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REVIEW ARTICLE

Mitapivat: A Novel Drug Discovery for the Treatment of Hereditary Hemolytic Anaemias

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Abstract:

Mitapivat (AG-348) is a novel, first-in-class oral small-molecule allosteric activator of the pyruvate kinase enzyme. Mitapivat has been shown to significantly upregulate both wild-type and numerous mutant forms of erythrocyte pyruvate kinase (PKR), increasing adenosine triphosphate (ATP) production and reducing levels of 2,3-diphosphoglycerate. Given this mechanism, mitapivat has been evaluated in clinical trials in a wide range of hereditary hemolytic anaemias, including pyruvate kinase deficiency (PKD), sickle cell disease, and thalassemia.

The technique entails searching numerous search engines, such as PubMed, Science Direct, and Sci Finder, for relevant citations to the current subject matter. This is done in order to obtain the data that is required. In relation to medicine, mitapivat has been examined for its ability to cure a wide variety of inherited haemolytic anaemias in clinical investigations. Some examples of these conditions include pyruvate kinase deficiency (PKD), sickle cell disease, and thalassemias.

It has been demonstrated that mitapivat is both safe and effective in treating adults with PKD in two phases III clinical trials, and the development of the medicine is very close to being finished. Based on these findings, mitapivat may end up becoming the very first medication in the history of the world to receive regulatory approval.

Allosteric activator of pyruvate kinase mitapivat has shown promise in treating various hereditary hemolytic anemias, including sickle cell disease, PKD, and alpha- and beta-thalassemia.

Keywords: Mitapivat, Haemolytic anaemia, Clinical trials, Sickle cell disease, FDA, Thalassemia, Pyruvate kinase deficiency.

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1. INTRODUCTION

Pyruvate kinase deficiency is a rare inherited disorder which affects the red blood cells and glycolysis, ultimately leading to persisting haemolytic anaemia. The Embden-Meyerhof glycolytic pathway is a process in the body that breaks down glucose to produce energy in the form of ATP. The final step of this pathway is carried out by an enzyme called pyruvate kinase, which converts phosphoenolpyruvate into pyruvate, generating ATP. In mammals, there are four different types of pyruvate kinase enzymes, each encoded by two genes. While most cells in the human body can generate ATP through aerobic metabolism, some cells, such as red blood cells, lack the ability to do so and rely on anaerobic glycolysis for energy production. If there is a deficiency in ATP production, red blood cells may be destroyed prematurely, leading to hemolytic anaemia. Pyruvate kinase deficiency is the most common congenital nonspherocytic hemolytic anaemia worldwide, resulting from a family of glycolytic enzyme defects. Other conditions that cause increased stress and energy utilization in red blood cells may also lead to a lack of ATP production, resulting in premature cell death [1]. Therefore, treatments that can increase ATP production in red blood cells may be helpful in various types of hemolytic anaemias.

Mitapivat (AG-348) is a type of medication that activates the pyruvate kinase enzyme, which is involved in producing ATP in the body. This medication is taken orally as a small molecule allosteric activator, which means it binds to a different site on the enzyme than fructose bisphosphate (FBP), activating both normal and mutant forms of the enzyme. This is especially promising for individuals with pyruvate kinase deficiency (PKD) and other hemolytic anaemias with increased energy demands. Many clinical trials have been conducted, are currently ongoing, and are planned to evaluate the effectiveness of Mitapivat in treating these conditions. These trials will help researchers better understand how mitapivat works and its potential for treating a wide range of hereditary hemolytic anaemias. In summary, mitapivat has shown promising results in preclinical studies and clinical trials,

