAS REGISTRY NO.: 1260075-17-9  
DERIVATIVE(S): 1260075-17-9mitapivat  
\*\*\*2151847-10-6\*\*\* mitapivat sulfate  
STRUCTURE:

PAGE 1-A





CLASSIFICATION: A16A Other Alimentary Tract And Metabolism Products;  
B3X Other Anti-anaemic Products  
HIGHEST DEV. PHASE: Phase III (50)  
ENTRY DATE: Entered STN: 3 Jan 2014  
Last Updated on STN: 28 Dec 2018

COMPANY INFORMATION:

| Type       | Company | Nationality | Corporation           |
|------------|---------|-------------|-----------------------|
| Originator | Agios   | USA         | Agios (United States) |

| Electronic Acknowledgement Receipt   |                                                     |
|--------------------------------------|-----------------------------------------------------|
| EFS ID:                              | 42425378                                            |
| 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:                        | 12-APR-2021                                         |
| Filing Date:                         | 12-JUN-2020                                         |
| Time Stamp:                          | 15:17:33                                            |
| Application Type:                    | Utility under 35 USC 111(a)                         |

| Payment information:   |                       |                                                                                                            |                                          |                  |                  |
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| Submitted with Payment |                       |                                                                                                            | no                                       |                  |                  |
| File Listing:          |                       |                                                                                                            |                                          |                  |                  |
| Document Number        | Document Description  | File Name                                                                                                  | File Size(Bytes)/ Message Digest         | Multi Part /.zip | Pages (if appl.) |
| 1                      | Non Patent Literature | 20210212_AGI-<br>PKR-16US_2020_June13_EHA_<br>presentation_Proof_of_concep<br>t_for_the_oral_pyruvate_.PDF | 418778                                   | no               | 14               |
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| 2                                                                                                                                                                                                          | Non Patent Literature | EHA_25_POC_6-17-2020.PDF                                                                 | 100052                                    | no | 1  |
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| 3                                                                                                                                                                                                          | Non Patent Literature | 20210203_AGI-PKR-16US_2019_Nov7_EHA_An_ongoing_openlabel_multicenter_study.pdf           | 176458                                    | no | 2  |
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| 4                                                                                                                                                                                                          | Non Patent Literature | 20210212_AGI-PKR-16US_2019_Nov8_presentation_An_ongoing_open-label_multicenter_study.pdf | 1680812                                   | no | 11 |
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| 5                                                                                                                                                                                                          | Non Patent Literature | BBTS_Poster_10-4-2018.PDF                                                                | 282430                                    | no | 1  |
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| 6                                                                                                                                                                                                          | Non Patent Literature | Layton_BBTS_Abstract_10-4-2018.PDF                                                       | 230694                                    | no | 3  |
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| 7                                                                                                                                                                                                          | Non Patent Literature | 20210212_AGI-PKR-16US_2017_Feb4_presentation_New_drugs_for_enzymatic_defects.pdf         | 2239594                                   | no | 23 |
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| 9                                                                                                                                                                                                          | Non Patent Literature | 20210203_AGI-PKR-16US_2015_ASH_Abstract_Population_Pharmacokinetics_and_Pharmacodynamics_o.pdf | 214807                                    | no | 2  |
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| 11                                                                                                                                                                                                         | Non Patent Literature | 20210212_AGI-PKR-16US_2019_Dec3_Abstract-Mitapivat_AG-348_in_adults_with_pyruvate_kinase.pdf   | 151324                                    | no | 2  |
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| 13                                                                                                                                                                                                         | Non Patent Literature | 20210203_AGI-PKR-16US_2016_June9_EHA_The_pyruvate_kinase_activator_AG-348_improves_murine_.pdf | 45706                                     | no | 2  |
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| 14                                                                                                                                                                                                         | Non Patent Literature | GRC_Poster_7-17-2019.PDF                                                                       | 196249                                    | no | 1  |
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| 16           | Non Patent Literature | 20210212_AGI-<br>PKR-16US_2020_Jan23_Haematologica_Publication.pdf                                 | 2821064                                  | no | 12 |
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| 18           | Non Patent Literature | 20210212_AGI-<br>PKR-16US_2019_Dec3_Abstract_Deceased_activity_and_stability_of_pyruvate_kina.pdf  | 217611                                   | no | 3  |
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| 20           | Non Patent Literature | 20210209_AGI-<br>PKR-16US_2019_June13_Abstract_Deceased_activity_and_stability_of_pyruvake_ki.pdf  | 162713                                   | no | 2  |
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| 22           | Non Patent Literature | 20210209_AGI-<br>PKR-16US_2017_March15_Presentation_Efficacite_SF17_DRI_VE_PK_Rose_at_al_submi.pdf | 89306                                    | no | 2  |
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| 23           | Non Patent Literature | 20210212_AGI-PKR-16US_2017_March_15_Effiacite_dun_activateur_de_la_pyruvate_kinase.pdf | 4080802                                   | no | 32 |
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| 24           | Non Patent Literature | Stahl_Handbook_of_Pharmaceutical_Salts_212_2002.PDF                                    | 687804                                    | no | 6  |
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| 25           | Non Patent Literature | Iqvia_Database_Imsresearch_Accession_no_2014-8_2018.PDF                                | 40850                                     | no | 5  |
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| 26           | Non Patent Literature | 20210209_AGI-PKR-16US_2019_June13_Abstact_design_of_a_phase_2.pdf                      | 128983                                    | no | 1  |
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| 27           | Non Patent Literature | vanBeersetalBlood2019_134suppl1_A4791.PDF                                              | 186533                                    | no | 3  |
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| 28           | Non Patent Literature | EHA_SWG_11-7-2019.PDF                                                                  | 397262                                    | no | 2  |
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| 29           | Non Patent Literature | EHA_22_Poster_6-22-2017.PDF                                                            | 771573                                    | no | 1  |
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| 30                                                                                                                                                                                                         | Non Patent Literature | 20210209_AGI-<br>PKR-16US_2017_June22_Abstr<br>act_EHA_Ex_vivo_treatment_o<br>f_red_blood_cells.pdf        | 240381                                       | no | 2  |
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| 31                                                                                                                                                                                                         | Non Patent Literature | ASH_56_Poster_12-6-2014.PDF                                                                                | 184821                                       | no | 1  |
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| 34                                                                                                                                                                                                         | Non Patent Literature | 20210212_AGI-<br>PKR-16US_2015_June12_prese<br>ntation_Phase_1_multiple_asc<br>ending_dose_study_of_th.pdf | 1844551                                      | no | 19 |
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| 35                                                                                                                                                                                                         | Non Patent Literature | 20210203_AGI-<br>PKR-16US_2015_June11_EHAP<br>hase_1_multiple_ascending_d<br>ose_study_of_the_safety_t.pdf | 145625                                       | no | 2  |
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| 36                                                                                                                                                                                                         | Non Patent Literature | 20210209_AGI-<br>PKR-16US_2019_July25_Abstra<br>ct_Phase_1_single_and_multip<br>le.pdf                     | 149803                                       | no | 1  |
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| 37                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Non Patent Literature | Yang_IDDST_17_Presentation_7-26-2019.PDF            | 1484937                                   | no | 19 |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                       |                                                     | c0a42b2282849d2f55a4ce1782e8f4ad6dec690a  |    |    |
| Warnings:                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                       |                                                     |                                           |    |    |
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| 38                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Non Patent Literature | Yang_Clin_Pharmacol_Drug_Development_8_246_2018.PDF | 98994                                     | no | 2  |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                       |                                                     | 2017f1d139f51fa7022f596ccafb4f510f975cd82 |    |    |
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| 39                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | Non Patent Literature | PCTUS2018062197_ISR_WO_2-20-2019.PDF                | 2412759                                   | no | 24 |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                       |                                                     | 27ef62f347594bebd7755004b9a2be77d728dd0d  |    |    |
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| Total Files Size (in bytes):                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                       |                                                     | 39983947                                  |    |    |
| <p><b>This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.</b></p> <p><b><u>New Applications Under 35 U.S.C. 111</u></b><br/>If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.</p> <p><b><u>National Stage of an International Application under 35 U.S.C. 371</u></b><br/>If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.</p> <p><b><u>New International Application Filed with the USPTO as a Receiving Office</u></b><br/>If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.</p> |                       |                                                     |                                           |    |    |



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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 |             |                           | EXAMINER            |                  |
| 265 Franklin Street            |             |                           | KOSTURKO, GEORGE W  |                  |
| Boston, MA 02110               |             |                           |                     |                  |
|                                |             |                           | 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.<br>16/900,610 | Applicant(s)<br>Iyer et al. |                          |
|                       | Examiner<br>GEORGE W KOSTURKO | Art Unit<br>1628            | AIA (FITF) Status<br>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](http://www.uspto.gov/patents/init_events/pph/index.jsp) or send an inquiry to [PPHfeedback@uspto.gov](mailto: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 19<sup>th</sup>, 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](http://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](http://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

|                                   |                                       |  |                                                           |             |
|-----------------------------------|---------------------------------------|--|-----------------------------------------------------------|-------------|
| <i>Notice of References Cited</i> | Application/Control No.<br>16/900,610 |  | Applicant(s)/Patent Under<br>Reexamination<br>Iyer et al. |             |
|                                   | Examiner<br>GEORGE W KOSTURKO         |  | Art Unit<br>1628                                          | Page 1 of 1 |

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|                       | D |                                                  |                 |                        |                    |                   |
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|                          | N | WO-2012015712-A1                                 | 02-2012         | WO      | GHOSAL A  | A61K38/06          |
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|                          | Q |                                                  |                 |         |           |                    |
|                          | R |                                                  |                 |         |           |                    |
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| *                    |   | Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages) |  |  |  |  |
|                      | U |                                                                                           |  |  |  |  |
|                      | V |                                                                                           |  |  |  |  |
|                      | W |                                                                                           |  |  |  |  |
|                      | X |                                                                                           |  |  |  |  |

\*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)  
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

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: April 12, 2021  
Electronic Signature for Mei Bai, Ph.D.: /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: 1653

For: MITAPIVAT THERAPY AND MODULATORS  
OF CYTOCHROME P450

Examiner: Not Yet Assigned

INFORMATION DISCLOSURE STATEMENT (IDS)

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

Dear Sir:

Pursuant to 37 C.F.R. §§ 1.56, 1.97, and 1.98, the attention of the United States Patent and Trademark Office is hereby directed to the references listed on the attached PTO/SB/08. It is respectfully requested that the information be expressly considered during the prosecution of the above-identified application, and that the references be made of record therein and appear among the “References Cited” on any patent to issue therefrom.

This Information Disclosure Statement is filed before the mailing date of a first Office Action on the merits as far as is known to the undersigned (37 C.F.R. § 1.97(b)(3)).

In accordance with 37 C.F.R. § 1.98(a)(2)(ii), Applicant has not submitted copies of the U.S. patent and U.S. patent application. Applicant submits herewith copies of the foreign patent and non-patent literature in accordance with 37 C.F.R. § 1.98(a)(2).

In accordance with 37 C.F.R. § 1.97, Applicant hereby cites the following additional patent application directed to related subject matter:

ME1 36183356v.1

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /G.W.K/

Application No.: 16/900,610

Docket No.: 128748-01502

| U.S. Application | Filing Date  | Publication No. | Status    | Atty. Docket No. |
|------------------|--------------|-----------------|-----------|------------------|
| 16/765,456       | May 19, 2020 | 2020-0277279    | Published | 128748-00403     |

In accordance with 37 C.F.R. § 1.97(g), the filing of this Information Disclosure Statement shall not be construed as a representation that a search has been made. In accordance with 37 C.F.R. § 1.97(h), the filing of this Information Disclosure Statement shall not be construed to be an admission that the information cited in this Information Disclosure Statement is, or is considered to be, material to patentability as defined in 37 C.F.R. § 1.56(b).

It is submitted that the Information Disclosure Statement is in compliance with 37 C.F.R. § 1.98, and the Examiner is respectfully requested to consider the listed references.

Applicant believes no fee is due with this response.

Dated: April 12, 2021

Respectfully submitted,

Electronic signature: /Mei Bai, Ph.D./  
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Attorney/Agent For Applicant

/GEORGE W KOSTURKO/  
07/08/2022

ME1 36183356v.1

Receipt date: 04/12/2021

16/900,610 -- GAU: 1628

Used in Lieu of PTO/SB/08A/B  
(Based on PTO 10-07 version)

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|---------------------------------------------------------------------------------------------------------------------------------------------------|---|----|---|------------------------|---------------------------|
| <div>Substitute for form 1449/PTO</div> <div>INFORMATION DISCLOSURE<br/>STATEMENT BY APPLICANT</div> <div>(Use as many sheets as necessary)</div> |   |    |   | Complete if Known      |                           |
|                                                                                                                                                   |   |    |   | Application Number     | 16/900,610                |
|                                                                                                                                                   |   |    |   | Filing Date            | June 12, 2020             |
|                                                                                                                                                   |   |    |   | First Named Inventor   | Varsha Venkatachalam Iyer |
|                                                                                                                                                   |   |    |   | Art Unit               | 1653                      |
|                                                                                                                                                   |   |    |   | Examiner Name          | Not Yet Assigned          |
| Sheet                                                                                                                                             | 1 | of | 8 | Attorney Docket Number | 128748-01502              |

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| Examiner<br>Initials* | Cite<br>No. <sup>1</sup> | Document Number                           | Publication Date<br>MM-DD-YYYY | Name of Patentee or<br>Applicant of Cited Document | Pages, Columns, Lines, Where<br>Relevant Passages or Relevant<br>Figures Appear |
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|                          |                          | Country Code <sup>3</sup> -Number <sup>4</sup> -Kind Code <sup>5</sup> (if known) |                                   |                                                    |                                                                                 |                |
|                          | B1                       | WO-2016/201227                                                                    | 12-15-2016                        | Agios Pharmaceuticals, Inc                         |                                                                                 |                |

\*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. \* CITE NO.: Those application(s) which are marked with an single asterisk (\*) next to the Cite No. are not supplied (under 37 CFR 1.98(a)(2)(iii)) because that application was filed after June 30, 2003 or is available in the IFW. <sup>1</sup> Applicant's unique citation designation number (optional). <sup>2</sup> See Kinds Codes of USPTO Patent Documents at [www.uspto.gov](#) or MPEP 901.04. <sup>3</sup> Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). <sup>4</sup> For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. <sup>5</sup> Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST.16 if possible. <sup>6</sup> Applicant is to place a check mark here if English language Translation is attached.

|                       |                     |                    |            |
|-----------------------|---------------------|--------------------|------------|
| Examiner<br>Signature | /GEORGE W KOSTURKO/ | Date<br>Considered | 07/08/2022 |
|-----------------------|---------------------|--------------------|------------|

ME1 36183359v.1

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /G.W.K/

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|                                                                                                                                               |   |    |   | Application Number     | 16/900,610                |
|                                                                                                                                               |   |    |   | Filing Date            | June 12, 2020             |
|                                                                                                                                               |   |    |   | First Named Inventor   | Varsha Venkatachalam Iyer |
|                                                                                                                                               |   |    |   | Art Unit               | 1653                      |
|                                                                                                                                               |   |    |   | Examiner Name          | Not Yet Assigned          |
| Sheet                                                                                                                                         | 2 | of | 8 | Attorney Docket Number | 128748-01502              |

