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Updates and advances in pyruvate kinase deficiency

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Abstract

Mutations in the *PKLR* gene lead to pyruvate kinase (PK) deficiency, causing chronic hemolytic anemia secondary to reduced red cell energy, which is crucial for maintenance of the red cell membrane and function. Heterogeneous clinical manifestations can result in significant morbidity and reduced health-related quality of life. Treatment options have historically been limited to supportive care, including red cell transfusions and splenectomy. Current disease-modifying treatment considerations include an oral allosteric PK activator, mitapivat, which was recently approved for adults with PK deficiency, and gene therapy, which is currently undergoing clinical trials. Studies evaluating the role of PK activators in other congenital hemolytic anemias are ongoing. The long-term effect of treatment with disease-modifying therapy in PK deficiency will require continued evaluation.

Recent advances in pyruvate kinase deficiency

Pyruvate kinase deficiency (see Glossary), a congenital **hemolytic anemia** caused by a glycolytic pathway defect, was first described in the 1960s. Over the past decade, through registry studies, our understanding of the clinical and genetic heterogeneity, symptoms, and potential complications has expanded. Despite this progress, diagnosing and managing patients with PK deficiency remains challenging because of difficulties in the diagnostic evaluation and heterogeneity of clinical manifestations. Supportive care with blood transfusions, splenectomy, and iron chelation have historically been the main management strategies. A recently approved **PK activator**, mitapivat, offers an innovative disease-directed approach that may considerably improve the disease burden and quality of life of many patients with PK deficiency. The safety and efficacy of mitapivat in adults with PK deficiency have been demonstrated with successful Phase 2 and Phase 3 clinical trials,

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and pediatric trials are underway. In addition, as a disease due to a single gene defect, gene therapy is a promising disease-modifying and potentially curative treatment. In the modern era of disease-targeted therapies, recognizing the symptoms and diagnosing patients with PK deficiency is even more critical. This review highlights the recent advances in the diagnosis and natural history of PK deficiency, as well as guidance for monitoring and supportive care, newly approved targeted treatments, and treatments in development.

Diagnosis, symptoms, monitoring, and supportive treatment approaches

Pathophysiology

Mature red blood cells lack a nucleus and mitochondria, thus relying on glycolysis for **adenosine triphosphate (ATP)** production in order to maintain structure and function. **Pyruvate kinase** is a key glycolytic enzyme and catalyzes the conversion of **phosphoenolpyruvate** to pyruvate with the production of ATP (Figure 1). PK deficiency causes red cell dehydration and disturbs the red cell membrane, leading to ineffective erythropoiesis and hemolysis. **Reticulocytes**, the youngest circulating red blood cells, require higher ATP levels than mature red cells and therefore are the most susceptible to PK deficiency, particularly in the splenic environment [1–3]. As PK is the terminal enzyme in glycolysis, the proximal glycolytic intermediates such as **2,3-biphosphoglycerate (2,3-BPG)** are often increased [4,5]. This causes a right shift in the hemoglobin–oxygen dissociation curve, leading to increased tissue oxygenation which can result in better anemia tolerance in some patients [5,6].

The ***PKLR*** gene, located on chromosome 1q21, encodes the liver and red cell PK isoenzymes. PK deficiency is autosomal recessive, caused by pathogenic compound heterozygous or homozygous mutations in *PKLR*. Pathogenic mutations in *PKLR* affect the PK enzyme by altering its affinity for phosphenolpyruvate (its substrate) or fructose 1,6-bisphosphate (its allosteric activator). *PKLR* variants can also result in decreased protein stability or altered stability of PK homotetramers [7,8].

Epidemiology

PK deficiency is genetically heterogeneous, with over 300 pathogenic *PKLR* variants having been described. The variants include single-nucleotide substitutions, as well as intronic and exonic deletions and insertions. The most common mutations include Arg510Gln, which is found in Northern Europe and the USA, followed by Arg486Trp in the Southern European population. A particularly high frequency exists among the Pennsylvania Amish (Arg479His) and Romany communities (1149 base pair deletions resulting in the loss of Exon 11) [9–11]. Most patients have at least one **missense** *PKLR* mutation. Highly symptomatic clinical phenotypes are generally associated with mutations that cause premature stop codons, frameshifts, or large deletions; however, there is relatively little predictive value between genotype and phenotype and the severity of the clinical course [12].