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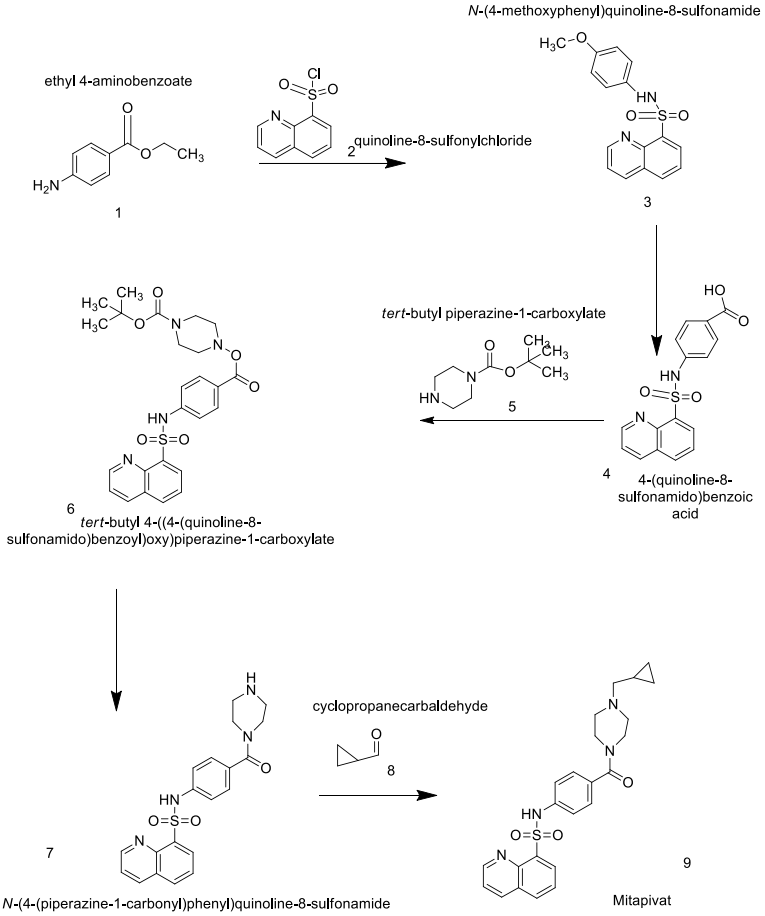
making it a potentially useful medication for individuals with PKD and other hemolytic anaemias with increased energy demands.

2. CHEMISTRY

Mitapivat is available as a salt form of sulphate with the chemical formula $C_{48}H_{60}N_8O_{13}S_3$, and its molecular weight is 450.56 g·mol−1. It is prepared by (Scheme **1**) mitapivat, which is a compound with a complex chemical name. It is known as N-(4-(4-(Cyclopropylmethyl)piperazine-1-carbonyl) Phenyl quinoline-8-sulfonamides [2]. It is used in tablet forms, and Some more important properties of mitapivat are provided in Fig. (**1**) and Table **1**.



Scheme (1). Synthesis pathway of Mitapivat [2].

Table 1. Some important properties of Mitapivat.

Synonyms	**N4JTA67V3O, Pyrukynd**
Trade names	Pyrukynd
Mechanism of action	Mitapivat is a pyruvate kinase enzyme activator.
Use	Anaemia in adults with pyruvate kinase (PK) deficiency.
Route of administration	Oral
Dose	5 mg twice daily
Pharmacokinetics	Rapid oral absorption, good oral bioavailability, and a high volume of distribution at steady state
Adverse reaction	Discomfort or pain in the back area, watery bowel movements, Constipation, dry mouth.
Chemical formula	$C_{48}H_{60}N_8O_{13}S_3$
Molecular weight	450.56 g·mol−1

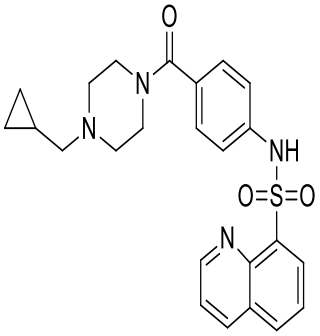


Fig. (1). Mitapivat structure.

3. MECHANISM OF ACTION

Pyruvate kinase is an essential enzyme in the Embden-Meyerhof glycolytic pathway, generating ATP. It plays a vital role in converting phosphoenolpyruvate to pyruvate, producing adenosine triphosphate (ATP) necessary for cellular function. Among its isoforms, erythrocyte pyruvate kinase (PKR) is found in red blood cells. Since red blood cells lack the ability for aerobic glucose metabolism and ATP production, they rely on anaerobic glycolysis to generate ATP [1]. Abnormalities in glycolytic enzymes like pyruvate kinase can lead to energy deprivation, resulting in chronic haemolytic anaemia, ineffective erythropoiesis, and premature destruction of red blood cells. Pyruvate kinase deficiency is a rare genetic condition caused by mutations in the PKLR gene, responsible for both the red blood cell (PKR) and liver-specific (PKL) isoforms of pyruvate kinase. Insufficient pyruvate kinase activity is associated with elevated levels of 2,3-disphosphoglycerate (2,3-DPG), a glycolysis metabolite, and low ATP levels. Erythrocyte pyruvate kinase is regulated by allosteric control, with fructose bisphosphate (FBP) acting as an allosteric activator [2, 3]. Mitapivat, although binding to a different allosteric site on the PKR tetramer compared to FBP, functions as an allosteric activator of pyruvate kinase. It can activate both wild-type and mutant forms of erythrocyte pyruvate kinase, even when FBP cannot activate the enzyme. Mitapivat enhances the enzyme's affinity for its substrate, phosphoenolpyruvate, and stabilizes the active tetrameric form of the enzyme. As a result, mitapivat increases erythrocyte pyruvate kinase activity and ATP production and reduces 2,3-DPG levels [4]. Fig. (**2**) mentions mitapivat action.