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| Examiner Initials               | Cite No. <sup>1</sup> | Include name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), volume-issue number(s), publisher, city and/or country where published.                                                                                                                                                          | T <sup>2</sup> |                 |            |
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| Examiner Signature              | /GEORGE W KOSTURKO/   |                                                                                                                                                                                                                                                                                                                                                                                                                          |                | Date Considered | 07/08/2022 |

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| <div>Substitute for form 1449/PTO</div> <div>INFORMATION DISCLOSURE<br/>STATEMENT BY APPLICANT</div> <div>(Use as many sheets as necessary)</div> |   |    |   | Complete if Known      |                           |
|                                                                                                                                                   |   |    |   | Application Number     | 16/900,610                |
|                                                                                                                                                   |   |    |   | Filing Date            | June 12, 2020             |
|                                                                                                                                                   |   |    |   | First Named Inventor   | Varsha Venkatachalam Iyer |
|                                                                                                                                                   |   |    |   | Art Unit               | 1653                      |
|                                                                                                                                                   |   |    |   | Examiner Name          | Not Yet Assigned          |
| Sheet                                                                                                                                             | 3 | of | 8 | Attorney Docket Number | 128748-01502              |

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|                    |                     |                 |            |
|--------------------|---------------------|-----------------|------------|
| Examiner Signature | /GEORGE W KOSTURKO/ | Date Considered | 07/08/2022 |
|--------------------|---------------------|-----------------|------------|

ME1 36183359v.1

ALL REFERENCES CONSIDERED EXCEPT WHERE LINED THROUGH. /G.W.K/

Receipt date: 04/12/2021

16/900,610 -- GAU: 1628

Used in Lieu of PTO/SB/08A/B  
(Based on PTO 10-07 version)

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|-----------------------------------------------------------------------------------------------------------------------------------------------|---|----|---|------------------------|---------------------------|
| <div>Substitute for form 1449/PTO</div> <div>INFORMATION DISCLOSURE STATEMENT BY APPLICANT</div> <div>(Use as many sheets as necessary)</div> |   |    |   | Complete if Known      |                           |
|                                                                                                                                               |   |    |   | Application Number     | 16/900,610                |
|                                                                                                                                               |   |    |   | Filing Date            | June 12, 2020             |
|                                                                                                                                               |   |    |   | First Named Inventor   | Varsha Venkatachalam Iyer |
|                                                                                                                                               |   |    |   | Art Unit               | 1653                      |
|                                                                                                                                               |   |    |   | Examiner Name          | Not Yet Assigned          |
| Sheet                                                                                                                                         | 4 | of | 8 | Attorney Docket Number | 128748-01502              |

|  |     |                                                                                                                                                                                                                                                                                                            |  |
|--|-----|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--|
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| Examiner Signature | /GEORGE W KOSTURKO/ | Date Considered | 07/08/2022 |
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| Registration Number: | 68901                        |

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| 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                                              |
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| Receipt Date:                        | 18-NOV-2022                                         |
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| 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: |                  |

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| 1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | Amendment/Request for Reconsideration-After Non-Final Rejection | Response_to_Non-Final_Office_Action.pdf | 52262                                     | no               | 9                |
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| Warnings:                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                                                                 |                                         |                                           |                  |                  |
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| Total Files Size (in bytes):                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                                                                 |                                         | 90657                                     |                  |                  |
| <p>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.</p> <p><b><u>New Applications Under 35 U.S.C. 111</u></b><br/>If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.</p> <p><b><u>National Stage of an International Application under 35 U.S.C. 371</u></b><br/>If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.</p> <p><b><u>New International Application Filed with the USPTO as a Receiving Office</u></b><br/>If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.</p> |                                                                 |                                         |                                           |                  |                  |

Docket No.: 128748-01502  
(PATENT)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

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

Application No.: 16/900,610

Confirmation No.: 1054

Filed: June 12, 2020

Art Unit: 1628

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For: MITAPIVAT THERAPY AND MODULATORS  
OF CYTOCHROME P450

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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 mitapivrit induces CYP3A4/5, which has been found to metabolize mitapivrit 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 mitapivrit. Applicant disagrees.

However, to facilitate prosecution, the pending claims are no longer directed to administering an inhibitor of CYP3A4/5 with mitapivrit, 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 mitapivrit 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 mitapivrit 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 of 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 associated symptoms. *See id at 10.* From this, the Examiner contends that it would have been obvious to administer dexamethasone with mitapivat at higher dosages of 700 mg to reduce nausea and emesis. *See id.* Applicant disagrees.

Reiterated from 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 mitapivat 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 mitapivat is metabolized by CYP3A4/5. The combination of mitapivat 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 mitapivat (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 Jannetta. 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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| 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                  |



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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 |             |                           | EXAMINER            |                  |
| 265 Franklin Street                        |             |                           | KOSTURKO, GEORGE W  |                  |
| Boston, MA 02110                           |             |                           | 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



**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 18<sup>th</sup> 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**

|                                   |                                       |  |                                                           |             |
|-----------------------------------|---------------------------------------|--|-----------------------------------------------------------|-------------|
| <i>Notice of References Cited</i> | Application/Control No.<br>16/900,610 |  | Applicant(s)/Patent Under<br>Reexamination<br>Iyer et al. |             |
|                                   | Examiner<br>GEORGE W KOSTURKO         |  | Art Unit<br>1628                                          | Page 1 of 1 |

| U.S. PATENT DOCUMENTS |   |                                                  |                 |      |                    |                   |
|-----------------------|---|--------------------------------------------------|-----------------|------|--------------------|-------------------|
| *                     |   | Document Number<br>Country Code-Number-Kind Code | Date<br>MM-YYYY | Name | CPC Classification | US Classification |
|                       | A |                                                  |                 |      |                    |                   |
|                       | B |                                                  |                 |      |                    |                   |
|                       | C |                                                  |                 |      |                    |                   |
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| FOREIGN PATENT DOCUMENTS |   |                                                  |                 |         |           |                    |
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| *                        |   | Document Number<br>Country Code-Number-Kind Code | Date<br>MM-YYYY | Country | Name      | CPC Classification |
|                          | N | WO-2016201227-A1                                 | 12-2016         | WO      | AGRESTA S | A61K31/496         |
|                          | O | WO-2019104134-A1                                 | 05-2019         | WO      | GUO L     | A61K31/496         |
|                          | P |                                                  |                 |         |           |                    |
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|                          | R |                                                  |                 |         |           |                    |
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|                          | T |                                                  |                 |         |           |                    |

| NON-PATENT DOCUMENTS |   |                                                                                                          |
|----------------------|---|----------------------------------------------------------------------------------------------------------|
| *                    |   | Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)                |
|                      | U | Kassa (BMC Hematology pages 1-11 published 2016) (Year: 2016)                                            |
|                      | V | Chen (Annals of Clinical Microbiology and Antimicrobials Vol. 5 pages 1-11, published 2006) (Year: 2006) |
|                      | W |                                                                                                          |
|                      | X |                                                                                                          |

\*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)  
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

RESEARCH ARTICLE

Open Access



# Effect of anti-tuberculosis drugs on hematological profiles of tuberculosis patients attending at University of Gondar Hospital, Northwest Ethiopia

Eyuel Kassa<sup>1</sup>, Bamlaku Enawgaw<sup>1</sup>, Aschalew Gelaw<sup>2</sup> and Baye Gelaw<sup>2\*</sup>

## Abstract

**Background:** Tuberculosis (TB) treatment may present significant hematological disorder and some anti-TB drugs also have serious side effects. Although many other diseases may be reflected by the blood and its constituents, the abnormalities of red cells, white cells, platelets, and clotting factors are considered to be primary hematologic disorder as a result of tuberculosis treatment. The aim of this study was to determine hematological profiles of TB patients before and after intensive phase treatment.

**Objective:** The aim of this study was to determine hematological profiles of TB patients before and after intensive phase treatment.

**Methods:** Smear positive new TB patients were recruited successively and socio-demographic characteristics were collected using pre-tested questionnaire. About 5 ml of venous blood was collected from each patient and the hematological profiles were determined using Mindry BC 3000 plus automated hematology analyzer.

**Result:** The hematological profiles of TB patients showed statistically significant difference in hematocrit (38.5 % versus 35.7 %), hemoglobin (12.7 g/l versus 11.8 g/l) and platelet ( $268 \times 10^3/\mu\text{l}$  versus  $239 \times 10^3/\mu\text{l}$ ) values of patients before initiation of treatment and after completion of the intensive phase of tuberculosis treatment, respectively ( $P < 0.05$ ). The red cell distribution width (RDW) of treatment naïve TB patients was by far lower ( $17.6 \pm 7.09$  %) than the corresponding RDW ( $31.9 \pm 5.19$  %) of intensive phase treatment completed patients. Among TB patients that had high platelet distribution width (PDW) ( $n = 11$ ) before initiation of TB treatment, 10 demonstrated lower PDW values after completion of the intensive phase. There was no significant difference on total white blood cell count among TB patients before and after completion of the 2 month treatment.

**Conclusion:** The levels of hemoglobin, hematocrit and platelet count of the TB patients were significantly lowered after completion of the intensive phase of TB treatment. Significant variation of the RDW and PDW were also observed among treatment naïve and treatment completed patients. Hematological abnormalities resulted from TB treatment should be assessed continuously throughout the course of tuberculosis therapy.

**Keywords:** Tuberculosis, Haematological profile, Intensive phase, Anti-TB drugs

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## Background

Tuberculosis (TB) is a contagious disease that can affect almost any tissue and organs of the human body but mainly cause infection of the lungs [1]. Tuberculosis is a major public health problem throughout the world. About a third of the world's population is estimated to be infected with tubercle bacilli and hence at risk of developing active TB disease. The burden of TB is highest in Africa, Asia, India and China together accounting for almost 40 % of the world's TB cases [2]. About 2.4 million new TB cases and 540,000 TB-related deaths occur in sub-Saharan Africa annually [3]. Ethiopia is among the countries most heavily affected by TB [4]. The annual TB incidence of Ethiopia is estimated to be 341/100,000. Tuberculosis mortality rate is 73/100,000 and the prevalence of all forms of TB is estimated to be 546/100,000 [5].

TB is a curable disease although drug resistance is one of the major challenges in the treatment, prevention and control of the disease. However, the use of anti-TB drugs is not free from side effects. Allergic reactions, fever, rash, vasculitis, nausea, vomiting, hepatotoxicity, hepatocellular inflammation, peripheral neuropathy and others are some of the common side effects associated with TB treatment [6]. In addition, a wide range of hematological abnormalities has been reported as a result of administration of anti-TB drugs. The major hematological disorder due to anti-TB drug is aplastic anemia. Aplastic anemia is a hypo-regenerative bone marrow disorder characterized by a reduction in the amount of haemopoietic bone marrow and pancytopenia. It has been reported that pancytopenia due to aplastic anemia of moderate severity was observed in a patient while receiving anti-tuberculosis therapy, probably caused by an idiosyncratic reaction to streptomycin. Although the total leukocyte and platelet counts could be depressed after cessations of drugs, physicians rarely suspect anti-tuberculosis induced hematological complications which may have fatal consequences [7].

Hematological disorders arise through a variety of mechanisms and etiologies. Drug-induced hematological disorders can span almost the entire spectrum of hematology, affecting red cells, white cells, platelets, and the coagulation system. The wide spectrum of drug-induced hematologic syndromes is mediated by a variety of mechanisms, including immune effects, interactions with enzymatic pathways, and direct inhibition of hematopoiesis. Drug-induced syndromes include hemolytic anemia, red cell aplasia, sideroblastic anemia, megaloblastic anemia, polycythemia, aplastic anemia, leukocytosis and others. There are four possible relationships of tuberculosis to hematologic disease. These are the hematologic disease predisposes to tuberculosis reactivation. Drugs may cause idiosyncratic reactions, malabsorption, interference with

iron metabolism, and hemolysis in patients with red blood cell enzyme deficiencies. Idiosyncratic reactions manifested by depression of any or all of the three cellular blood elements (white cells, red cells and platelets) together with the coagulation system may be caused by any of the anti-tuberculosis drugs [8, 9].

The magnitude of drug induced hematological abnormalities had been investigated in different parts of the world. For example, leucopenia as a result of rifampicin and isoniazid therapy was reported in Japan [10]. Anti-TB drug induced normocytic normochromic anemia was the most common abnormality observed in Malaysia [11] and a 74 % prevalence of anemia together with 26 % Leukocytosis, 24 % Thrombocytosis was reported in India [12]. In a study conducted in South Africa, a 15 % leucopenia, 23 % thrombocytopenia and 87 % lymphopenia was reported as a result of anti-TB drug treatment [13]. By another study conducted in Nigeria, anti-TB drug induced hematological abnormality were reported 93.6, 22.3, 45.2 and 4.8 % for anemia, leukocytosis, neutrophilia, and lymphopenia, respectively [14]. Moreover, in Tanzania an 86 % anti-TB drug induced anemia was reported [15].

Different reports showed that altered hematopoiesis occurs in TB patients. Hematological changes associated with tuberculosis treatments have been investigated in many parts of the world. However, to the best of our knowledge, there is no comprehensive study assessed the hematological abnormalities among TB patients in Ethiopia in general and in Gondar in particular. Hence, this study was designed to determine the effect of anti-TB drugs on hematological profile among TB patients. In the present study, hematological findings among TB patients before initiation and after completion of the intensive phase of tuberculosis treatment were assessed.

## Methods

### Study design, area and setting

A longitudinal prospective study was conducted from February to June 2014 at the University of Gondar (UoG) hospital, Northwest Ethiopia. The hospital is found in Gondar town. Gondar town is located in the North Gondar Zone of the Amhara Region, North of Lake Tana, Northwestern Ethiopia, and 748 km far from Addis Ababa. The town has a latitude and longitude of 12°36'N 37°28'E with an elevation of 2133 m above sea level. Based on figures from the Central Statistical Agency in 2008, Gondar has an estimated total population of 231,977. University of Gondar hospital is a referral hospital for Northwestern Ethiopia with more than 400 beds serving a population of about 5 million. The Hospital is one of the biggest tertiary level referral and teaching hospitals in the region. A large number of people from the surrounding zones and nearby regions

visit the hospital both for inpatient and as an outpatient treatment.

#### Populations

The source population was all TB suspected patients (both pulmonary and extra pulmonary) attending for health service at the University of Gondar Hospital. The study population were smear positive pulmonary tuberculosis patients and all confirmed extra-pulmonary TB patients who took all the intensive phase of treatment at the Directly Observed Treatment Short course (DOTS) clinic of University of Gondar hospital during the study period. In this study, TB patient less than 5 years of age and greater than 65 years of age, retreatment cases, patient with known organ impairment, malignant cases (cancer and chronic patients) and HIV patients were excluded from the study.

#### Sample size determination and sampling technique

Time delimited consecutive sampling technique was employed to recruit the study subjects. All newly diagnosed TB patients who were confirmed for tuberculosis infection and seeking for anti-TB treatment at the University of Gondar Teaching Hospital DOTS clinic during the study period were included. By using the inclusion and exclusion criteria's set for the study, a total of 168 new TB patients were recruited and involved in this study.

#### Socio-demographic data

Socio-demographic characteristics such as age, sex, marital status, residence and clinical information such as WHO clinical stage, use of myelo-suppressive drugs, loss of appetite, weight loss, night sweats and fever were gathered using pre-tested and structured questionnaire. A trained clinical nurse and laboratory technologist working at the DOTS clinic of the University of Gondar hospital collected the socio-demographic characteristics of the TB patients.

