CRITICAL REVIEW
Pyruvate Kinase Deficiency and Developments
In its Treatment

ARTIST
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ABSTRACT
Pyruvate kinase (PK) is an enzyme involved in ATP production in the glycolytic pathway. PK deficiency is a rare, autosomal recessive disorder that manifests with hemolytic anemia of variable severity, partly due to an insufficiency of ATP production in erythrocytes. Its exact prevalence is unknown due to lack of awareness and challenges in making the diagnosis of PK deficiency. Current treatments for PK deficiency include blood transfusion and splenectomy, which focus on temporarily alleviating anemia and reducing hemolysis, respectively. Due to the rarity of this condition and its variable severity, a systematic treatment protocol for novel therapies is difficult to compile and validate. Oral administration of mitapivat, an allosteric activator of erythrocyte PK, is a novel treatment. Despite its promising effects on the hemoglobin levels in PK deficient patients, mitapivat’s efficacy and safety profiles must be determined through randomized controlled trials.

INTRODUCTION
Pyruvate kinase (PK) deficiency results in impaired PK activity in erythrocytes, and is caused by mutations in the PKLR gene. It presents with hemolytic anemia, which occurs when erythrocytes are lysed at a faster rate than they are produced, reducing erythrocyte numbers.1 Due to hemolytic anemia, an individual with PK deficiency can experience lethargy, pallor and jaundice. Other complications of this condition include splenomegaly, gallstones, and pulmonary hypertension.2,3

As PK deficiency is rare, its true prevalence is currently unknown.4 In addition, current prevalence estimates of PK deficiency are skewed due to errors in diagnosis. Diagnostic accuracy is an important consideration when analyzing prevalence, as PK deficiency is only diagnosed when physicians purposefully evaluate erythrocyte assays. Furthermore, errors in diagnosis persist due to the failure to associate hemolytic anemia with a genetic disorder such as PK deficiency.4 Even with the presence of enzyme assays and genetic testing, the variability and complexity of clinical presentation often result in delayed PK deficiency diagnosis.

Although the true prevalence of PK deficiency remains elusive, it has been established that PK deficiency has a worldwide distribution, with greater occurrence in northern Europe and regions of China. As PK deficiency is an autosomal recessive disorder, it is equally prevalent in male and female populations. However, women between the ages of 21 and 30 have a higher than basal prevalence; this increase is attributed to pregnancy, which is hypothesized to worsen anemia in those with PK deficiency.5 PK deficiency is also more prevalent in areas or social groups with higher rates of consanguinity, which can occur in isolated populations where the "founder effect" significantly reduces genetic variance.6,7

The diagnosis of PK deficiency requires confirmation of hemolytic anemia, accompanied by low PK activity and/or positive genetic testing for mutations in the PKLR gene. This usually involves genetic screening or complete blood count assays and peripheral blood smears. These diagnostic tests are performed in individuals with unexplained anemia or hemolysis, particularly if there is a family history of congenital hemolysis coupled with PK deficiency.

Initial work-up for hemolysis usually involves complete blood count, peripheral blood smear, and reticulocyte count, along with measurement of bilirubin, haptoglobin and a direct antiglobulin test. This helps to assess the severity of hemolytic anemia and aids in the elimination of other acquired and inherited disorders with similar presentations.2,3,7 The differential diagnosis of PK deficiency includes acquired hemolysis and congenital anemias.

This review discusses the nature of PK deficiency, current treatments and mitapivat, a novel therapy that aims to address the symptoms of PK deficiency.

PATHOPHYSIOLOGY
PK is responsible for transferring a phosphate group from phosphoenolpyruvate (PEP) to ADP, ultimately yielding 2 pyruvates and 2 ATPs per glucose molecule.8 The enzyme is expressed by either the PKLR or PKM genes in a tissue-specific manner. The PKLR gene codes for the PK enzyme in the liver and red blood cells (R-enzyme), whereas PKM codes for PK in muscle tissues and during fetal development (M2-enzyme). The M2-PK enzyme eventually persists in white blood cells and platelets.3
PK deficiency is an autosomal recessive disorder, meaning a child must inherit one mutated allele from each parent to display clinical features. While there are patients with homozygous mutations, most patients show compound heterozygosity, where each inherited mutation is different from the other. Clinical PK deficiency can be caused by over 250 mutations on the PKLR gene. These include missense, nonsense, insertion, and deletion mutations. Many of these mutations are geographically specific, such as the R486W and R510Q mutations in the PKLR gene, which are highly prevalent in Southern and Northern European populations, respectively.\(^1\)\(^2\)