3.1. Pharmacodynamics

3.1.1. Exposure-response Relationship

Although it hasn't been examined, the exposure-response relationship for Mitapivat after topical treatment is unlikely because systemic exposure after topical use is hardly detectable.

3.2. Pharmacokinetics

3.2.1. Absorption

After a single dose, mitapivat has an approximate absolute bioavailability of 73%. The exposure to mitapivat increases proportionally with the dose. At steady state, when Mitapivat was administered orally twice daily at doses of 5 mg, 20 mg, and 50 mg, the mean (CV%) Cmax (maximum plasma concentration) was 101.2 (17%) ng/mL, 389.9 (18%) ng/mL, and 935.2 (18%) ng/mL, respectively. The mean (CV%) AUC (area under the concentration-time curve) was 3591.4 (28%) ng x h/mL, 450.4 (28%) ng x h/mL, and 1623.0 (28%) ng x h/mL, respectively. The median Tmax (time to reach maximum concentration) at steady state ranged from 0.5 to 1.0 hours post-dose across the dosage range of 5 mg to 50 mg twice a day [5]. Compared to fasting, administering mitapivat with a high-fat meal resulted in a 42% lower Cmax and a 2.3-hour delay in Tmax in healthy participants. However, it also slowed down the absorption rate of mitapivat.

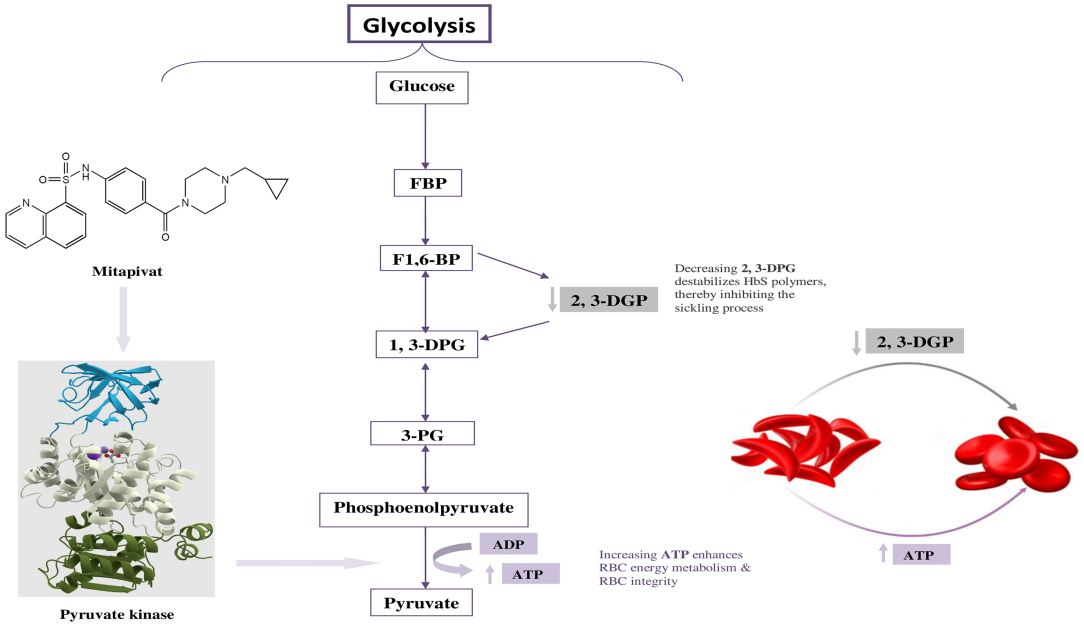


Fig. (2). Molecular mechanism of action Mitapivat.