#### Sputum sample collection and microscopy

Sputum samples were collected using dry, clean, leak proof, translucent and screw-capped plastic containers with a capacity of 30 ml. Smears were prepared and air dried then stained with Ziehl-Neelsen (ZN) stain. Briefly, smears were gently heat fixed and each smear was flooded with carbolfuchsin solution. On each preparation, heat was applied until steaming and allowed to stand for 5 min. After washing with tap water, smear was decolorized with acid alcohol for 1 min and washed with tap water and counter stained with methylene blue for 30 s, washed and air dried. Slides were examined under 100× oil immersion objective with bright field illumination. Microscopy results were recorded following

the WHO reporting methods as negative, actual number of acid-fast bacilli (AFB), 1+, 2+ and 3+.

#### Blood sample collection and determination of hematological parameters

About 5 ml of venous blood was collected aseptically by using EDTA tube from each of the selected study subjects. Following collection, the EDTA tube was labeled with code number. The blood sample was collected from each study subject before initiation of anti-TB drugs and after completion of the 2 month intensive phase treatment. Hematologic profiles such as Red blood cell count, hemoglobin level, hematocrit (HCT), Red blood cell indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), Red cell distribution width (RDW), total white blood cell count (WBC), WBC differential count, Platelet count, Mean Platelet Volume (MPV) and platelet cell distribution width (PDW) were determined using Mindray automated hematology analyzer following the manufacturers instruction and the Standard Operational Procedures (SOP) of the University of Gondar hospital laboratory.

#### Quality control

The reliability of the study findings were guaranteed by implementing quality control (QC) measures throughout the whole process of the laboratory work. All materials, equipment and procedures were adequately controlled. Blood samples were collected free of hemolysis, clot, with sufficient quantity (5 ml) and correctly labeled with patients identification number. The performance of the hematology analyzer was checked by running normal, low and high blood controls. During a condition where flags were found with automation hematological parameters, manual differential count was performed. Sputum samples with an AFB score of 1+ ( $n = 10$ ) was used to assess the reliability of microscopic examinations. These samples were stained by Ziehl-Neelsen stain and examined by two laboratory technologists blinded from each other. The coefficient of variation (CV) was found 0.016 which shows that the result of the two laboratory technologists was 99.984 % consistent. The questionnaire was prepared in English, translated to local Amharic language and then translated back to English to check for consistency. Data on Socio-demographic and TB related medical history were collected by trained nurses under the supervision of the investigators.

#### Data analysis

Data were checked, sorted, categorized and coded manually then transferred to SPSS version 20 statistical packages for analysis. Frequencies and cross tabulations were used to summarize descriptive statistics. Paired *t* test

was used in the analysis to compare the hematologic values before initiation and after completion of the intensive phase of tuberculosis treatment. A *P* values less than 0.05 were considered statistically significant.

#### Ethical consideration

Ethical approval was obtained from an ethical review committee organized by the School of Biomedical and Laboratory Sciences which was mandated by the College of Medicine and health Sciences, University of Gondar. A permission and support letter was also obtained from University of Gondar hospital and both verbal and written consent was obtained from each participant. All the consent procedures were approved by the ethical approval committee of the School of Biomedical and Laboratory Sciences. In the case of children, both verbal and written consent were taken from the guardian that was also approved by the ethical review committee. The purpose and importance of the study was explained to each study participants. To ensure confidentiality of participants information, anonymous typing was applied where by the name of the participant and any identifier of participants were not written on the questionnaire, and during the interview to keep the privacy, they were interviewed alone. The laboratory findings of each study participant were communicated with the responsible clinician assigned at TB clinic. Above all the data was collected after full written consent was obtained from each study participants.

#### Results

##### Characteristics of the study participants

A total of 168 HIV negative new TB patients were included in this study. Ninety six (56.5 %) of the TB patients were males and the other 73 (43.5 %) were females. The mean age of the study participants was  $34.8 \pm 15.3$  years ranging from 5 to 65 years. Sixty-two (36.9 %) TB patient were within the age group 5–25, 62 (36.9 %) within the age group 26–44 and the other 44 (26.2 %) within 45–65 years of age. Forty-seven percent ( $n = 79$ ) were urban dwellers and the other 53 % ( $n = 89$ ) were living in rural areas. The clinical and laboratory data of the TB patients showed that 87 (51.8 %) and 81 (48.2 %) were pulmonary and extra pulmonary cases.

##### Association between hematological parameters with the age and sex of the TB patients before and after completion of the intensive phase treatment

Tuberculosis confirmed patients were investigated to assess their hematological profile prior initiation of anti-TB treatment and after completion of the intensive phase treatment. The hematological profiles assessed were red blood cell count, hemoglobin concentration, hematocrit, red cell indices, total white blood cell count,

white blood cell differential count, red blood cell distribution width, platelet distribution width, platelet count and mixed cells (eosinophile, monocyte and basophile) count. Analysis of the demographic characteristics of the TB patients such as age and sex versus hematological parameters demonstrated that there was significant difference on Hgb and Hct concentrations ( $P < 0.001$ ) before initiation and after completitions of the intensive phase treatment. In addition, a significant difference on RBC among patients with the age group 45–65 years was observed ( $P = 0.030$ ). The RDW-CV parameter of female patients and the PLT count of male patients were also significantly different ( $P = 0.002$  and  $0.031$  respectively) (Table 1). However, there was no significant difference in WBC parameters and PDW versus the age and sex of the TB patients.

##### Assessment of anemia

The magnitude of anemia among TB patients before initiation and after completion of the intensive phase of treatment was assessed using red blood cell count (RBC), hemoglobin (Hgb) and hematocrit (Hct) values. The average RBC of the TB patients before initiation of anti-TB treatment was relatively similar to that of RBC after completion of the intensive phase of tuberculosis treatment ( $4.25 \times 10^3 \pm 0.83$  versus  $4.42 \times 10^3 \pm 0.824$ , respectively) (Table 2). Many of the TB patients that had normal RBC before initiation of tuberculosis treatment ( $n = 72$ ) demonstrated also normal RBC after completion of the intensive phase ( $n = 69$ ) of treatment. However, 54 % of the TB patients had lower RBC before initiation and after completion of the intensive phase of treatment. Nevertheless, data showed no statistically significant difference on the RBC of TB patients before initiation and after completion of the intensive phase of tuberculosis treatment. The average Hgb concentration of the TB patients before initiation of tuberculosis treatment was slightly higher ( $12.7 \pm 2.09$  g/dl) than the corresponding Hgb concentration determined after completion of the intensive phase of treatment ( $11.8 \pm 1.68$  g/dl). Similarly, the average packed cell volume (Hct) was also relatively higher before initiation of treatment ( $38.5 \pm 2.42$  %) than the corresponding concentration after completion of the 2 month treatment ( $35.7 \pm 2.31$  %). In this study, 55 % of the TB patients ( $n = 92$ ) had low Hgb concentration and the Hct values of 86 TB patients (51 %) was found low before initiation of anti-tuberculosis treatment. The proportion of TB patients with low Hgb and Hct concentration was by far increased (72 %) after completion of the intensive phase of TB treatment. Among the TB patients that had higher Hct concentration ( $n = 13$ ) before initiation of anti-TB treatment, only 4 resulted similarly higher concentration. On the other hand, among 69 and 76 patients that had normal Hct and Hgb concentration

**Table 1** Hematological parameters that demonstrated significant difference based on the age and sex of TB patients before initiation of anti-TB drugs compared with after completion of the intensive phase of treatment

|                            |     |         | Before treatment | After treatment | P-value |
|----------------------------|-----|---------|------------------|-----------------|---------|
| RBC × 10 <sup>6</sup> / μl | Sex | Male    | 4.27 ± 0.92      | 4.43 ± 0.92     | 0.235   |
|                            |     | Female  | 4.24 ± 0.72      | 4.42 ± 0.68     | 0.143   |
|                            | Age | 5 – 25  | 4.26 ± 0.77      | 4.42 ± 0.89     | 0.320   |
|                            |     | 26 – 44 | 4.37 ± 0.75      | 4.33 ± 0.68     | 0.747   |
|                            |     | 45 – 65 | 4.09 ± 1.01      | 4.58 ± 0.92     | 0.030*  |
| Hgb (g/dl)                 | Sex | Male    | 12.7 ± 2.1       | 11.8 ± 1.5      | 0.000*  |
|                            |     | Female  | 12.8 ± 2.1       | 11.8 ± 1.9      | 0.000*  |
|                            | Age | 5 – 25  | 12.9 ± 2         | 11.8 ± 1.8      | 0.000*  |
|                            |     | 26 – 44 | 12.9 ± 1.8       | 12 ± 1.4        | 0.001*  |
|                            |     | 45 – 65 | 12.4 ± 2.5       | 11.5 ± 1.8      | 0.000*  |
| HCT (%)                    | Sex | Male    | 38.6 ± 6         | 35.8 ± 4.4      | 0.000*  |
|                            |     | Female  | 38.9 ± 5.5       | 35.7 ± 5.4      | 0.000*  |
|                            | Age | 5 – 25  | 39.1 ± 5.7       | 35.7 ± 5.5      | 0.000*  |
|                            |     | 26 – 44 | 38.9 ± 4.9       | 36.6 ± 4        | 0.001*  |
|                            |     | 45 – 65 | 37.9 ± 6.8       | 34.7 ± 5        | 0.000*  |
| MCV (fl)                   | Sex | Male    | 88.4 ± 6.9       | 89.4 ± 9.1      | 0.036*  |
|                            |     | Female  | 89.4 ± 6.7       | 90.9 ± 5.1      | 0.143   |
|                            | Age | 5 – 25  | 89.4 ± 7.3       | 90.2 ± 5.1      | 0.486   |
|                            |     | 26 – 44 | 88.3 ± 5.8       | 91.2 ± 5.8      | 0.013*  |
|                            |     | 45 – 65 | 88.7 ± 7.6       | 90.4 ± 5.2      | 0.241   |
| MCH (pg)                   | Sex | Male    | 28.9 ± 3.3       | 29.6 ± 2.3      | 0.143   |
|                            |     | Female  | 29.2 ± 2.6       | 30.1 ± 2        | 0.040*  |
|                            | Age | 5 – 25  | 29.3 ± 3         | 29.7 ± 2.1      | 0.427   |
|                            |     | 26 – 44 | 29.1 ± 3.1       | 29.7 ± 2.5      | 0.253   |
|                            |     | 45 – 65 | 28.5 ± 3         | 30 ± 2          | 0.012*  |
| MCHC (%)                   | Sex | Male    | 32.7 ± 2         | 32.7 ± 1.3      | 0.878   |
|                            |     | Female  | 32.6 ± 1.4       | 33.1 ± 1.1      | 0.049*  |
|                            | Age | 5 – 25  | 32.6 ± 1.6       | 32.9 ± 1.3      | 0.357   |
|                            |     | 26 – 44 | 33 ± 1.9         | 32.7 ± 1.3      | 0.390   |
|                            |     | 45 – 65 | 32.2 ± 1.6       | 33.1 ± 1        | 0.003*  |
| RDW CV (%)                 | Sex | Male    | 14.4 ± 1.4       | 14.2 ± 1.2      | 0.311   |
|                            |     | Female  | 14.5 ± 1.7       | 13.9 ± 1.2      | 0.02*   |
|                            | Age | 5 – 25  | 14.4 ± 1.4       | 14.1 ± 1.3      | 0.143   |
|                            |     | 26 – 44 | 14.3 ± 1.4       | 14.1 ± 1.2      | 0.477   |
|                            |     | 45 – 65 | 14.6 ± 1.8       | 14 ± 1.1        | 0.059   |
| PLT × 10 <sup>3</sup> /μl  | Sex | Male    | 267 ± 106        | 232 ± 109       | 0.031*  |
|                            |     | Female  | 270 ± 101        | 250 ± 86        | 0.169   |
|                            | Age | 5 – 25  | 258 ± 105        | 250 ± 116       | 0.697   |
|                            |     | 26 – 44 | 274 ± 101        | 246 ± 72        | 0.085   |
|                            |     | 45 – 65 | 275 ± 105        | 216 ± 108       | 0.006*  |

\* = significant association

**Table 2** Hematological profiles of tuberculosis patients before and after initiation of anti-TB treatment at University of Gondar Teaching hospital, 2014

| Variable     | Before treatmentof TB Mean (SD) | After treatmentof TB Mean (SD) | P-Value |
|--------------|---------------------------------|--------------------------------|---------|
| RBC/ $\mu$ l | $4.25 \times 10^6$ (0.83)       | $4.42 \times 10^6$ (0.824)     | 0.072   |
| WBC/ $\mu$ l | $7.5 \times 10^3$ (3.76)        | $7.08 \times 10^3$ (3.27)      | 0.224   |
| Hgb (g/dl)   | 12.7 (2.09)                     | 11.8 (1.68)                    | 0.000*  |
| HCT (%)      | 38.5 (2.42)                     | 35.7 (2.31)                    | 0.000*  |
| MCV (fl)     | 88.8 (6.8)                      | 89.7 (8.4)                     | 0.268   |
| MCH (Pg)     | 30.13 (5.0)                     | 29.03 (3.0)                    | 0.018*  |
| MCHC (g/dl)  | 30.13 (2.91)                    | 29.43 (7.1)                    | 0.000*  |
| RDW-CV       | 17.6 (7.09)                     | 31.9 (5.19)                    | 0.000*  |
| PLT/ $\mu$ l | $268 \times 10^3$ (103.2)       | 239.93 (99.8)                  | 0.01*   |
| PDW          | 17.01 (4.79)                    | 15.7 (0.61)                    | 0.001*  |
| Lymphocyte   | $1.96 \times 10^3$ (0.94)       | $2.08 \times 10^3$ (1.36)      | 0.375   |
| Mixed cell   | $0.74 \times 10^3$ (0.422)      | $0.69 \times 10^3$ (0.425)     | 0.272   |
| Neutrophil   | $4.37 \times 10^3$ (2.42)       | $4.08 \times 10^3$ (2.31)      | 0.230   |

RBC red blood cell, WBC White blood cell, HCT Haematocrit, HGB Haemoglobin, MCV mean corpuscular volume, MCH mean haemoglobin, MCHC mean haemoglobin concentration, RDW-CV red cell distribution width, PLT platelet, PDW Platelet distribution width, fl femto litter ( $10^{-15}$ L), pg pico-gram ( $10^{-12}$ g), \* significant association

before initiation of anti-TB treatment, only 42 and 47 demonstrated normal concentrations respectively after completion of the intensive phase treatment (Table 3). Moreover, data showed a significant decreased level of Hgb concentration and Hct value after completion of the 2 month tuberculosis treatment compared to the pre-treatment level ( $P = 0.00$ ).

Leukocytosis versus leukopenia

The average total WBC of TB patients before treatment ( $7.5 \times 10^3 \pm 3.76$ ) was slightly lower than the corresponding average WBC after completion of the 2 month treatment ( $7.08 \times 10^3 \pm 3.27$ ). The total WBC of 24 TB patients (14.3 %) enumerated before initiation of TB treatment was low but that of 115 (68.5 %) and 29 (17.3) patients were normal and high, respectively. The proportion of tuberculosis patients with low WBC was slightly raised 18.5 % ( $n = 31$ ) when examined after completion of the intensive phase of treatment. However, there was no statistically significant difference on total WBC among TB patients before initiation and after completion of the 2 month tuberculosis treatment. The lymphocyte, neutrophil and mixed cell (eosinophil, monocyte and basophil) differential WBC count of the TB patients showed no statistically significant difference among TB patients before and after initiation of tuberculosis treatment. However, the proportion of TB patients with high neutrophil count was raised from 7.7 % ( $n = 13$ ) of treatment naïve phase to 14.3 % ( $n = 24$ ) after completion of the 2 month tuberculosis treatment.