The estimated prevalence of PK deficiency is 1 to 8 per 1,000,000 [13–15]. PK deficiency has a worldwide distribution, with a possibly higher frequency in malaria-endemic regions

and in isolated populations because of a founder effect. A large number of patients remain undiagnosed for various reasons including difficulties in accessing diagnostic testing, misdiagnosis, and mild disease [16–18].

Molecular and biochemical diagnosis

Testing for PK deficiency should be considered in patients with chronic hemolysis when more common causes have been excluded, including autoimmune hemolytic anemia, red cell membranopathies, and hemoglobinopathies (Figure 2). Diagnosing PK deficiency requires high clinical suspicion, given the widely variable clinical phenotype and nonspecific red cell morphology.

Given the potential limitations of both PK enzyme activity and *PKLR* genetic testing, the diagnosis of PK deficiency is made through a combination of both tests, if available. Low PK enzyme activity can be found in both carriers and disease states. Falsely normal PK activity can be seen in transfused patients as a result of contamination with healthy red cells or leukocytes, increased reticulocytes, or nonphysiologic substrate concentrations [19,20]. In the presence of reticulocytosis, comparing PK activity with the activity of other age-dependent red cell enzymes, such as hexokinase, increases the sensitivity of PK enzyme testing [21,22]. Somewhat counterintuitively, the measured PK enzyme activity does not correlate with the severity of anemia or hemolysis [1,23,24].

Genetic testing for congenital hemolytic anemias is increasingly available, including through commercial laboratories using next-generation sequencing [25]. Up to 20% of individuals may have newly described *PKLR* variants of uncertain significance; in these cases, low PK activity can provide additional support for the diagnosis [3,26,27]. Additionally, up to 10% of individuals with reduced PK enzyme activity and a clinical presentation consistent with PK deficiency may have falsely normal genotyping in one or more *PKLR* alleles if only exon sequencing is performed, due to a missed deep intronic mutation or large deletion [28].

Heterogeneous spectrum of clinical and laboratory manifestations

Laboratory and clinical manifestations of PK deficiency are variable and are secondary to the symptoms and complications of chronic hemolysis (Figure 3).

Newborns may present with wide-ranging manifestations from unrecognized mild compensated anemia to neonatal hyperbilirubinemia and anemia requiring exchange transfusions to fulminant liver failure [13,23]. Severe manifestations can occur in utero, resulting in restricted growth, hydrops fetalis, prematurity, and/or fetal demise [29,30].

Infants and children generally have jaundice and scleral icterus, splenomegaly, and anemia, with symptoms that may include poor feeding and growth, low energy levels, and irritability. Older children and adolescents can have poor concentration, fatigue, or shortness of breath during activity. Acute exacerbations of anemia may occur during episodes of illness, including infections. Most young children will require regular or intermittent blood transfusions [13]. Severe anemia, iron overload states, persistent jaundice, and genotypes with the presence of two drastic *PKLR* mutations are associated with a lower quality of life [31]. Reported manifestations in adults include jaundice, pallor, fatigue, poor concentration,

and shortness of breath. Pregnancy can increase hemolysis and result in a transient need for transfusions in patients who are not otherwise regularly transfused [2,13,32,33]. The clinical variability is evident in adults, with some individuals experiencing few symptoms of anemia and others, having a significant impact on their everyday quality of life [34]. In general, increased transfusion needs and complications of PK deficiency, such as iron overload requiring chelation or pulmonary hypertension, are associated with a lower quality of life [31]. With advancing age, despite stable laboratory findings, symptoms can increase in the presence of additional comorbidities.

Supportive treatment strategies and associated complications

Transfusions—Initiating red blood cell transfusion therapy depends on growth in children, daily symptoms that impact quality of life, complications, and, to a lesser extent, the nadir hemoglobin. The need for transfusions decreases with age, likely because of decreased hemolytic episodes from viral infections and the timing of splenectomy [13]. Approximately half of children less than 5 years old are regularly transfused at an average interval of 5 weeks. In one large cohort, regular transfusions were required in 30% of children aged 5–12 years and 14% aged 12–18 years [2,13]. Postsplenectomy transfusions are typically required only during intermittent hemolytic episodes, although a subset who undergo splenectomy will continue to require regular transfusions. Chronic anemia may impact quality of life, leading to the reinitiation of regular transfusions in some adults. Regular transfusions are associated with iron overload, the risk of infection, need for intravenous access, and a risk of allosensitization, as well as personal and financial costs. For these reasons, individuals may choose not to be transfused, despite having symptoms of anemia.