3.2.2. Volume of Distribution

At a state of equilibrium, the substance's average distribution volume (Vss) was determined to be 42.5 litres.

3.2.3. Protein Binding

Mitapivat has a 0.37 RBC-to-plasma ratio and is 97.7% bound to plasma proteins.

3.2.4. Metabolism

In vitro investigations have shown that CYP3A4 is primarily responsible for the metabolism of mitapivat. Additionally, mitapivat acts as a substrate for CYP1A2, CYP2C8, and CYP2C9.1. In studies involving healthy volunteers, the main circulating substance in plasma after a single oral dose of 120 mg of radiolabelled Mitapivat was unaltered mitapivat.

3.2.5. Route of Elimination

Pyrukynd, also known as Mitapivat, is primarily eliminated through hepatic metabolism. In studies involving healthy participants, a single oral administration of radiolabelled Mitapivat resulted in an overall recovery of 89.2% of the radioactive dose administered. Approximately 49.6% of the radioactivity was recovered in the urine, with only 2.6% excreted as unchanged mitapivat [6]. About 39.6% of the radioactivity was found in the faeces, with less than 1% of it being the unaltered drug.

3.2.6. Half-life

In individuals with pyruvate kinase deficiency who received multiple doses of 5 mg to 20 mg of mitapivat twice daily, the average effective half-life (t1/2) of the drug ranged from 3 to 5 hours.

3.2.7. Clearance

According to population pharmacokinetics, the median CL/F at steady state for the 5 mg twice daily, 20 mg twice daily, and 50 mg twice daily regimens were 11.5, 12.7, and 14.4 L/h, respectively.

3.3. Clinical Study

3.3.1. PKD Background

PKD is a rare type of inherited anaemia that occurs in about 1 in every 20,000 to 1 in every 300,000 people. In some areas where malaria is common, the prevalence might be even higher. The disease has a lot of genetic variations, with over 350 identified mutations in the PKLR gene, mainly due to changes in a single amino acid. Diagnosis of PKD involves measuring the activity of certain enzymes or conducting molecular tests [7]. Symptoms and the severity of the disease can vary widely among patients. Some individuals may have mild anaemia without noticeable symptoms, while others require lifelong blood transfusions from birth. In addition to the effects of chronic anaemia, such as low energy levels, reduced ability to exercise, difficulties with memory, and fatigue, PKD patients can also experience long-term complications related to the breakdown of red blood cells and problems with blood cell production. These complications include iron overload, abnormal growth of red blood cells in other body parts, gallstones, weakened bones, hormonal issues, delayed puberty, and leg ulcers. Currently, there are no approved medications specifically for treating PKD. Removing the spleen can help improve anaemia and slightly increase haemoglobin levels for some patients. However, this is not effective for everyone. Another option, haematopoietic stem cell transplantation, is considered risky and is mainly used for severe cases that require frequent blood transfusions [8]. Most patients receive supportive care, which includes taking folic acid supplements, receiving red blood cell transfusions (to alleviate symptoms rather than targeting a specific haemoglobin level), and managing complications associated with PKD, such as iron chelation therapy and bisphosphonates for bone health.

3.3.2. Phase II DRIVE-PK Study

After promising results in preclinical and phase I studies, a phase II clinical trial called DRIVE-PK was conducted to investigate the safety and efficacy of mitapivat in adults with pyruvate kinase deficiency (PKD) who did not require regular blood transfusions [9, 10]. The study involved 52 anaemic adults (38% female) who had received three or fewer units of red blood cell transfusions in the 12 months prior to starting mitapivat treatment, with no transfusions in the 4 months preceding treatment.