Association of tuberculosis treatment with platelet count

The mean thrombocyte count among TB patients before administration of anti-TB drugs was  $268 \times 10^3 \pm 103.2$ . The corresponding platelet counts among TB patients after completion of the 2 month treatment was slightly lower ( $239 \times 10^3 \pm 99.8$ ). However, data showed no statistically significant difference on platelet count among TB patients before initiation and after completion of the intensive phase tuberculosis treatment. The proportion of TB patients with low platelet count was slightly increased after completion of tuberculosis treatment ( $n = 25$ ; 14.9 %) compared to the corresponding platelet count among tuberculosis treatment naïve patients ( $n = 22$ ; 13.1 %). On the other hand, significantly reduced proportion of TB patients with high platelet count was observed (8.9 %;  $n = 15$ ) after completion of the intensive phase of TB treatment compared to the proportion of TB patients with high platelet count (19.6 %;  $n = 33$ ) among treatment naïve TB patients. Moreover, data showed significant difference in the proportion of TB patients with both low and high platelet count after completion of the 2 month treatment compared with treatment naïve patients ( $P = 0.01$ ).

Red blood cell indices during tuberculosis treatment

The average MCV of the TB patients before initiation of tuberculosis treatment and after completion of the 2 month treatment was nearly similar ( $88.8 \pm 6.8$  versus  $89.7 \pm 8.4$ , respectively). A slightly reduced MCH ( $29.03 \pm 3.0$ ) was observed after completion of the intensive phase tuberculosis treatment compared with the average MCH before initiation of treatment ( $30.13 \pm 5.0$ ).

**Table 3** The proportion of TB patients with low, normal and high hematological profile before initiation and after completion of the intensive phase tuberculosis treatment at University of Gondar hospital, 2014

| Parameters | Category | Before TB treatment | After TB treatment | Reference Intervals                                                            | P-Value |
|------------|----------|---------------------|--------------------|--------------------------------------------------------------------------------|---------|
| RBC        | Low      | 91 (54.2)           | 92 (54.8)          | M = $4.5-6.2 \times 10^6/\mu\text{l}$<br>F = $4.0-5.5 \times 10^6/\mu\text{l}$ | 0.92    |
|            | Normal   | 72 (42.9)           | 69 (41.1)          |                                                                                |         |
|            | High     | 5 (3)               | 7 (4.2)            |                                                                                |         |
| WBC        | Low      | 24 (14.3)           | 31 (18.5)          | 4.0 – $10.0 \times 10^3/\mu\text{l}$                                           | 0.262   |
|            | Normal   | 115 (68.5)          | 113 (67.3)         |                                                                                |         |
|            | High     | 29 (17.3)           | 24 (14.3)          |                                                                                |         |
| HCT        | Low      | 86 (51.2)           | 122 (72.6)         | M = 40 -52 %<br>F =36 – 47 %                                                   | 0.000*  |
|            | Normal   | 69 (41.1)           | 42 (25.0)          |                                                                                |         |
|            | High     | 13 (7.7)            | 4 (2.4)            |                                                                                |         |
| HGB        | Low      | 92 (54.8)           | 121 (72)           | M = 13 – 18 g/dl<br>F = 12–16 g/dl                                             | 0.000*  |
|            | Normal   | 76 (45.2)           | 47 (28)            |                                                                                |         |
|            | High     | 0 (0)               | 0 (0)              |                                                                                |         |
| MCV        | Low      | 13 (7.7)            | 10 (6)             | 80 – 100 fl                                                                    | 0.589   |
|            | normal   | 129 (76.8)          | 130 (77.4)         |                                                                                |         |
|            | high     | 26 (15.5)           | 28 (16.7)          |                                                                                |         |
| MCH        | low      | 37 (22)             | 18 (10.7)          | 27 – 32 pg                                                                     | 0.000*  |
|            | Normal   | 96 (57.1)           | 105 (62.5)         |                                                                                |         |
|            | High     | 35 (20.8)           | 45 (26.8)          |                                                                                |         |
| MCHC       | low      | 75 (44.6)           | 163 (98.2)         | 31.5 – 36 g/dl                                                                 | 0.000*  |
|            | normal   | 90 (53.6)           | 3 (1.8)            |                                                                                |         |
|            | High     | 118 (70.2)          | 9 (5.4)            |                                                                                |         |
| RDW_CV     | Normal   | 50 (29.8)           | 159 (94.6)         | 11.5 - 14.5 %                                                                  | 0.000*  |
|            | high     | 22 (13.1)           | 25 (14.9)          |                                                                                |         |
|            | normal   | 113 (67.3)          | 128 (76.2)         |                                                                                |         |
| PLT        | High     | 33 (19.6)           | 15 (8.9)           | 150 - $450 \times 10^3/\mu\text{l}$                                            | 0.01*   |
|            | Normal   | 157 (93.5)          | 167 (99.4)         |                                                                                |         |
|            | high     | 11 (6.5)            | 1 (0.6)            |                                                                                |         |
| PDW        | low      | 4 (2.4)             | 6 (3.6)            | 10 -17 %                                                                       | 0.000*  |
|            | Normal   | 160 (95.2)          | 156 (92.9)         |                                                                                |         |
|            | high     | 4 (2.4)             | 6 (3.6)            |                                                                                |         |
| Lymphocyte | Normal   | 160 (95.2)          | 165 (98.2)         | 1.5 - $4.5 \times 10^3/\mu\text{l}$                                            | 1.000   |
|            | high     | 4 (2.4)             | 3 (1.8)            |                                                                                |         |
|            | Normal   | 160 (95.2)          | 165 (98.2)         |                                                                                |         |
| Mixed cell | high     | 8 (4.8)             | 124 (73.8)         | 0.26 - $1.3 \times 10^3/\mu\text{l}$                                           | 0.960   |
|            | Low      | 13 (7.7)            | 20 (11.9)          |                                                                                |         |
|            | Normal   | 136 (81)            | 124 (73.8)         |                                                                                |         |
| Neutrophil | High     | 19 (11.3)           | 20 (11.9)          | 2.0 – $7.0 \times 10^3/\mu\text{l}$                                            | 0.233   |
|            | Normal   | 136 (81)            | 124 (73.8)         |                                                                                |         |
|            | High     | 19 (11.3)           | 20 (11.9)          |                                                                                |         |

\*= significant association. Hematological parameter values less than and greater than the reference intervals are categorized as low and high respectively

Similarly, the MCHC was also slightly reduced after completion of the 2 month treatment compared with before initiation of treatment ( $29.43 \pm 7.1$  versus  $30.13 \pm 2.91$ , respectively). The difference in MCH and MCHC among TB treatment naïve and TB patients completed

the intensive phase was found statistically significant ( $P \leq 0.018$ ). In this study, 7.7 % ( $n = 13$ ) of the TB patients had low MCV before initiation of anti-TB treatment. The proportion of TB patients with low MCV was slightly reduced (6 %;  $n = 10$ ) after completion of

the intensive phase treatment. The majority of the TB patients (57.1 %;  $n = 96$ ) had normal MCH before initiation of treatment and the proportion of TB patients with normal MCH risen to 108 (62.5 %) after completion of the intensive phase treatment. Forty-five percent ( $n = 75$ ) of the TB patients had low MCHC before initiation of treatment. The proportion of TB patients that had low MCHC was significantly raised (98.2 %;  $n = 163$ ) after completion of the intensive phase treatment. The proportion of TB patients with normal MCHC before initiation of TB treatment was 53.6 % ( $n = 90$ ). However, only 1.8 % ( $n = 3$ ) of the TB patients that had normal MCHC before initiation of tuberculosis treatment had also normal MCHC after completion of the intensive phase treatment.

#### Red blood cell and Platelet distribution width during tuberculosis treatment

The mean red blood cell distribution width (RDW) among treatment naïve TB patients ( $17.6 \pm 7.09$  %) was by far lower than the corresponding RDW ( $31.9 \pm 5.19$  fl) of TB patients determined after completion of the intensive phase of treatment. This difference was also statistically significant ( $P = 0.00$ ). Contrary to RDW, the mean platelet distribution width (PDW) among treatment naïve TB patients ( $17.01 \pm 4.79$  %) was higher than that of TB patients completed the intensive phase of treatment ( $15.7 \pm 0.61$  %). Moreover, data also showed statistically significant difference on PDW among treatment naïve TB patients compared with TB patients completed the intensive phase of treatment ( $P = 0.00$ ) (Table 2).

In this study, 70.2 % ( $n = 118$ ) of TB patients had normal RDW before initiation of anti-TB treatment. However, the proportion of TB patients that had normal RDW was by far reduced to 5.4 % ( $n = 9$ ) after completion of the intensive phase treatment. On the other hand, the proportion of TB patients with high RDW which was 29.8 % ( $n = 50$ ) was raised to 94.6 % ( $n = 159$ ) after completion of the intensive phase of tuberculosis treatment. Moreover, data showed statistically significant difference on the proportion of TB patients with high/low RDW before initiation and after completion of the intensive phase of tuberculosis treatment ( $P = 0.00$ ) (Table 3).

The proportion of TB patients that had normal PDW (93.5 %;  $n = 157$ ) before initiation of treatment was relatively similar to the corresponding proportion of TB patients that had normal PDW (99.4 %;  $n = 167$ ) after completion of the 2 month treatment. Eleven TB patients (6.5 %) had high PDW before initiation of tuberculosis treatment but only 1 patient showed high PDW after completion of the intensive phase of treatment and the difference was statistically significant ( $P = 0.00$ ).

#### Discussion

Tuberculosis exerts incredible varieties of hematologic effects and hematologic abnormality is a common finding among TB patients. These abnormalities involves both cell lines and plasma components. Anti-tuberculosis therapy has its own spectrum of hematologic toxicity and blood cell abnormalities [16]. The current study compared the hematologic profile of HIV negative TB patients before initiation of treatment and after completion of the intensive phase of tuberculosis treatment.

In the current study, the average RBC of the TB patients before initiation of anti-TB treatment was relatively similar to that of RBC after completion of the intensive phase of tuberculosis treatment. The average RBC of the TB patients were  $4.25 \times 10^6 \pm 0.83$  versus  $4.42 \times 10^6 \pm 0.824$  before treatment and after completion of the intensive phase treatment, respectively, which is nearly similar to the lower limits of the reference values for male and female adults (4.7 and 4.2 million cells per microliter (cells/ $\mu$ L), respectively). Previously Tsegay et al. [17] reported reference range of erythrocyte counts,  $5.1 \times 10^{12}/l$  (males) and  $4.5 \times 10^{12}/l$  (females) for healthy adult Ethiopians.

The hemoglobin concentration and packed cell volume were slightly higher among treatment naïve TB patients compared with those received anti-TB treatment for 2 months. However, the proportion of TB patients with low hemoglobin and hematocrit concentration was significantly increased (72 %) after completion of the intensive phase of TB treatment. All chronic infections including TB can cause anemia [18]. Various pathogenesis have been suggested in TB-associated anemia, but most studies have been shown suppression of erythropoiesis by inflammatory mediators as a cause of anemia [19]. Nutritional deficiency and malabsorption syndrome can deepen the severity of anemia [20]. The possible mechanisms for the development of anemia during tuberculosis infection may be due to nutritional insufficiency, impaired iron utilization, mala-absorption, bone marrow granuloma and shortened duration of RBC survival [21]. Weiss [18], Means [22] and Nemeth et al. [23] explain the mechanism behind the occurrence of anemia in pulmonary tuberculosis patients saying that the invasion of bacteria leads to activation of T-lymphocyte and macrophages, which induce the production of the cytokines like interferon gamma (IFN- $\gamma$ ), tumor necrosis factor alpha (TNF- $\alpha$ ), Interlukin-1 (IL-1) and interleukin-6 (IL-6) which with their products will cause diversion of iron into iron stores in the reticulo-endothelial system resulting in decreased iron concentrations in the plasma thus limiting its availability to red cells for hemoglobin synthesis, inhibition of erythroid progenitor cell proliferation and in appropriate production and activity of erythropoietin which may lead to anemia and suboptimal

response of the bone marrow to anemia, respectively. The high proportion of TB patients with reduced hemoglobin and hematocrit concentration after completion of the intensive phase of tuberculosis treatment may suggest drug induced anemia among TB patients. Drugs can induce variety of hematological disorders affecting RBCs, WBCs and platelets. Drug induced syndromes includes hemolytic anemia, methemoglobinemia, red cell aplasia, sideroblastic anemia, megaloblastic anemia, polycythemia and aplastic anemia [8].

The total WBC count of TB patients before initiation of anti-TB drugs was relatively similar to that of total WBC count after completion of the intensive phase of treatment. However, the proportion of TB patients with low total WBC count was raised from 14.3 % before initiation of treatment to 18.5 % after completion of the intensive phase treatment. On the other hand, the proportion of TB Patients with high neutrophil differential count was increased from 7.7 % before treatment to 14.3 % after completion of the intensive phase of treatment. Nevertheless, there was no leukocytosis neither before initiation of treatment nor after completion of the 2 month treatment. In a study designed to assess the hematological abnormalities of TB patients, Singh et al. [24] reported a 25 % leukopenia and a 22 % neutropenia among TB patients. Drug induced neutropenia was also reported in association with various analgesics, psychotropic's, anti-convulsions, anti-thyroid drugs, anti-histaminics, anti-rheumatics, gastro-intestinal drugs, anti-microbials and cardiovascular drugs [25].

The result of the current study showed that platelet count was another important hematological profile that showed significant difference when the count before initiation of tuberculosis treatment was compared to that of platelet count after completion of the 2 month tuberculosis treatment ( $P = 0.010$ ). Almost half of the TB patients that had high platelet count before treatment showed decreased platelet count after completion of the intensive phase. This finding is supported by a report from India and was suggested that decrease in platelet count could possibly be due to the effect of anti-TB drugs and immune destruction of platelets [26]. Classical causes of drug-induced thrombocytopenia are the quinine and quinine-like drugs [27]. The thrombocytopenia induced by these drugs is caused by antibody that is non-reactive in the absence of drug, but binds to epitopes on platelet membrane, glycoproteins IIb/IIIa or Ib/IX, when the sensitizing drug is present. Vancomycin can also be associated with marked thrombocytopenia and demonstrable drug-dependent antibodies in the serum [28]. Other drugs associated with thrombocytopenia include antimicrobials (sulfanomides, rifampin, linezolid), anti-inflammatory drugs, anti-neoplastics, anti-depressants, benzodiazepines, anti-convulsants (carbamazepine, phenytoin,

valproic acid) as well as cardiac and anti-hypertensive drugs [29, 30].

In the current study, the MCV values before and after treatments were not significantly changed. However, the differences in the MCH and MCHC values were significantly different when the values were compared before and after completion of the intensive phase of TB treatment. Previous reports showed that the red cell indices in the untreated male pulmonary TB patients showed lower values as compared to normal males. In addition, it was also found that the MCH and MCHC among male pulmonary TB patients were significantly lower when compared with normal males [31, 32]. Different study reports showed that after anti-tuberculosis treatment with streptomycin, rifampicin and isoniazid, the RBC indices were affected and reached closer to normal values. Moreover, RBC morphology in pulmonary TB patients was found to be mainly normocytic normochromic type and during medication, the blood film showed normochromic pictures [31, 33, 34] which suggests the effects of tuberculosis treatment on the restoration of RBC morphology.