Splenectomy—In individuals with PK deficiency who are frequently transfused or have poor quality of life, splenectomy is a supportive treatment typically considered in childhood [35]. Splenectomy only partially alleviates anemia and is not effective for all patients [36–38]. After a splenectomy, most patients have improvements in their hemoglobin and transfusion burden, with a paradoxical increase in the reticulocyte count. Hemolysis persists after splenectomy; therefore, many complications are not resolved by a splenectomy. Splenectomy is typically avoided before the age of 5 years to reduce the risk of postsplenectomy sepsis. In one large cohort, by 12 years of age, nearly half (40%) had undergone a splenectomy and, by the age of 18 years, 70% had done so [13]. Those with less severe hemolysis with a higher presplenectomy hemoglobin and lower indirect bilirubin level are the most likely to benefit from splenectomy [13,34]. In addition to infection, there is a substantially increased lifetime risk of thrombosis postsplenectomy. After a splenectomy, patients must adhere to guidelines regarding vaccines, prophylactic antibiotics, and thromboprophylaxis. Many individuals with PK deficiency still undergo splenectomy in early childhood. As access to newly approved treatments for adults expands and trials are completed for disease-directed therapies in children, fewer individuals with PK deficiency may undergo splenectomy in the future.

Complications—Complications are caused by chronic hemolysis and/or a result of supportive treatment. Iron overload can occur both in transfused and non-transfused patients. In non-transfused patients, increased intestinal iron absorption occurs because of chronic

hemolysis and ineffective erythropoiesis [13,39,40]. Iron overload can cause significant morbidity, including chronic liver disease and cirrhosis, bone fractures, cardiac dysfunction, heart failure, and endocrine dysfunction. The toxic effects of circulating free iron can be seen after 10 to 14 red cell transfusions. Regular monitoring allows for adequate treatment via iron chelation [41]. Monitoring of regular ferritin levels is recommended, with annual magnetic resonance imaging (MRI) surveillance (Table 1).

Pigmented gallstones secondary to hemolysis and bilirubin accumulation occurs in nearly half of patients [13,42,43]. Splenectomy does not decrease this risk; therefore, cholecystectomy is often considered at the time of a splenectomy. Anemia may worsen in the setting of parvovirus infections, resulting in aplastic crisis. Pulmonary hypertension is a rare manifestation that significantly impacts quality of life when it occurs [31].

Patients with PK deficiency have a high rate of reduced bone density, with an earlier onset than in the general population. Reduced bone density may begin in adolescence or early adulthood, and most older adults with PK deficiency have osteopenia or osteoporosis [44]. Leg ulcers and extramedullary hematopoiesis are rare but potentially morbid complications in adults [45–47]. The impact of PK deficiency on quality of life can result in mental health issues; clinicians should monitor for these symptoms and provide psychological support. Monitoring guidance for individuals with PK deficiency is included in Table 1.

Targeted therapeutic strategies in PK deficiency

Mitapivat for treating PK deficiency

Mitapivat (AG-348, Agios Pharmaceuticals, Figure 4) is an oral small molecule that allosterically activates erythrocyte PK. Mitapivat binds to a separate allosteric site from fructose 1,6-bisphosphate on the red cell PK tetramer. Two Phase 3 clinical trials have demonstrated the safety and efficacy of mitapivat for adults with PK deficiency, leading to recent FDA and European Medicines Agency approval [48]. Although additional PK activators are currently in clinical development for the treatment of congenital hemolytic anemias, mitapivat is the only PK activator evaluated to date for PK deficiency. Mitapivat has excellent oral bioavailability with or without food, with a steady state reached after 1 week with dosing of every 12 h. Mitapivat is eliminated via hepatic metabolism by cytochrome P450 enzymes and is a mild to moderate aromatase inhibitor [49]. Clinical studies of mitapivat have not demonstrated meaningful endocrinologic effects to date [50,51]. Ex vivo human studies have demonstrated both increased wild-type and variant PKR enzyme activity after exposure to mitapivat [8].