During the 24-week core study period, the participants were randomly assigned to receive either 50mg or 300mg of mitapivat twice daily [11]. The study's primary objective was to evaluate the safety profile and potential side effects of mitapivat. Rigorous monitoring was conducted through frequent laboratory tests, electrocardiography, and physical examinations to detect any acute or subacute toxicities associated with the drug [12]. Dual-energy X-ray absorptiometry (DEXA) scans were also performed periodically to monitor changes in bone density. This monitoring was important to assess any effects on bone mineral density resulting from the drug's off-target inhibition of aromatase, as well as potential positive effects on bone density due to the reduction in ineffective erythropoiesis and expansion of red blood cells [13, 14]. The study's secondary objectives included analyzing the pharmacokinetics and pharmacodynamics of mitapivat. Additionally, the clinical effectiveness of mitapivat was assessed by measuring changes in haemoglobin levels and markers of hemolysis. Participants had the option to participate in the long-term extension of the study, which allowed for further evaluation of mitapivat's effects beyond the core study period. During the DRIVE-PK study, the participants generally tolerated mitapivat well. The most commonly reported adverse events were mild headaches, experienced by 24 patients, followed by insomnia in 22 patients and nausea in 21 patients. Fortunately, the majority of these events resolved within a week after starting the medication [15]. Some serious adverse events potentially related to mitapivat were observed in multiple patients, including hypertriglyceridemia in four patients and rebound hemolysis in two patients.

In terms of efficacy, approximately half of the patients (26 out of 52) experienced an increase in haemoglobin levels of 1.0g/dl or more compared to their baseline levels. The average maximum increase in haemoglobin was 3.4g/dl, with a range of 1.1g/dl to 5.8g/dl. The median time for haemoglobin increase was just 10 days, and the improvements were sustained in the majority of patients who continued the treatment. The response to mitapivat varied depending on the specific genetic mutations of the patients [16]. Those with two significant mutations or two copies of a specific mutation did not show a response to the treatment. However, patients with two other types of mutations were more likely to respond positively. The initial level of the PKR protein in red blood cells at the beginning of the study also correlated with the response in haemoglobin levels. Markers of haemolysis, such as reticulocyte count, indirect bilirubin, and haptoglobin, also improved in patients who experienced a haemoglobin response. The pharmacokinetics and pharmacodynamics of mitapivat in patients with PK deficiency were similar to what was observed in previous phase I studies involving healthy volunteers [17, 18]. Considering the potential impact of mitapivat on bone mineral density, it is important to assess its effect on PKD patients, who already have a high occurrence of osteopenia and osteoporosis. Analysis of long-term data from the DRIVE-PK study, involving patients treated for up to 56 months, showed that bone mineral density remained stable over time in adults with PKD who received mitapivat. This stability is promising, as PKD patients typically experience progressive worsening of bone mineral density [19]. Further studies with longer follow-up periods are needed to fully understand the potential impact of mitapivat on bone health.

3.3.3. Phase III ACTIVATE Study

The ACTIVATE study was a phase III clinical trial designed to evaluate the effectiveness and safety of mitapivat in adults with PKD who did not require regular transfusions. The study included patients who had experienced four or fewer transfusion episodes in the year before enrollment. Eligible patients needed to have specific genetic mutations and a baseline haemoglobin level of less than 10.0g/dl. Patients who had undergone splenectomy or hematopoietic stem cell transplantation were excluded [20, 21]. The participants were randomly assigned to receive either mitapivat or a placebo. The treatment period consisted of a 12-week individualized dose escalation phase followed by a 12-week fixed-dose period. Patients who completed the study had the option to continue in an ongoing open-label extension study. The primary objective of the ACTIVATE study was to assess the haemoglobin response to mitapivat, defined as a sustained increase of at least 1.5g/dl at multiple assessments during the fixed-dose period [22]. Secondary endpoints included changes in haemoglobin levels, reticulocyte count, and markers of haemolysis. Patient-reported outcome measures were also used to evaluate the impact on quality of life. The study enrolled 80 patients, with a balanced distribution between the mitapivat and placebo groups.

Mitapivat demonstrated positive results in meeting both the primary and secondary endpoints. Forty per cent of patients in the mitapivat group achieved a haemoglobin response, compared to none in the placebo group. The average increase in haemoglobin from baseline was significant in the mitapivat group, while it slightly decreased in the placebo group. Other markers of disease severity showed improvements with mitapivat treatment [23]. Patients receiving mitapivat reported enhancements in their quality of life. In terms of safety, mitapivat was well-tolerated, with the most common adverse events being nausea and headache, which were more frequent in the placebo group. No adverse events led to treatment discontinuation. Overall, the results of the ACTIVATE study were promising, demonstrating the effectiveness of mitapivat in improving haemoglobin levels, reducing disease markers, and enhancing the quality of life for PKD patients. The safety data supported its use as a well-tolerated treatment option [24, 25].