The mean RDW of TB patients determined after completion of the intensive phase of treatment ( $31.9 \pm 5.19$  %) was significantly different from the corresponding RDW of treatment naïve TB patients ( $17.6 \pm 7.09$  %). Opposite to the RDW, the PDW among treatment naïve TB patients ( $17.01 \pm 4.79$  %) was higher than the PDW of TB patients measured after completing the intensive phase of treatment ( $15.7 \pm 0.61$  %). Moreover, TB patients that had high PDW before treatment ( $n = 10$ ) showed reduced PDW after completion of the 2 month anti-TB treatment. Red cell distribution width and PDW measure the variation of RBC and platelet volume. The normal reference range of RDW-CV in adult humans was reported 11.5 to 14.5 % [35] while that of PDW 9–16.56 % for men and 8–13.3 % for women [36]. Nowadays, RDW is used to diagnose anemia. Domingo et al. [37] reported greater decrease in hemoglobin concentration in patients with higher values for RDW. Red cell distribution width is often used to differentiate anemia of mixed causes from anemia of a single cause. There are reports that documented deficiency of vitamin B12, or folate produce macrocytic anemia (large cell anemia) in which the RDW is elevated in roughly two-thirds of all cases [38]. However, varied size distribution of RBC is the hallmark of iron deficiency anemia, and as such shows increased RDW in virtually all cases [39]. Long term exposure to anti-tuberculosis medication increases the risk of adverse drug reactions and toxicity. Isoniazid and rifampicin may directly cause hemolytic anemia, as can pyrazinamide cause sideroblastic anemia [40]. Others have suggested that anemia is seen as part of the clinical manifestation of tuberculosis and as a consequence of a

chronic disease. In general, tuberculosis patients have a higher predisposition to develop gastro-intestinal absorption problems, consequently leading to anemia [20] and elevated RDW is associated with anemia. At a condition when there is microcytic RBCs produced prior to treatment and normocytic RBCs produced after completion of treatment in the circulation leads to high size variation (increase RDW value). Lee et al. [20] showed that anemia is a common hematological abnormality in patients and Baynes et al. [19] states that RDW values in chronic inflammatory disorder like tuberculosis similar with the RDW values occurring in iron-deficiency anemia which is in line with the current study.

Platelet distribution width directly measures the variability in platelet size and has been used to differentiate disorders of platelets such as essential thrombocythemia from reactive thrombocytosis [41]. In the present study, PDW of TB patients was significantly reduced after taking anti-TB drugs for 2 months. Previously, Tozkoparan et al. [42] reported that there were significantly higher PDW values ( $40 \pm 23.5$ ) among TB patients which decreased significantly with anti-tuberculosis therapy. Thrombocytopenia is a serious side effect that potentially caused by anti-TB drugs which occurs mostly due to rifampicin (RIF) [43]. The main mechanisms of thrombocytopenia are decreased production or increased destruction of platelets. The drug binds non-covalently to membrane glycol-proteins to produce compound epitopes or induce conformational changes which antibodies are specific. In addition, RIF-dependant antibodies attach to thrombocytes and cause increased destruction [44]. However, the exact mechanism of INH-induced thrombocythemia is not known.

The result of this study showed that the Hgb concentration, HCT, PLT and PDW values were reduced after completion of the intensive phase of tuberculosis treatment. This is due to the fact that iron is critical for *Mycobacterium tuberculosis* growth in macrophages in turn causes iron deficiency anemia. After the intensive phase treatment, there will be sufficient iron in the body and starts production of normal erythrocytes. This study finding is supported by Tozkoparan et al. [42] that indicates significantly lower PDW values with anti-tuberculosis therapy. Also another study by Sahin et al. [45] indicates that reactive thrombocytosis and PDW develop frequently in PTB and there is a relation with acute phase reactants, which is the inflammatory response and decreased after treatment.

## Conclusion

The levels of hemoglobin, hematocrit and platelet count of the TB patients were significantly lowered after completion of the intensive phase of TB treatment compared with the corresponding values before initiation of anti-

TB treatment. The RDW and PDW also showed significant variation before initiation and after completion of the 2 month tuberculosis treatment. The varied hematological abnormalities observed in TB patients after intensive phase tuberculosis therapy suggests the need for continuous monitoring and evaluation of TB patients for adverse hematological abnormalities during tuberculosis treatment. Anemia was found to be one of the most common hematological abnormalities among patients taking anti-tuberculosis treatment. Thus anemia and thrombocytopenia should be regularly checked during tuberculosis treatment.

## Limitation of the study

The limitation of this study was the relatively small sample size which is the result of the nature of the study as longitudinal study are time consuming and costly. We were also unable to determine the effect of anti-TB drugs after completion of the 6 month treatment.

## Competing interest

The authors declare that they have no competing interests.

## Author's contribution

This work was accomplished in collaboration between all authors. Author EK designed the study, wrote the proposal and collected laboratory data. Author BG commented the protocol, analyzed the data and prepared the manuscript for publication. Author BE managed the literature search and involved in data analysis. Author AG participated in data analysis, literature search and laboratory investigation. All authors read and approved the final manuscript.

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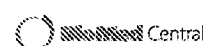
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Review

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## Roles of rifampicin in drug-drug interactions: underlying molecular mechanisms involving the nuclear pregnane X receptor

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### Abstract

Rifampicin, an important drug in the treatment of tuberculosis, is used extensively despite its broad effects on drug-drug interactions, creating serious problems. The clinical importance of such interactions includes autoinduction leading to suboptimal or failed treatment. The concomitantly administered effects of rifampicin on other drugs can result in their altered metabolism or transportation that are metabolised by cytochromes P450 or transported by p-glycoprotein in the gastrointestinal tract and liver. This review paper summarises recent findings with emphases on the molecular mechanisms used to explain these broad drug-drug interactions. In general, rifampicin can act on a pattern: rifampicin activates the nuclear pregnane X receptor that in turn affects cytochromes P450, glucuronosyltransferases and p-glycoprotein activities. This pattern of action may explain many of the rifampicin inducing drug-drug interactions. However, effects through other mechanisms have also been reported and these make any explanation of such drug-drug interactions more complex.

### Introduction

Multiple drug therapy is often used with a single patient. For example, in individuals infected with HIV and tuberculosis, both anti-tuberculosis and antiretroviral agents have to be used [1]. During treatment, drug-drug interactions occur when the presence of one drug affects the bio-availability of another through absorption, metabolism or disposition, and therefore affects the treatment and drug toxicity in the patient. The estimated incidence of drug-drug interactions that have a clinical significance ranges from 3% to 20%, depending on how many drugs are taken [2]. These adverse reactions are sometimes life-threatening. Such adverse effects caused by drug-drug interactions could be eliminated by more rational drug use, in which patients receive their medications in appropriate doses [3]. To achieve this use, an understanding of

the mechanism of drug-drug interactions is important, so that dosages can then be adjusted correspondingly.

Rifampicin is an effective antibiotic against gram-positive bacteria including mycobacteria, being frequently used currently in the chemotherapy of tuberculosis along with isoniazid, pyrazinamide and ethambutol/streptomycin [4,5]. Most patients can be cured with this, but some present with suboptimal or failed treatment [6,7]. Such failures could, in part, be explained by well known autoinduction leading to decreased drug efficacy whereby the drug stimulates its own metabolism into inactive metabolites and thus loses its effectiveness [8,9]. Suboptimal or failed treatment may also be arise from interactions with other drugs and foodstuffs that affect the metabolism of rifampicin [9-11]. For example, Trikatu, a commonly used

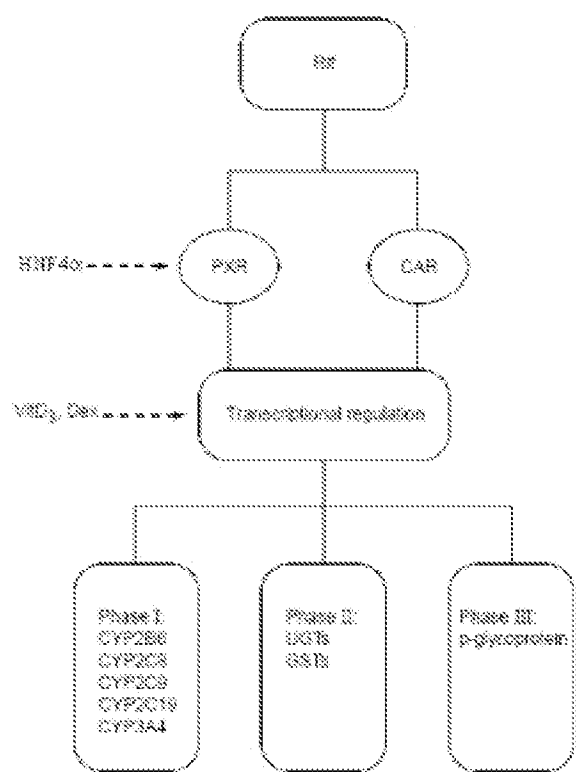


**Figure 1**  
Target genes activated by rifampicin via PXR and CAR. Rifampicin (Rif) induces nuclear receptors PXR and CAR that in turn activate a set of target genes including phase I enzymes such as CYP2B6, CYP2C8, CYP2C9 and CYP3A4, and phase II enzymes such as UGTs, GSTs and phase III enzymes such as p-glycoprotein. PXR and CAR are also regulated by HNF4α and these target genes are also regulated by Vitamin D3, and dexamethasone (Dex).

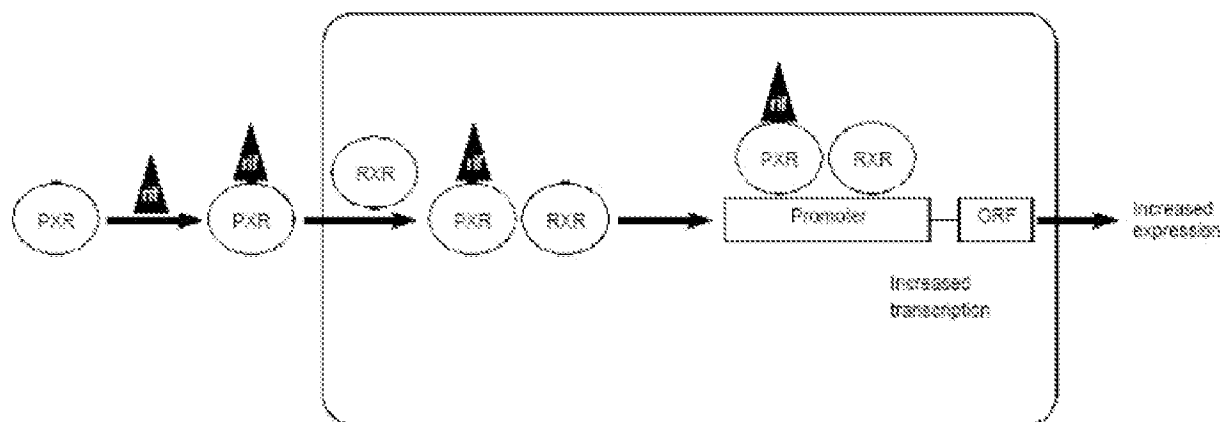
herb in Ayurvedic medicine, can significantly reduce the peak concentration of rifampicin probably by slowing stomach emptying and gastrointestinal motility [12]. In addition, rifampicin may also interfere with the metabolism of other concomitantly administered drugs [13]. Thus, rifampicin has been used extensively in clinical studies as a prototypical inducer of drug-metabolizing enzymes and transporters, due not only to autoinduction by itself or induction by other drugs and foodstuffs, but also its broad effects on drug-drug interactions. Many earlier reviews have discussed different aspects of rifampicin metabolism and its effects on the actions of other drugs [13-20]. However; application of molecular biology techniques has led to a rapid accumulation of new knowledge. Nuclear pregnane X receptor (PXR) and constitutive androstane receptor (CAR) have now been discovered and their roles in rifampicin-mediated drug-drug interactions

demonstrated [21-28]. Another important protein, p-glycoprotein, has also been shown to interplay with CYP3A4 in rifampicin inducing drug-drug interactions [29]. This paper reviews these recent findings, focusing on the molecular mechanisms of rifampicin-inducing drug metabolism.

**History of rifampicin-inducing drug-drug interactions**  
Rifampicin-inducing drug-drug interactions were recorded 25 years ago, and those affected include anticoagulants, hypoglycaemia agents and contraceptives [30-32]. The average elimination half-life of hexobarbital was decreased from 624 to 262 min and that of tolbutamide from 292 to 160 min following rifampicin treatment in patients with cirrhosis or cholestasis [31]. Rifampicin at 600 mg/day caused about a 3 fold increase in propranolol's clearance [33]. During concurrent treatment with prednisolone, rifampicin increased the plasma clearance of prednisolone by 45% and reduced areas under curves (AUC) by 66%, and these interactions have been reviewed recently [19,34].

The rifampicin-inducing drug-drug interactions were first discovered by Remmer (1972) to be caused by increased levels of cytochrome P450 (CYP), a large group of heme-containing monooxygenase isoenzymes encoded by a gene superfamily [35-37]. Total human CYP was markedly increased by rifampicin in needle biopsy samples taken from human liver. It was found that concomitant administration of rifampicin and oral contraceptives could lead to failure of the antifertility effect of the latter [38]. Further studies elucidated the interaction to be caused by increased estradiol metabolism [39,40]. Increased metabolism of estradiol in the presence of rifampicin was caused by the induction of estrogen-2-hydroxylation, which is catalysed mainly by CYP1A2 and also CYP1A1, CYP1B1 and CYP3A4 [41-43]. So far, many different CYPs have been identified. Thus, each individual CYP is assigned to a family, subfamily and number, so CYP1A2 stands for family 1, subfamily A, and isoform 2 [36]. Within the same family, all CYP proteins share at least 40 % sequence identity in their amino acid sequence, and within the same subfamily, at least 55% [37].

In 1998, a member of the nuclear receptor superfamily-PXR was found and named, based on its activation by both natural and synthetic C21 steroids (pregnanes) [15-17]. PXR was related to CYP3A4 and both are highly coexpressed in the liver and intestine. PXR is also activated by CYP3A4 inducers including drugs such as rifampicin, dexamethasone, indinavir, paclitaxel, pesticides such as trans-nonachlor, chlordane, endocrine disruptors such as phthalic acid, nonylphenol and other environmental contaminants such as polychlorinated biphenols [21]. Further experiments have demonstrated that PXR

**Figure 2**

Transcriptional activation of rifampicin target genes. Pregnane X receptor (PXR) binds with rifampicin in the cytoplasm and enters into nucleus to form a heterodimer with retinoic acid receptor (RXR). The heterodimer binds to the promoter of a target gene to activate the transcription of its open reading frame (ORF).

transcriptionally regulates CYP3A4. Thus, PXR can bind to CYP3A4 promoters to activate reporter genes, a process that is disregulated in PXR-null mice [19,44].

In addition to CYP3A4, PXR also upregulates a large number of genes involved in xenobiotic detoxification, including enzymes of phase I, II and III drug metabolism (Fig 1). These have been characterised by gene profiling studies performed with pregnenolone 16 $\alpha$ -carbonitrile in wild-type and PXR-null mice [45]. The genes regulated by PXR include those encoding CYP enzymes, p-glycoprotein, aldehyde dehydrogenases, UDP-glucuronosyltransferases (UGTs), sulfonyletransferases and glutathione-S-transferases (GSTs).