Two Phase 1 randomized, placebo-controlled, double-blind studies in healthy volunteers assessed the pharmacokinetics, pharmacodynamics, and safety of mitapivat [49]. In a multiple ascending dose study with six healthy volunteer cohorts randomized to receive a placebo or mitapivat, the maximum increase in ATP by day 14 was 60% and the maximum decrease in 2,3-BPG was 47%. There were no serious treatment-emergent adverse events. Treatment-emergent adverse events were more common at high doses of mitapivat (≥700 mg) including headaches, nausea, and/or vomiting. Pharmacodynamic studies and the safety profile supported mitapivat trials in adults with PK deficiency.

The open-label randomized Phase 2 DRIVE-PK trial evaluated the safety and efficacy of mitapivat in adults with PK deficiency who were not receiving regular transfusions, defined as less than four transfusion episodes in the prior 12 months (NCT02476916) [5]. Patients (n = 52) were randomized to 50 mg or 300 mg of mitapivat twice daily for a 24-week period. The primary endpoint of this study was a safety assessment. The secondary objectives were characterizing the pharmacokinetic and pharmacodynamic profile and demonstrating its clinical efficacy, as measured by hemoglobin and hemolysis markers. The therapy was well-tolerated, with the most common adverse events being transient mild headaches, insomnia, and nausea. One individual experienced acute hemolysis when mitapivat was discontinued without tapering. Other rare adverse events included reduced bone mineral density, elevated transaminases, and increased triglycerides. Of the 52 patients, 26 had an increase in hemoglobin of ≥1 g/dl from the baseline, with a mean maximum increase of 3.4 g/dl (range: 1.1–5.8 g/dl). The increase in hemoglobin was rapid (a median duration of 10 days), with a durable response seen with continued dosing. A genotype–response relationship was observed, such that most patients with at least one non-R479H **missense mutation** had a hemoglobin response, whereas patients with two non-missense mutations or homozygous R479H mutations had poor or no responses [50].

The ACTIVATE trial was a Phase 3 placebo-controlled trial, in which 80 adults with PK deficiency who were not receiving regular transfusions were randomized to receive a placebo or mitapivat twice daily, with potential dose escalation (from 5 mg to 50 mg twice daily) for 24 weeks (NCT03548220) [51]. The inclusion criteria required two *PKLR* mutations, including at least one non-R479H missense mutation. The primary endpoint was a sustained hemoglobin increase of ≥1.5 g/dl at two or more assessments at weeks 16, 20, or 24 of the trial, a higher response threshold than in the DRIVE-PK study. Secondary endpoints included a change from the baseline in the hemoglobin level, markers of hemolysis and hematopoiesis, and patient-reported outcome measures. The therapy was tolerated well, with similar overall adverse event (AE) rates between the mitapivat and placebo arms, and most AEs being minor and transient. The most common AEs observed with mitapivat, nausea and headache, were both more common in patients receiving the placebo [52]. Mitapivat significantly increased the hemoglobin level, decreased hemolysis, and improved patient-reported outcomes. In this trial, 40% of the patients (16/40) receiving mitapivat met the primary endpoint versus 0% in the placebo arm. Data from an open-label extension study suggested additional benefits, including decreases in ineffective erythropoiesis and iron overload, and stable bone health [53,54].

A Phase 3 single-arm, open-label trial, ACTIVATE-T, evaluated the efficacy and safety of mitapivat in adults with PK deficiency who were regularly transfused (≥6 transfusions in the prior 12 months, NCT03559699) [55]. Eligible patients began with a 16-week dose escalation period followed by a 24-week fixed dose period. The primary endpoint was a reduction in the burden of transfusions, defined as a 33% reduction in transfusion requirements. Secondary endpoints included the proportion of transfusion-free responders and the annualized number of red cell units transfused. Twenty out of 27 enrolled patients completed the study. The primary endpoint was met, with 37% experiencing a reduction in

their transfusion burden and 22% achieving a transfusion-free response. Mitapivat was also tolerated well in this trial, with no major adverse events leading to treatment discontinuation.