3.3.4. Phase III ACTIVATE-T Study

The ACTIVATE-T study was a phase III, single-arm, open-label study that evaluated the effectiveness and safety of mitapivat in adults with PKD who required regular transfusions. These patients had undergone six or more transfusion episodes in the year before the study. The study enrolled patients with specific genetic mutations and excluded those who had undergone splenectomy within the previous year. The treatment consisted of a 16-week individualized dose-escalation period followed by a 24-week fixed-dose period [26]. The primary endpoint of the ACTIVATE-T study was a reduction in transfusion burden, defined as a 33% decrease in transfusion requirements during the 24-week fixed-dose period compared to the patient's historical transfusion burden over the same duration [27]. Secondary endpoints included the proportion of transfusion-free responders and the annualized number of red blood cell units transfused. A total of 27 patients were enrolled, with 20 completing the study. Ten patients (37%) achieved a reduction in transfusion burden of at least 33%, and six patients (22%) remained transfusion-free during the fixed-dose period. The annualized number of red blood cell units transfused decreased by 39%. The treatment with mitapivat was well-tolerated, and no treatment-emergent adverse events led to treatment discontinuation [28]. These results suggest that mitapivat has the potential to reduce transfusion requirements and improve outcomes in transfusion-dependent patients with PKD.

In a phase II study of mitapivat in non-transfusion-dependent thalassemia, including beta-thalassemia, haemoglobin E/beta-thalassemia, and haemoglobin H disease, 20 patients were enrolled. The study aimed to evaluate the efficacy and safety of mitapivat in this patient population. The treatment period lasted for 24 weeks, with a potential dose escalation to 100mg twice daily after 6 weeks [29, 30]. The primary endpoint was a haemoglobin response, defined as an increase of 1.0g/dl or more from baseline at any time between weeks 4 and 12. The study successfully met its primary endpoint, with 16 patients (80%) achieving a haemoglobin response. The response was observed in patients with beta-thalassemia as well as alpha-thalassemia. Improvements in haemoglobin levels were sustained in patients who continued treatment, and markers of erythropoiesis and hemolysis also showed improvements. The safety profile of mitapivat in this

study was similar to previous studies, with most adverse events being mild or moderate [31]. These findings suggest that mitapivat has the potential to improve haemoglobin levels and provide clinical benefits for patients with non-transfusion-dependent thalassemia, including both beta-thalassemia and alpha-thalassemia.

3.3.5. Phase I and II Studies of Mitapivat in Sickle Cell Disease

The phase I multiple ascending dose study of mitapivat in sickle cell disease enrolled 17 patients with HbSS sickle cell disease. The study aimed to evaluate the safety, tolerability, and efficacy of mitapivat in this patient population. Patients were required to have a baseline haemoglobin level of at least 7.0g/dl and had not received transfusions or erythropoietin therapy in the preceding 3 months [32]. Stable doses of hydroxyurea and/or l-glutamine were allowed during the study. Patients received three or four ascending doses of mitapivat (5, 20, 50, and 100mg twice daily) for 2 weeks each during the study. The primary objective was to assess the safety and tolerability of mitapivat, while secondary endpoints included changes in haemoglobin levels, haemolytic markers, 2,3-DPG and ATP levels, and markers of Hb S polymerization (p50). The results indicated that mitapivat was generally safe and well-tolerated [33, 34]. Only one serious treatment-emergent adverse event (TEAE), a vaso-occlusive crisis during the drug tapering period, was possibly related to the study drug.

In terms of efficacy, at the dose of 50mg twice daily, the mean change in haemoglobin was +1.2g/dl, with a range of -0.3 to +2.9g/dl. However, haemoglobin levels returned to baseline after tapering the drug [35, 36]. Nine out of 16 patients achieved a haemoglobin response at any dose level, defined as an improvement of 1.0g/dl or more relative to baseline. Improvements were also observed in haemolytic markers, including lactate dehydrogenase, total bilirubin, reticulocytes, and aspartate aminotransferase, with normalization after discontinuation of mitapivat. Furthermore, mean 2,3-DPG levels decreased, ATP levels increased in a dose-dependent manner, and decreases in p50 (a marker of Hb S polymerization) were observed. These findings suggest that mitapivat showed promising safety and tolerability in patients with sickle cell disease. It also demonstrated potential for improving haemoglobin levels and modifying key disease-related parameters, including hemolysis, 2,3-DPG levels, ATP levels, and Hb S polymerization markers [15]. However, further research is necessary to establish the long-term efficacy and safety profile of mitapivat in sickle cell disease.