#### **Mechanism of induction of rifampicin- role of PXR**

The mechanism of rifampicin-mediated induction of CYP enzymes is now better understood. As mentioned earlier, this induction is mediated by the activation of PXR, which functions as a ligand-activated transcription factor [21,22]. Thus, when a PXR ligand binds to PXR, it in turn activates transcription of CYP 3A4 and several other genes (Fig 2).

The mechanism of PXR in regulating CYP3A4 involves its binding to response elements of the CYP3A4 gene that confers transactivation. PXR binds to CYP3A promoters together with the 9-cis retinoic acid receptor (RXR) as a heterodimer to ER6 (everted repeat with a 6 bp spacer) elements [25,46]. In the CYP3A4 gene, two PXR elements (distant and proximal) have been identified that are sepa-

rated by more than 7000 nucleotides. Disruption of the distant and proximal elements causes a 73% and 53% decrease in the respective reporter activities. However, *in vitro* binding studies showed that the proximal element was preferred [42]. Chromatin immunoprecipitation experiments showed that the genomic fragment harbouring the proximal element was preferably precipitated over the fragment containing the distant element in the CYP3A4 gene [47]. The fact that CYP3A4 is disregulated in PXR-null mice further demonstrates a role for PXR in CYP3A4 activation [44].

Thus, rifampicin has been clearly identified as a PXR activator by binding to PXR [48]. It also modulates PXR binding to PXR elements of the CYP3A4 promoter [47]. Studies by X-ray crystallography indicated the overall architecture of PXR binding to rifampicin comprised 12  $\alpha$ -helices that fold to form a hydrophobic pocket in the lower portion of the protein [20]. The large volume of the PXR ligand-binding pocket characterised by a smooth, elliptical shape explains why PXR acts as a broad-specificity sensor of lipophilic xenobiotics.

Another receptor, constitutive androstane receptor (CAR), is also involved in CYP3A4 transcriptional regulation. CAR is a member of the steroid/retinoid/thyroid hormone receptor superfamily. It has been demonstrated that CAR can trans-activate CYP3A4 gene expression both *in vitro* and *in vivo* [49]. The CAR response elements are also bound by PXR, indicating their interplay in CYP3A4 expression.

CAR has been demonstrated to cross-talk with PXR, i.e. they share some ligands capable of binding to each other's DNA response elements [44,45,49]. It was also confirmed in chimeric mice with humanized mice liver in which both human CYP3A4 mRNA and protein production was dramatically increased by rifampicin [50]. In receptor-null mice, PXR and CAR regulate overlapping but distinct sets of genes [45]. The significance of cross-talk could be that CAR and PXR together compensate for the loss or malfunction of each other to a certain extent. However, rifampicin has a much lower effect on CAR than PXR [45].

#### **Effect of rifampicin on CYPs through PXR**

CYPs are mainly found on the smooth endoplasmic reticulum membranes in liver hepatocytes and along the intestinal tract mucosal surface [51]. CYPs play an important role in protecting organisms from potentially toxic compounds, by their ability to biotransform lipophilic substrates of diverse structures into more water-soluble metabolites that are subsequently excreted from the body [37,52]. They are also essential for eukaryotic life because of their roles in the metabolism of sterols such as cholesterol, bile acids, fatty acids, prostaglandins, leukotrienes, retinoids as well as biogenic amines [51,53].

These CYPs are important in drug metabolism as they catalyse metabolic reactions involving thousands of substrates in over 60 different types of reactions [54]. Rifampicin can induce many CYPs including CYP2A, CYP2B, CYP2C and CYP3A [51]. Among the many different subsets of these enzymes, CYP3A4 is the most abundant, in both the liver and the intestinal tract. CYP3A4 is responsible for the oxidative metabolism of a wide variety of substrates, involving steroid catabolism and metabolism of foreign compounds, with many of the clinically used drugs being substrates for CYP3A4. It is also a key enzyme in chemical carcinogenesis in both the liver and extrahepatic tissues [55].

The reactions catalysed by CYP3A4 include N-oxidation, C-oxidation, N-dealkylation, O-dealkylation, nitro-reduction, dehydration and C-hydroxylation [55]. CYP3A4 is the primary catalyst of steroid 6 $\beta$ -hydroxylation [56]. These broad reactions are explained by the structure of the CYP3A4 active site which recently has been characterised by X-ray crystallography [57].

CYP3A4 is more efficiently induced than other CYPs [51,58-60]. In primary human hepatocytes, 20  $\mu$ M rifampicin increased CYP3A4 mRNA by 14 fold, but CYP2B6 by only 2.1 fold [61]. CYP3A4 appears to be regulated transcriptionally by a variety of structurally diverse compounds and hormones [46,62]. In cultured hepatocytes, triiodothyronine decreased CYP3A4 enzyme activity as well as protein and mRNA levels, while dexamethasone

and growth hormone increased CYP3A4 gene expression [63].

Rifampicin is involved in drug-drug interaction by its induction of CYPs, especially CYP3A4, and CYP3A4 mRNA is induced by rifampicin in blood mononuclear and liver HepG2 cells [64-66]. The CYP3A4 gene has also been isolated and cloned. A 20-bp region residing -150 bp upstream of the transcription initiation site has been shown to confer responsiveness to rifampicin [67]. The relative potency in inducing CYP3A4 by the rifamycins is rifampicin > rifapentine > rifabutin [10].

Rifampicin can induce both gut (enterocytic) and liver (hepatic) CYP, especially CYP3A4, and increase its own metabolism (autoinduction), but the induced metabolic pathway is still not clear [27]. In healthy volunteers, repeated oral administration of rifabutin (450 mg daily for 10 days) led to lower AUC and minimal blood concentration (C<sub>min</sub>) values, compared to the corresponding theoretical values, with an unchanged half-life [26]. This indicates induced metabolism, although it is unclear as to which specific enzymes are involved. Rifabutin, its derivatives and metabolites, are likely to be metabolised by CYPs, particularly by CYP3A4 [29,68]. If CYPs are involved in rifampicin metabolism, how they act is not clear [29].

There are marked individual variations in the expression of CYP that affect the biological response (efficacy and/or toxicity) of many drugs as well as individual responses to CYP inducers [62,69]. In humans, a 10-fold variation exists among individuals for rifampicin inducing CYP3A4 expression [70,71]. In the liver, substantial interindividual differences in CYP3A4 expression have been detected that could not be explained by environmental factors [70]. A similar situation was found in the intestine whereby CYP3A4 mRNA levels in biopsies of mucosa of five healthy subjects varied by about 10 fold [71]. These differences could in part account for interpatient differences in the oral bioavailability of CYP3A4 substrates.

It has been well demonstrated that rifampicin-induced CYP2C9 is mediated by PXR [72]. Deletion and mutagenesis studies showed that the functional PXR-responsive element is located between -1839/-1824 base pairs upstream from the translation start site previously identified to be CAR-responsive element, and essential for rifampicin inducibility. A second CAR-responsive element located at -2899/-2883 also has a cooperative effect.

In addition, PXR and CAR activate CYP2B6 by transcriptional regulation, although not all CYP2B6 inducers activate PXR [73]. A phenobarbital-responsive enhancer module (PBREM) has been identified, although for

human CYP2B6, PBREM effects only a weak response to PXR and CAR. A novel nuclear receptor response module that strongly binds to both PXR and CAR has been described and is known as the xenobiotic-responsive enhancer module (XRE) [74].

#### **Effect of rifampicin on glucuronosyltransferases through PXR**

Rifampicin can induce UDP-glucuronosyltransferase 1A (UGT1A) that catalyses the glucuronidation of a wide range of xenobiotics and endogenous substrates [48,75,76]. UGT1A is regulated by many factors in a tissue-specific manner [77,78]. Both PXR and CAR mediate expression of UGT1A [79-81]. Transgenic mice expressing a constitutively active form of human PXR show a markedly increase of UGT activity [79]. A XRE in the UGT1A gene was thought to be responsible for this induction [80]. The rifampicin mediated-UGT increase may lead to increased clearance of steroids, heme, environmental toxins and drugs [79].

#### **Effect of rifampicin on p-glycoprotein through PXR**

Studies in humans have long indicated that drug transporters are involved in rifampicin hepatic and intestinal uptake. p-Glycoprotein is a plasma membrane-bound drug efflux (multidrug resistance pump), a 170 kDa phosphorylated and glycosylated protein, 1280 amino acids long, which consists of two homologous halves of 610 amino acids joined by a flexible 60 amino acid linker [82]. p-Glycoprotein belongs to the ATP binding cassette superfamily of transport proteins encoded by the multidrug resistance genes (MDR) and was found primarily in drug-eliminating organs, including the kidney, liver, brain, intestinal lumen, testes, adrenal gland, pregnant uterus, tumour cells and epithelial cells [83,84]. They play an important role in drug absorption and disposition, as a biological barrier by extruding toxins and xenobiotics from cells rather than by enhancing the excretion of drugs from hepatocytes and renal tubules. Human p-glycoprotein plays an important role in drug efflux through these barriers. Recent paired human tissue studies have shown p-glycoprotein and CYP3A4 protein contents in intestine were higher than in liver, which might indicate an important role for the intestine in drug metabolisms [85].

Should the p-glycoprotein be induced by rifampicin, for example, then the drug's therapeutic usefulness will decline as more drugs are pumped out of the cells. p-Glycoprotein has only recently been identified and thus the potential risk of p-glycoprotein-mediated drug interactions has probably been underestimated in the past. Many drug interactions have been shown to involve both p-glycoprotein and CYP3A4 due to overlapping substrate specificity between them, and similarities in their inhibitors and inducers, as for example, with the coinduction of CYP3A4

and p-glycoprotein by rifampin in human LS180 colon carcinoma cells [86]. Furthermore, p-glycoprotein can control drug access to intracellular CYP3A4 through repeated cycles of drug absorption and efflux, as demonstrated by the studies with cyclosporine, a p-glycoprotein and CYP3A4 inhibitor and GG918, a p-glycoprotein inhibitor only [23]. After inhibition of GG918, K77, a substrate for p-glycoprotein and CYP3A4, accumulated in the cell and metabolism was increased, but felodipine, a substrate of CYP3A4 but not p-glycoprotein was not changed [23]. Cyclosporine decreased the metabolisms of both K77 and felodipine. In another study, inhibition of p-glycoprotein increased both intestinal uptake and blood concentrations of verapamil but with no changes in its rate of metabolism [87].

Rifampicin both induces p-glycoprotein expression and reduces digoxin concentrations during concomitant therapy [88,89]. In healthy volunteers, oral administration of digoxin with rifampicin showed lower plasma concentrations than in those without. This was less pronounced if digoxin was given intravenously. Correspondingly, p-glycoprotein content was 3.5 fold higher after rifampicin treatment [88]. Any regulation is likely to take place at the transcriptional level since increased mRNA levels were detected [90-92]. In healthy subjects, administration of 600mg rifampicin for 9 days upregulated p-glycoprotein mRNA synthesis [92].

The induction of MDR1, a gene encoding p-glycoprotein, is also activated by PXR which binds to PXR response elements located upstream of the MDR1 gene [93]. Only one DR4 (direct repeat with a 4 bp spacer) motif (at about -8 kilobase pairs) was shown to be necessary for the induction by rifampicin, although several other binding sites were identified. It has been demonstrated in mouse hepatoma cell culture and rats that dexamethasone induces the expression of MDR1 and MDR3, with two genes encoding for p-glycoprotein in the mouse [94,95].

#### **Other factors**

PXR is also regulated by a hepatic nuclear factor 4 $\alpha$ (HNF4 $\alpha$ ), which regulates many genes preferentially expressed in liver [22]. HNF4 $\alpha$  is stimulated by hepatic maturation factors, such as oncostatin and matrigel, and plays an important role in early embryogenesis [96]. In a fetal hepatocyte primary culture system, inactivation of HNF4 $\alpha$  by infection with an adenovirus containing the Cre gene leads to suppression of PXR [97]. An HNF4 $\alpha$  antisense RNA has been transduced into human hepatocytes, which resulted in the reduction of HNF4 $\alpha$  protein and CYP3A4 [98].

An HNF4 $\alpha$  binding site has been characterised in the PXR promoter that is necessary for expression of PXR in fetal

Table 1: Example drugs affected by rifampicin through different mechanisms

| Mediated proteins |                               | p-glycoprotein                                             | CYP1A2                                                                                             | CYP2B6                                   | CYP2C9                                                                                               | CYP2C19                                                                        | CYP3A4                                                                                                                                                                                                                    |
|-------------------|-------------------------------|------------------------------------------------------------|----------------------------------------------------------------------------------------------------|------------------------------------------|------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Affected drugs    | Anesthetics:                  |                                                            | Ropivacaine [118]                                                                                  |                                          |                                                                                                      | Cyclophosphamine [74]                                                          | Alfentanyl [13]<br>Ropivacaine [118]<br>Codeine [19]<br>Dapsone [19]<br>Mexiletine [119]<br>Propafenone [120]<br>Quinidine [19]                                                                                           |
|                   | Analgesics:                   |                                                            | Mexiletine [119]                                                                                   |                                          |                                                                                                      |                                                                                |                                                                                                                                                                                                                           |
|                   | Anti-arrhythmics:             |                                                            |                                                                                                    |                                          |                                                                                                      |                                                                                |                                                                                                                                                                                                                           |
|                   | Anti-cancer drugs:            |                                                            | Cyclophosphamide [74]<br>Ifosfamide [74]                                                           | Cyclophosphamide [74]<br>Ifosfamide [74] | Cyclophosphamide [74]                                                                                |                                                                                | Cyclophosphamide [74]<br>Imatinib [121]<br>Tamoxifen [19]                                                                                                                                                                 |
|                   | Anti-epileptics:              |                                                            |                                                                                                    |                                          | Mephenytoin [122]                                                                                    |                                                                                |                                                                                                                                                                                                                           |
|                   | Anti-histamines:              | Fexofenadine [123]<br>Terfenadine [123]                    |                                                                                                    |                                          |                                                                                                      |                                                                                | Fexofenadine [123]<br>Terfenidine [123]<br>Clarithromycin [13]<br>Erythromycin [124]<br>Itraconazole [13]<br>Ketoconazole [13]<br>Miconazole [13]<br>Alprazolam [13]<br>Diazepam [13]<br>Midazolam [13]<br>Triazolam [13] |
|                   | Anti-microbials:              | Erythromycin [124]                                         |                                                                                                    |                                          |                                                                                                      |                                                                                | Nifedipine [13]<br>Propranolol [13]<br>Verapamil [19]                                                                                                                                                                     |
|                   | Benzodiazepines:              |                                                            |                                                                                                    |                                          |                                                                                                      |                                                                                |                                                                                                                                                                                                                           |
|                   | Cardiac drugs:                | Digoxin* [88]<br>Quinidine [13]                            | Propranolol [13]<br>Verapamil [19]                                                                 | Bupropion* [114]<br>Methadone [19]       | Propranolol [13]<br>Warfarin* [113]                                                                  | Propranolol [12]                                                               |                                                                                                                                                                                                                           |
|                   | CNS drugs:                    |                                                            | Amitriptyline [125]<br>Bupropion [114]<br>Clomipramine [125]<br>Clozapine [19]<br>Ondansetron [19] |                                          | Amitriptyline [125]<br>Clomipramine [125]                                                            | Amitriptyline [119]<br>Clomipramine [119]<br>Phenytoin [120]<br>Clozapine [19] | Amitriptyline [125]<br>Buspirone [19]<br>Clomipramine [125]<br>Zolpidem [19]                                                                                                                                              |
|                   | Gastro-enterology drugs:      |                                                            |                                                                                                    |                                          |                                                                                                      |                                                                                |                                                                                                                                                                                                                           |
|                   | HIV Antivirals:               | Indinavir [13]<br>Nelfinavir [13]<br>Ritonavir [13]        |                                                                                                    | Efavirenz [13]                           | Nelfinavir [13]                                                                                      | Nelfinavir [12]                                                                | Amprenavir [13]<br>Delavirdine [13]<br>Indinavir [13]<br>Nelfinavir [13]<br>Nevirapine [13]<br>Ritonavir [13]<br>Saquinavir [13]                                                                                          |
|                   | HMG CoA Reductase Inhibitors: |                                                            |                                                                                                    |                                          | Fluvastatin [109]<br>Losartan [109]                                                                  | Cerivastatin [103]                                                             | Atorvastatin [109]<br>Cerivastatin [109]<br>Lovastatin [109]<br>Simvastatin * [108]<br>Pioglitazone [127]                                                                                                                 |
|                   | Hypoglycemics                 |                                                            |                                                                                                    | Pioglitazone [127]                       | Glibenclamide [13]<br>Glipizide [13]<br>Nateglinide [13]<br>Rosiglitazone* [110]<br>Tolbutamine [13] | Repaglinide [12]                                                               |                                                                                                                                                                                                                           |
|                   | Immune Modulators:            | Cyclosporine [105]<br>Tacrolimus [17]<br>Methotrexate [19] |                                                                                                    |                                          |                                                                                                      |                                                                                | Cyclosporine* [105]<br>Sirolimus [17]<br>Tacrolimus [19]                                                                                                                                                                  |
|                   | Steroids:                     | Cortisol [19]<br>Corticosterone [19]<br>Dexamethason [19]  | Estradiol [19]                                                                                     |                                          |                                                                                                      |                                                                                | Estradiol [19]<br>Ethinylloestradiol [19]<br>Hydrocortisone [19]                                                                                                                                                          |