R510Q, R486W, and other mutations on the PKLR gene may result in impaired formation of the enzyme's tetrameric structure and stability. Furthermore, these deleterious mutations can alter PK's affinity for PEP and allosteric modulators such as fructose 1,6-bisphosphate, resulting in PK deficiency.\(^3\)\(^4\) Impaired PK function leads to a decrease in glycolytic ATP production, as well as an accumulation of glycolytic intermediates. As erythrocytes primarily utilize ATP created by glycolysis, a decrease in the available energy is a contributing factor to hemolysis and the removal of erythrocytes from circulation.\(^5\) The effects of hemolytic anemia are partially offset by a accumulation of 2,3-bisphosphoglycerate (2,3-BPG), which binds to hemoglobin to improve oxygen delivery to tissues. 2,3-BPG levels are increased in patients with PK deficiency due to an accumulation of glycolytic intermediates, allowing for patients to better tolerate anemia-related symptoms.\(^6\)

### CURRENT TREATMENTS

Since PK deficiency is rare and prognosis varies greatly between individuals, there are no evidence-based management guidelines. Depending on the symptoms and severity presented by patients, healthcare providers can prescribe blood transfusions, surgical procedures, or investigational therapies including gene therapy and hematopoietic cell transplants.

The central feature of PK deficiency is hemolytic anemia.\(^7\) A common approach to alleviate the symptoms of hemolytic anemia and maintain hemoglobin levels is blood transfusion, the frequency of which depends on the severity of the symptoms. Children with severe PK deficiency may undergo frequent blood transfusions until splenectomy, which is typically deferred until the age of five when they can receive full immunization against Streptococcus pneumoniae. In a study with 250 PK deficient patients who underwent transfusions, 48% of patients aged five or younger received regular transfusions. In comparison, only 26% of patients between ages 5 to 12 received regular transfusions.\(^8\)

According to a medical record review, the most common reasons for splenectomy in PK deficiency were: to improve baseline anemia, decrease the burden of transfusions, and reduce jaundice.\(^9\) A review by Grace et al. suggests that PK deficiency treatment plans are often individualized on a case-by-case basis.\(^10\)

### FUTURE DIRECTIONS

Mitapivat is an orally administered allosteric activator of erythrocyte PK.\(^11\) A recent phase II multicenter study evaluated the efficacy and safety of mitapivat in 52 PK deficient patients who were not undergoing transfusions. This study randomly assigned patients to daily mitapivat doses of either 50mg or 300mg for a 24-week period.\(^12\) The results indicated that mild adverse events such as headaches (92%) and insomnia (47%) occurred at drug initiation and were resolved within a week.\(^13\) In the 29-month follow-up period, 16 out of the 52 patients experienced a grade 3 adverse event, while 3 patients experienced a grade 4 event. Furthermore, pharyngitis and hemolytic anemia occurred in only two patients.

The efficacy of this intervention was evaluated through monitoring changes in hemoglobin levels. 50% of patients had an increase of over 1.0 g/dL in their hemoglobin levels.\(^14\) Interestingly, this hemoglobin response was only found in patients who had at least one missense mutation. The 10 patients who had two non-missense mutations and 5 patients who were homozygous for R479H mutations did not have this response. This indicates that the response to mitapivat might depend on which mutations are causing the disease. Even though the mean maximum increase in hemoglobin was 3.4 g/dL, the study could not demonstrate a definitive relationship between mitapivat and hemoglobin levels.\(^15\)

Although these results are worth consideration, mitapivat does have side effects; Grace et al. did not depict the severity of the adverse outcomes. In the 29-month follow-up period, some patients had adverse

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outcomes including osteoporosis, ovarian cyst, renal-cell carcinoma, gastroenteritis, hernia, and thrombosis. While it is unclear which of these adverse events are a result of mitapivat as oppose to unrelated medical concerns, the effects are not transient and further study is required to evaluate the safety profile of the drug. Moreover, this uncontrolled phase II study does not provide sufficient quality of evidence to guide clinical treatment at this time. It is imperative that more rigorous studies, such as randomized controlled trials, be conducted to gain a better understanding of the efficacy and safety of mitapivat, as well as which patients are most likely to benefit from the treatment.

There is an ongoing, multicentre, open label study that is evaluating the long-term efficacy and safety of mitapivat. The primary outcomes of this study are adverse events while secondary outcomes include the change in concentrations of various relevant biomarkers (hemoglobin, bilirubin and erythrocyte count, etc.).

**CONCLUSION**

PK deficiency is a rare autosomal recessive disease that causes lifelong hemolytic anemia. The impaired functioning of PK in erythrocytes leads to a decrease in ATP production, contributing to hemolytic anemia. Current treatment options are limited to blood transfusion and splenectomy. Recent research suggests that mitapivat, an allosteric activator of PK, holds promise in improving anemia in some patients with PK deficiency. However, both its efficacy and safety require further study. Future research should focus on the safety of this drug and its efficacy for patients with different mutations in the PKLR gene. An example is the aforementioned extension study, which aims to evaluate adverse events of mitapivat administration, that is currently in the recruitment stage.

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