3.4. Safety

It has been observed that mitapivat does not appear to be toxic to the body as a whole, but some side effects are connected to mitapivat, such as hives, breathing problems, swelling of the face, lips, tongue, or throat, yellowing of the skin or eyes (jaundice), dark urine, dizziness, disorientation, fatigue, and shortness of breath, *etc*.

Mitapivat was given orally to male rats twice daily before and during mating in research on fertility and early embryonic development. At doses up to 300 mg/kg/day, or 45 times the MRHD (Macular hole retinal detachment) of 50 mg twice daily, the drug had no adverse effects on sperm count or reproductive function.

3.5. Dosage and Administration

Mitapivat can be taken with or without food and should be swallowed whole. The tablets should not be chewed, split, crushed, or dissolved [37]. PYRUKYND 5 mg can be used orally as a starter dose. To gradually raise haemoglobin, titrate PYRUKYND from 5 mg twice daily to 20 mg twice daily, then up to the maximum recommended dose of 50 mg twice daily, with each dose increment occurring every 4 weeks (Hb).

Some patients may obtain and maintain normal haemoglobin levels by taking 5 mg twice daily or 20 mg twice daily [38]. Based on the results of the haemoglobin and haemolysis laboratory tests, as well as the need for transfusions, discontinue PYRUKYND if no benefit has been seen after 24 weeks. PYRUKYND should be taken as soon as possible if a dose is missed by 4 hours or less. Do not give a replacement dose of PYRUKYND if the missing dose was missed by more than 4 hours; wait until the next dose is planned. Return to the regular dose regimen after that.

3.6. Adverse Effects

The most frequent adverse effects of Pyrukynd are erectile dysfunction, breast development in males, infertility in men, severe joint pain, redness and swelling, malformed joints, weariness, anxiety, impatience, melancholy, forgetfulness, sexual dysfunction, water retention, bone loss, fat buildup, excessive or frequent sleep, joint pain, and back discomfort [39]. The amount of data from PYRUKYND clinical studies that are now available is insufficient to assess the drug's potential link to significant birth abnormalities, miscarriage, or other undesirable maternal or foetal outcomes.

3.7. Storage and Handling

Mitapivat should be kept between 68°F and 77°F (20°C and 25°C) at room temperature, and blister wallets should be kept in their original carton until usage.

4. DRUG INTERACTIONS

Plasma concentrations of mitapivat were raised by administering PYRUKYND together with potent CYP3A inhibitors. The risk of negative effects with PYRUKYND may increase the higher plasma concentrations of mitapivat. Mitapivat plasma concentrations will rise when mild CYP3A inhibitors and PYRUKYND are administered together. Strong CYP3A inhibitors should not be used together with PYRUKYND. If you're on PYRUKYND, consider substituting medicines that do not moderately induce CYP3A [40]. Evaluate Hb and titrate beyond 50 mg twice daily, if necessary, without other medications, but keep doses under the maximum advised level of 100 mg twice daily.

Pyrukynd induces CYP3A. PYRUKYND can reduce the systemic levels of drugs like hormonal birth control with sensitive CYP3A substrates (*e.g*., ethinyl oestradiol). PYRUKYND activates the CYP2B6, CYP2C8, CYP2C9, and

CYP2C19 enzymes *in vitro*, which could reduce systemic levels of drugs that are sensitive substrates for these enzymes.

CONCLUSION

Mitapivat, an allosteric activator of pyruvate kinase, has shown promise in treating certain hereditary hemolytic anaemias, such as sickle cell disease, PKD, and alpha- and beta-thalassemia. Its mode of operation hints that it might be beneficial in treating a wide variety of hemolytic disorders and erythropoiesis that are not functioning properly. Mitapivat's efficacy and safety in treating PKD have been shown by finished human research, including a phase III randomized study in the condition. These trials have indicated that Mitapivat is well tolerated and improves haemoglobin levels, the need for transfusions, and markers of hemolysis and hematopoiesis.

LIST OF ABBREVIATIONS

PKR	=	Pyruvate Kinase
ATP	=	Adenosine Triphosphate
PKD	=	Pyruvate Kinase Deficiency

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

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