hepatocytes [97]. Thus, HNF4 $\alpha$  is identified as the key transcriptional factor for PXR. A specific cis-acting element of gene enhancer in CYP3A4 has also identified which confers HNF4 $\alpha$  binding and permits PXR- and CAR-mediated gene activation [22].

The molecular mechanisms that underlie transcriptional activation of CYP3A4 are complex involving regulatory factors other than PXR and CAR. These include glucocorticoid and Vitamin D receptors. The CYP3A4 promoter may also be regulated through mechanisms other than PXR effects, including glucocorticoid-induced CYP3A4 gene expression, through the established PXR-dependent pathway and a PXR-independent pathway [99]. Disruption of a responsive element in the CYP3A4 proximal promoter for hepatocyte nuclear factor 3 and CCATT-enhancer binding protein alpha affects its induction by glucocorticoids, dexamethasone and hydrocortisone, but not that by rifampicin. Vitamin D3 has also been shown to induce CYP3A4 independently of PXR [100-102].

#### Explanation of drug-drug interactions

Some of the drugs affected by rifampicin are listed in Table 1. Theoretically, any drugs or foods that affect the activation or inhibition of PXR, and/or CYP3A4 and/or p-glycoprotein will interact. CYP3A4 catalyse the metabolism of more than 60% of all drugs currently in use including contraceptive steroids, immunosuppressive agents, imidazole antimycotics and macrolide antibiotics. "Foods" such as grapefruit juice, black pepper inhibit CYP3A4 so causing increased drug levels with resulting therapeutic complications [89,103,104].

The drugs affected by or those that affect rifampicin have been extensively reviewed elsewhere [6-11,13,14]. However, the mechanisms for these interactions are not well studied, especially now that considerable progress has been made in understanding the molecular mechanisms of rifampicin. The following examples are selected to represent the major clinically significant rifampicin-inducing drug-drug interactions mainly via the target genes CYP3A4, CYP2C9, CYP2B6 and p-glycoprotein, and these are labelled with \* in Table 1.

#### Cyclosporine (CYP3A4)

Interaction of the immunosuppressant cyclosporine and rifampicin is well documented. Doses of cyclosporine had to be increased about 3 fold to maintain its therapeutic blood concentration when coadministered with rifampicin [105]. A case report has shown that intravenous rifampicin injection led to failure of bone marrow transplantation resulting from subtherapeutic cyclosporine blood levels [106].

Cyclosporine is mainly metabolized by CYP3A4 in human liver [107]. Dexamethasone-induction of cyclosporine metabolism is inhibited by anti-CYP3A4 antibodies *in vitro* and so it is quite likely that rifampicin-inducing cyclosporine metabolism is mediated via CYP3A4 although this has not yet been confirmed by *in vitro* experiments [71].

#### Simvastatin (CYP3A4)

Simvastatin is used to treat hypercholesterolemia and is metabolised to inactive metabolites by CYP3A4. Rifampicin has been shown to reduce its effective concentration while diltiazem, a CYP3A4 inhibitor increases its concentration [108]. In healthy volunteers, 5 day pre-treatment with rifampicin at a dose of 600 mg daily following a single 40 mg dose of simvastatin led to reduced plasmid concentrations of simvastatin of 87 % compared with the control [109]. Thus, concomitant administration of rifampicin and simvastatin can lead to greatly reduced cholesterol lowering efficiency of simvastatin.

#### Rosiglitazone (CYP2C9)

Rosiglitazone is a novel thiazolidinedione antidiabetic drug. Its interaction with rifampicin has been studied in a clinical trial [110]. When ten healthy subjects were administered 8 mg of rosiglitazone after a 6 day pre-treatment of rifampicin at a dose of 600 mg daily, rifampicin decreased the plasma concentration of rosiglitazone by 65% and its elimination half-life from 3.9 to 1.5 hrs. Because rosiglitazone is metabolised by CYP2C8 and CYP2C9, it is proposed that the induction of these CYPs is the basis for this interaction [110,111].

#### Warfarin (CYP2C9)

Warfarin is an anticoagulant drug metabolized by CYP2C9 to 7-hydroxywarfarin [72,112]. Rifampicin increases the rate of clearance of warfarin, thus reducing its effect. In a patient receiving concomitant treatment with rifampicin and warfarin, a 233% increase in warfarin dosage over 4 months was insufficient to attain a therapeutic level and more aggressive upward titration of dose was needed [14,13]. This may be the result of rifampicin induced CYP2C9, which in turn accelerated warfarin metabolism.

#### Bupropion (CYP2B6)

Bupropion, an antidepressant drug, is metabolized by CYP2B6. Bupropion is hydroxylated by microsomes containing cDNA-expressed CYP2B6 and the reaction is subject to 95% inhibition by CYP2B6 inhibitory antibody [114]. High interindividual variability is thought to be a consequence of interactions between environmental and genetic influences on CYP2B6 gene function [115]. Thus, rifampicin induction of CYP2B6 should lead to increased

bupropion metabolism, but no data are available to support this view.

**Digoxin (p-glycoprotein)**

Clinical observations have shown that digoxin serum concentration decreased considerably in patients who were treated with rifampicin. This is of special interest since digoxin is generally considered to be renally eliminated [116,117]. In healthy volunteers, co-administration of digoxin and rifampicin orally resulted in plasma concentration of digoxin being much lower during rifampicin treatment. However, the effect was less pronounced after intravenous administration of digoxin [88].

The interaction is mainly mediated by p-glycoprotein in the intestine and intestinal p-glycoprotein content increased 3.5 fold on treatment with rifampicin, which correlated with the decrease in its plasma concentration after oral administration of digoxin [88]. Thus, induction of p-glycoprotein could explain the rifampicin-digoxin interaction.

**Rifampicin-inducing hepatotoxicity**

Rifampicin is very toxic to liver, this having been found both in the treatment of tuberculosis and cholestasis [128-130]. When it was used together with pyrazinamide, a 5.8% incidence of severe liver injury was reported, 2.6% with isoniazid and 1.1% alone [130]. The pathogenesis of hepatotoxicity is poorly understood although limited evidence has been obtained. Histopathological examination showed dose-related hepatic necrosis, ballooning degeneration and inflammatory infiltrates [130,131]. A possible explanation is the increased CYP related effects on calcium homeostasis [132]. It may also be that via oxidant stress, increased lipid peroxidation occurs [133]. In the case when rifampicin and isoniazid are used together, rifampicin may increase isoniazid toxicity though induction of CYPs because acetyl-isoniazid from isoniazid is converted to monoacetyl hydrazine, which is catalysed by CYPs to other hepato-toxic compounds. There were no reports if nuclear receptors were involved in this pathogenesis.

**Conclusion**

A common pattern for the molecular mechanisms of rifampicin-inducing drug-drug interaction is rifampicin – PXR/CAR – CYPs, UGTs, GSTs and p-glycoprotein. The possible pathways for rifampicin effects on other drugs are summarised in Table 1. However, other drugs may act in a similar way to rifampicin, or by other pattern as seen with dexamethasone. Therefore, how they act individually is probably complex.

More studies are needed to further increase our knowledge of rifampicin induced drug-drug interactions. Firstly,

aspects of the molecular mechanism including how rifampicin activates PXR and modulates their binding to the promoters of CYP3A4 and other target genes should be explored. Secondly, the characterisation of drugs affected by rifampicin and elucidation of their mechanisms of interactions are urgently needed.

**List of abbreviations**

- AUC: areas under curves
- CAR: constitutive androstane receptor
- CYP: cytochrome P450
- DR4: direct repeat with a 4 bp spacer
- ER6: everted repeat with a 6 bp spacer
- HNF 4 $\alpha$ : hepatic nuclear factor 4 $\alpha$
- GSTs: glutathione-S-transferases
- MDR: multidrug resistance genes
- PBREM: phenobarbital-responsive enhancer
- PXR: pregnane X receptor
- RXR: 9-cis retinoic acid receptor
- UGTs: UDP-glucuronosyltransferases
- UGT1A: UDP-glucuronosyltransferase 1A
- XRE: xenobiotic response element

**Competing interests**

The author(s) declare that they have no competing interests.

**Authors' contributions**

KR and JC discussed the most recent progress in the field and formed the concept for the review. JC did the literature search and prepared the first draft of the manuscript. KR then revised and approved this version of the paper to be submitted for publication.

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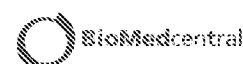
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| Electronic Acknowledgement Receipt   |                                                     |
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| EFS ID:                              | 47807967                                            |
| Application 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                                            |
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| 1                      | Applicant Arguments/Remarks Made in an Amendment | 128748_01502_Response_to_Final_OA_April_2023.PDF | 161103                                   | no               | 7                |
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| Information:                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |        |
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| <p>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.</p> <p><b><u>New Applications Under 35 U.S.C. 111</u></b><br/>If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.</p> <p><b><u>National Stage of an International Application under 35 U.S.C. 371</u></b><br/>If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.</p> <p><b><u>New International Application Filed with the USPTO as a Receiving Office</u></b><br/>If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.</p> |        |

Docket No.: 128748-01502  
(PATENT)

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

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

Application No.: 16/900,610

Confirmation No.: 1054

Filed: June 12, 2020

Art Unit: 1628

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For: MITAPIVAT THERAPY AND MODULATORS  
OF CYTOCHROME P450

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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 7<sup>th</sup> two-month date for responding to the subject Office Action. Nothing was issued by April 5<sup>th</sup> 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/  
Andrew R. Ehle  
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MCCARTER & ENGLISH, LLP  
265 Franklin Street  
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Attorney/Agent For Applicant

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

| <b>All Participants</b> (applicant, applicants representative, PTO personnel) | <b>Title</b>       | <b>Type</b> |
|-------------------------------------------------------------------------------|--------------------|-------------|
| 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/<br>Examiner, Art Unit 1621                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | /THEODORE R. HOWELL/<br>Primary Examiner, Art Unit 1628 |
| <b>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</b><br>Please further see:<br>MPEP 713.04<br>Title 37 Code of Federal Regulations (CFR) § 1.133 Interviews, paragraph (b)<br>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.

|                        |                               |                             |                          |
|------------------------|-------------------------------|-----------------------------|--------------------------|
| Notice of Allowability | Application No.<br>16/900,610 | Applicant(s)<br>Iyer et al. |                          |
|                        | Examiner<br>GEORGE W KOSTURKO | Art Unit<br>1621            | AIA (FITF) Status<br>Yes |

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

All claims being allowable, PROSECUTION ON THE MERITS IS (OR REMAINS) CLOSED in this application. If not included herewith (or previously mailed), a Notice of Allowance (PTOL-85) or other appropriate communication will be mailed in due course. **THIS NOTICE OF ALLOWABILITY IS NOT A GRANT OF PATENT RIGHTS.** This application is subject to withdrawal from issue at the initiative of the Office or upon petition by the applicant. See 37 CFR 1.313 and MPEP 1308.

1. ☒ This communication is responsive to 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](http://www.uspto.gov/patents/init_events/pph/index.jsp) or send an inquiry to [PPHfeedback@uspto.gov](mailto:PPHfeedback@uspto.gov).

4. ☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

**Certified copies:**

a) ☐ All      b) ☐ Some\*      c) ☐ None of the:

1. ☐ Certified copies of the priority documents have been received.

2. ☐ Certified copies of the priority documents have been received in Application No. \_\_\_\_\_ .

3. ☐ Copies of the certified copies of the priority documents have been received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

\* Certified copies not received: \_\_\_\_\_ .

Applicant has THREE MONTHS FROM THE "MAILING DATE" of this communication to file a reply complying with the requirements noted below. Failure to timely comply will result in ABANDONMENT of this application.  
**THIS THREE-MONTH PERIOD IS NOT EXTENDABLE.**

5. ☐ CORRECTED DRAWINGS (as "replacement sheets") must be submitted.  
☐ including changes required by the attached Examiner's Amendment / Comment or in the Office action of Paper No./Mail Date \_\_\_\_\_ .

**Identifying indicia such as the application number (see 37 CFR 1.84(c)) should be written on the drawings in the front (not the back) of each sheet. Replacement sheet(s) should be labeled as such in the header according to 37 CFR 1.121(d).**

6. ☐ DEPOSIT OF and/or INFORMATION about the deposit of BIOLOGICAL MATERIAL must be submitted. Note the attached Examiner's comment regarding REQUIREMENT FOR THE DEPOSIT OF BIOLOGICAL MATERIAL.

**Attachment(s)**

|                                                                                                                   |                                                                                      |
|-------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------|
| 1. <input type="checkbox"/> Notice of References Cited (PTO-892)                                                  | 5. <input checked="" type="checkbox"/> Examiner's Amendment/Comment                  |
| 2. <input type="checkbox"/> Information Disclosure Statements (PTO/SB/08),<br>Paper No./Mail Date _____.          | 6. <input checked="" type="checkbox"/> Examiner's Statement of Reasons for Allowance |
| 3. <input type="checkbox"/> Examiner's Comment Regarding Requirement for Deposit<br>of Biological Material _____. | 7. <input type="checkbox"/> Other _____.                                             |
| 4. <input checked="" type="checkbox"/> Interview Summary (PTO-413),<br>Paper No./Mail Date. _____.                |                                                                                      |

|                                                |                                                         |
|------------------------------------------------|---------------------------------------------------------|
| /GEORGE W KOSTURKO/<br>Examiner, Art Unit 1621 | /THEODORE R. HOWELL/<br>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

Application/Control Number: 16/900,610  
Art Unit: 1621

Page 4

(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**

Receipt date: 04/07/2023

16/900,610 -- GAU: 1621

OK TO ENTER: /G.W.K/

04/18/2023

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.



UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE  
United States Patent and Trademark Office  
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P.O. Box 1450  
Alexandria, Virginia 22313-1450  
www.uspto.gov

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](http://www.uspto.gov/PatentMaintenanceFees).

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P.O. Box 1450  
Alexandria, Virginia 22313-1450

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CURRENT CORRESPONDENCE ADDRESS (Note: Use Block 1 for any change of address)

190109 7590 05/03/2023  
McCarter & English / Agios Pharmaceuticals  
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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).<br><br><input type="checkbox"/> Change of correspondence address (or Change of Correspondence Address form PTO/AIA/122 or PTO/SB/122) attached.<br><br><input type="checkbox"/> "Fee Address" indication (or "Fee Address" Indication form PTO/AIA/47 or PTO/SB/47; Rev 03-02 or more recent) attached. <b>Use of a Customer Number is required.</b> | 2. For printing on the patent front page, list<br>(1) The names of up to 3 registered patent attorneys or agents OR, alternatively, 1 _____<br>(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed. 2 _____<br>3 _____ |
|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE (B) RESIDENCE: (CITY and STATE OR COUNTRY)

Please check the appropriate assignee category or categories (will not be printed on the patent) : ☐ Individual ☐ Corporation or other private group entity ☐ Government

4a. Fees submitted: ☐ Issue Fee ☐ Publication Fee (if required)

4b. Method of Payment: (Please first reapply any previously paid fee shown above)

☐ Electronic Payment via Patent Center or EFS-Web ☐ Enclosed check ☐ Non-electronic payment by credit card (Attach form PTO-2038)  
☐ The Director is hereby authorized to charge the required fee(s), any deficiency, or credit any overpayment to Deposit Account No. \_\_\_\_\_

5. Change in Entity Status (from status indicated above)

☐ Applicant certifying micro entity status. See 37 CFR 1.29  
☐ Applicant asserting small entity status. See 37 CFR 1.27  
☐ Applicant changing to regular undiscounted fee status.

NOTE: Absent a valid certification of Micro Entity Status (see forms PTO/SB/15A and 15B), issue fee payment in the micro entity amount will not be accepted at the risk of application abandonment.

NOTE: If the application was previously under micro entity status, checking this box will be taken to be a notification of loss of entitlement to micro entity status.

NOTE: Checking this box will be taken to be a notification of loss of entitlement to small or micro entity status, as applicable.

NOTE: This form must be signed in accordance with 37 CFR 1.31 and 1.33. See 37 CFR 1.4 for signature requirements and certifications.

Authorized Signature \_\_\_\_\_ Date \_\_\_\_\_  
Typed or printed name \_\_\_\_\_ Registration No. \_\_\_\_\_



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|--------------------------------------------|-------------|---------------------------|---------------------|------------------|
| APPLICATION NO.                            | FILING DATE | FIRST NAMED INVENTOR      | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
| 16/900,610                                 | 06/12/2020  | Varsha Venkatachalam Iyer | 128748-01502        | 1054             |
| 190109                                     | 7590        | 05/03/2023                | EXAMINER            |                  |
| McCarter & English / Agios Pharmaceuticals |             |                           | KOSTURKO, GEORGE W  |                  |
| 265 Franklin Street                        |             |                           | ART UNIT            |                  |
| Boston, MA 02110                           |             |                           | 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.
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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.

| 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: |                  |

|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                             |                                     |                                          |                  |                  |
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| 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                                   | no               | 1                |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                             |                                     | 139968079552245cb6fc52384065a8ec5064bbcd |                  |                  |
| Warnings:                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                             |                                     |                                          |                  |                  |
| Information:                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                             |                                     |                                          |                  |                  |
| 2                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            | Fee Worksheet (SB06)        | fee-info.pdf                        | 37934                                    | no               | 2                |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                             |                                     | 77850b6f2be52dabcc832ca2344cf7fb2f9661f  |                  |                  |
| Warnings:                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                    |                             |                                     |                                          |                  |                  |
| Information:                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                             |                                     |                                          |                  |                  |
| Total Files Size (in bytes):                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                 |                             |                                     | 223368                                   |                  |                  |
| <p>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.</p> <p><b><u>New Applications Under 35 U.S.C. 111</u></b><br/>If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.</p> <p><b><u>National Stage of an International Application under 35 U.S.C. 371</u></b><br/>If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.</p> <p><b><u>New International Application Filed with the USPTO as a Receiving Office</u></b><br/>If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.</p> |                             |                                     |                                          |                  |                  |

PART B–FEE(S) TRANSMITTAL

Complete and send this form, together with the applicable fee(s), by mail or fax, or via EFS-Web.

By mail, send to: Mail Stop ISSUE FEE  
Commissioner for Patents  
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Alexandria, VA 22313-1450

By fax, send to: (571) 273-2885

INSTRUCTIONS: This form should be used for transmitting the ISSUE FEE and PUBLICATION FEE (if required). Blocks 1 through 5 should be completed where appropriate. All further correspondence will be mailed to the current correspondence address as indicated unless corrected below or directed otherwise in Block 1, by (a) specifying a new correspondence address; and/or (b) indicating a separate "FEE ADDRESS" for maintenance fee notifications. **Because electronic patent issuance may occur shortly after issue fee payment, any desired continuing application should preferably be filed prior to payment of this issue fee in order not to jeopardize copendency.**

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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  | \$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)<br><br><input type="checkbox"/> Change of correspondence address (or Change of Correspondence Address form PTO/SB/122) attached.<br><input type="checkbox"/> "Fee Address" indication (or "Fee Address" Indication form PTO/AIA/47 or PTO/SB/47; Rev 03-09 or more recent) attached. <b>Use of a Customer Number is required.</b> | 2. For printing on the patent front page, list<br><br>(1) The names of up to 3 registered patent attorneys or agents OR, alternatively,<br><br>(2) The name of a single firm (having as a member a registered attorney or agent) and the names of up to 2 registered patent attorneys or agents. If no name is listed, no name will be printed. | 1. McCarter & English, LLP<br><br>2. Steven G. Davis, Ph.D.<br><br>3. |
|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------|

3. ASSIGNEE NAME AND RESIDENCE DATA TO BE PRINTED ON THE PATENT (print or type)

PLEASE NOTE: Unless an assignee is identified below, no assignee data will appear on the patent. If an assignee is identified below, the document must have been previously recorded, or filed for recordation, as set forth in 37 CFR 3.11 and 37 CFR 3.81(a). Completion of this form is NOT a substitute for filing an assignment.

(A) NAME OF ASSIGNEE  
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)

|                                                                                     |                                                                                                                                                                                                     |
|-------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <input type="checkbox"/> 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. |
| <input type="checkbox"/> 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.                                 |
| <input type="checkbox"/> 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         |

PTOL-85 Part B (03-23) Approved for use through 03/31/2023 OMB 0651-0033 U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

| 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   |                                                     |
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| 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)                         |

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| 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                                   | no               | 63               |
|                        |                      |                       | 5d42a58e31f4570fade6986ef18d8c58700a85f7 |                  |                  |
| Warnings:              |                      |                       |                                          |                  |                  |

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| Information:                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                |                                            |                                          |    |    |
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|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                |                                            | d774b89b97d89718b0a93f0fa990f2ed8269afc4 |    |    |
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| 3                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                   | Amendment after Notice of Allowance (Rule 312) | 128748_01502_312_Amendme nt_Aug_2023_F.pdf | 24094                                    | no | 3  |
|                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                                                |                                            | c548b2fa11cc599e2312e8e681c23390d695f04c |    |    |
| Warnings:                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                                                |                                            |                                          |    |    |
| Information:                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                |                                            |                                          |    |    |
| Total Files Size (in bytes):                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                                                |                                            | 534214                                   |    |    |
| <p><b>This Acknowledgement Receipt evidences receipt on the noted date by the USPTO of the indicated documents, characterized by the applicant, and including page counts, where applicable. It serves as evidence of receipt similar to a Post Card, as described in MPEP 503.</b></p> <p><b><u>New Applications Under 35 U.S.C. 111</u></b><br/>If a new application is being filed and the application includes the necessary components for a filing date (see 37 CFR 1.53(b)-(d) and MPEP 506), a Filing Receipt (37 CFR 1.54) will be issued in due course and the date shown on this Acknowledgement Receipt will establish the filing date of the application.</p> <p><b><u>National Stage of an International Application under 35 U.S.C. 371</u></b><br/>If a timely submission to enter the national stage of an international application is compliant with the conditions of 35 U.S.C. 371 and other applicable requirements a Form PCT/DO/EO/903 indicating acceptance of the application as a national stage submission under 35 U.S.C. 371 will be issued in addition to the Filing Receipt, in due course.</p> <p><b><u>New International Application Filed with the USPTO as a Receiving Office</u></b><br/>If a new international application is being filed and the international application includes the necessary components for an international filing date (see PCT Article 11 and MPEP 1810), a Notification of the International Application Number and of the International Filing Date (Form PCT/RO/105) will be issued in due course, subject to prescriptions concerning national security, and the date shown on this Acknowledgement Receipt will establish the international filing date of the application.</p> |                                                |                                            |                                          |    |    |

**MITAPIVAT THERAPY AND MODULATORS OF CYTOCHROME P450**

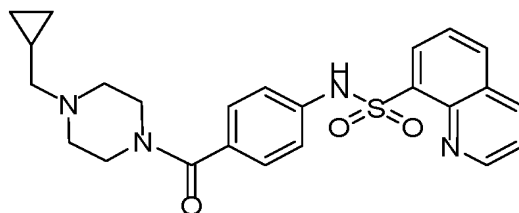
**RELATED APPLICATIONS**

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**

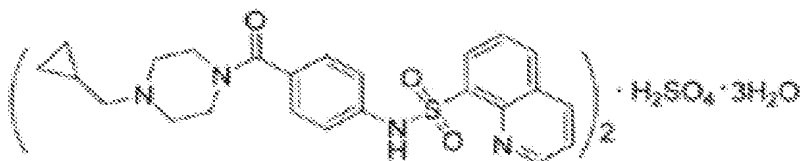
[0001] Pyruvate kinase deficiency (PKD) is a disease of the red blood cells caused by a deficiency of the pyruvate kinase R (PKR) enzyme due to recessive mutations of PKLR gene. PKR activators can be beneficial to treat PKD, thalassemia (*e.g.*, beta-thalassemia), abetalipoproteinemia or Bassen-Kornzweig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

“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 mitavipat or a pharmaceutically acceptable salt thereof (*e.g.*, mitavipat sulfate) is between about 0.01-100 mg/kg body weight/day of mitavipat or a pharmaceutically acceptable salt thereof (*e.g.*, mitavipat sulfate), *e.g.*, about 0.1-100 mg/kg body weight/day.

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

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

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

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

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

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

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

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

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

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

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

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

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

### **Compositions and Administration**

**[0033]**

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

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

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

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

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

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

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

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

#### **Doses and Dosing Regimens**

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

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

[illegible]

[illegible]

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

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

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

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

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

formulated for administration at a dose equivalent to about 200 mg to about 360 mg of mitapivat thereof once per day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 5 mg of mitapivat once every other day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof is formulated for administration at a dose equivalent to about 20 mg of mitapivat once every other day. In one aspect, mitapivat or a pharmaceutically acceptable salt thereof 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<sub>0-24</sub>, AUC<sub>0-t</sub>, AUC<sub>0-inf</sub>, AUC<sub>%estrap</sub>) and C<sub>max</sub> values (*i.e.*, the maximum (or peak) serum concentration that a drug achieves in a specified compartment or test area of the body after the drug has been administered and before the administration of a second dose). In one embodiment, step (a) comprises measuring and analyzing other PK parameters such as but not limited to C<sub>L</sub>/F (*i.e.*, apparent total body clearance of the drug from plasma), V<sub>Z</sub>/F (*i.e.*, apparent volume of distribution during

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

**[0082]** In one embodiment, the method of any one of the preceding embodiments comprises administering to the subject about 1 mg to about 2500 mg, or about 5 mg to 2500 mg, or about 30 mg to 2500 mg, or about 5 mg to about 700 mg, or about 15 mg to about 700 mg, about 5 mg to about 360 mg, about 5 mg to about 300 mg, about 50 mg to about 300 mg, about 120 mg to about 360 mg, about 120 mg to about 300 mg, about 5 mg to about 120 mg,

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

**[00107]** In one embodiment, in the method of any one of the suitable preceding embodiments, the inhibitor of CYP3A4/5 is selected from the group consisting of toamiodarone, amprenavir, aprepitant, atazanavir, chloramphenicol, clarithromycin, conivaptan, cyclosporine, darunavir, dasatinib, delavirdine, diltiazem, erythromycin,

fluconazole, fluoxetine, fluvoxamine, fosamprenavir, imatinib, indinavir, isoniazid, itraconazole, ketoconazole, lapatinib, miconazole, nefazodone, nelfinavir, posaconazole, ritonavir, quinupristin, saquinavir, tamoxifen, telithromycin, troleandomycin, verapamil, voriconazole, grapefruit, Seville oranges, limes, pomelos, fruit juices, and vegetables from mustard green family.

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

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

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

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

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

**[00113]** In one embodiment, in the method of any one of the suitable preceding embodiments, the inhibitor of CYP3A4/5 is itraconazole. In one aspect, about 0.1-1000 mg of itraconazole is administered to the subject. In one aspect, itraconazole is administered in the form of an oral solution. In one aspect, about 50-500 mg of itraconazole is administered to the subject.

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

**Diseases, Disorders and Conditions**

**[00115]** Pyruvate kinase deficiency (PKD) is a glycolytic enzymopathy that results in life-long hemolytic anemia. In certain embodiments, the subject having PKD is a patient having at least 2 mutant alleles in PKLR gene. In certain embodiments, the subject having PKD is a patient having at least 2 mutant alleles in PKLR gene and at least one is a missense mutation. See Canu. et al., Blood Cells, Molecules and Diseases **2016**, 57, pp. 100-109. In certain embodiments, a subject having PKD has an Hb concentration less than or equal to 10.0 g/dL. In certain embodiments, the subject having PKD is an adult not under regular transfusion (*e.g.*, having had no more than 4 transfusion episodes in the 12-month period up to the treatment). In certain embodiments, the subject having PKD is an adult transfusion independent (*e.g.*, having no more than 3 units of RBCs transfused in the 12-month period prior to the treatment). In certain embodiments, the subject having PKD is an adult under regular transfusion (*e.g.*, having had at least 4 transfusion episodes (*e.g.*, at least 6 transfusion episodes) in the 12-month period prior to the treatment). In certain embodiments, the subject having PKD has a total number of at least 5 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 10 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 15 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 20 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 25 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 30 transfusion episodes during the subject's lifetime. In certain embodiments, the subject having PKD has a total number of at least 40 transfusion episodes during the subject's lifetime. In certain

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

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

**[00117]** In certain embodiments, the subject with PKD achieves a hemoglobin response of at least 1.0 g/dL increase in Hb concentration after the treatment compared to the baseline of prior to the treatment. In certain embodiments, the subject with PKD achieves a hemoglobin response of at least 1.5 g/dL increase in Hb concentration from baseline prior to the treatment. In certain embodiments, the subject with PKD achieves a hemoglobin response of at least 2.0 g/dL increase in Hb concentration from baseline prior to the treatment.