With the recent FDA and EMA approvals of mitapivat, the first approved therapy for adults with PK deficiency, a treatment trial of mitapivat should be considered in all symptomatic adults with PK deficiency (Figure 5, Key figure). The approved dose ranges from 5 mg to 50 mg twice per day. Given the short onset of action, the response can be assessed over the first few months of treatment and is generally clear within a few weeks of titration to the highest dose of 50 mg twice daily. In those with a hemoglobin response, ongoing use of the drug is required for a durable effect, and abrupt discontinuation should be avoided because of the risk of hemolysis. Trials are currently ongoing in regularly transfused and non-regularly transfused children with PK deficiency (NCT05144256, NCT05175105).

PK activators in other hematologic diseases

PK activators increase the production of ATP which could alleviate the increased metabolic oxidative stress seen in other congenital hemolytic anemias and help maintain the red blood cell membrane and function. Early-phase clinical trials of PK activators, mitapivat and etavopivat, in adults with thalassemia and sickle cell disease have demonstrated an increase in hemoglobin and a reduction in the markers of hemolysis, and a significant decrease in the specific sickling point in the sickle cell population [56–60]. Phase 3 clinical trials of PK activators are ongoing or planned in these patient populations (NCT04770779, NCT04770753, NCT05031780, NCT04987489, and NCT04624659). The role of PK activators in the treatment of red cell disorders may be even broader, as preclinical studies have suggested the potential efficacy of PK activators in additional rare congenital hemolytic anemias, including hereditary spherocytosis [61]. A Phase 2 trial evaluating mitapivat in adults with hereditary spherocytosis and hereditary dyserythropoietic anemia is ongoing. A potent next-generation PK activator, AG-946, has demonstrated safety in a Phase 1 trial of healthy volunteers [62] and is currently being evaluated in sickle cell disease and low-grade myelodysplastic syndromes (NCT04536792 NCT05490446) [63,64].

Hematopoietic stem cell transplantation

Allogeneic hematopoietic stem cell transplantation (HSCT) is a curative treatment option for PK deficiency. However, no clearly described indications for a transplant in this patient population exist. Although HSCT is a curative option, the few published patient cohorts revealed a high rate of morbidity and mortality associated with transplants compared with current standard supportive care [65,66]. A global cohort study described 16 patients with PK deficiency who received transplants in Europe and Asia [67]. In this cohort, the median age at transplantation was 6.5 years, with median follow-up of 2.3 years. The results showed Grade 3–4 graft-versushost disease (GVHD) in a large group of patients (44%), with 5/16 patients (31%) dying from the complications of GVHD. The 2-year cumulative survival was 74%. Various factors such as donor types, conditioning regimens, GVHD and infection prophylaxis affected the prognosis of transplantation. Higher survival was noted in patients receiving transplants prior to 10 years of age, suggesting that HSCT is best considered early in life in the most severely afflicted patients (Figure 5). However, the emergence of PK

activators and the ongoing development of gene therapy has further dampened enthusiasm for HSCT in light of its considerable risks.

Gene therapy

Gene therapy has been successful in diseases caused by monogenic defects and other erythrocyte disorders such as thalassemia and sickle cell disease [68]. Both mouse and dog PK-deficient models have shown that gene therapy for PK deficiency can be efficacious; a mouse model demonstrated full correction of the PK deficiency genotype when >25% of genetically corrected cells were transplanted [69–71]. An open-label Phase 1 clinical trial is currently enrolling to evaluate the safety of a hematopoietic cell-based gene therapy for patients with PK deficiency using the Lentivirus-based RP-L301 gene therapy product containing autologous genetically modified CD34+ hematopoietic stem cells with corrected *PKLR* (NCT04105166). Secondary outcome measures include the genetic correction of cells, transfusion requirements, and reductions in hemolysis and anemia. The interim results of two adults who have undergone gene therapy reported the normalization of hemoglobin, improved hemolysis markers and quality of life, and no transfusions required at 24 months of follow-up. To date, there have been no serious adverse events [72].

Key figure

Algorithm for treatment considerations in pyruvate kinase deficiency

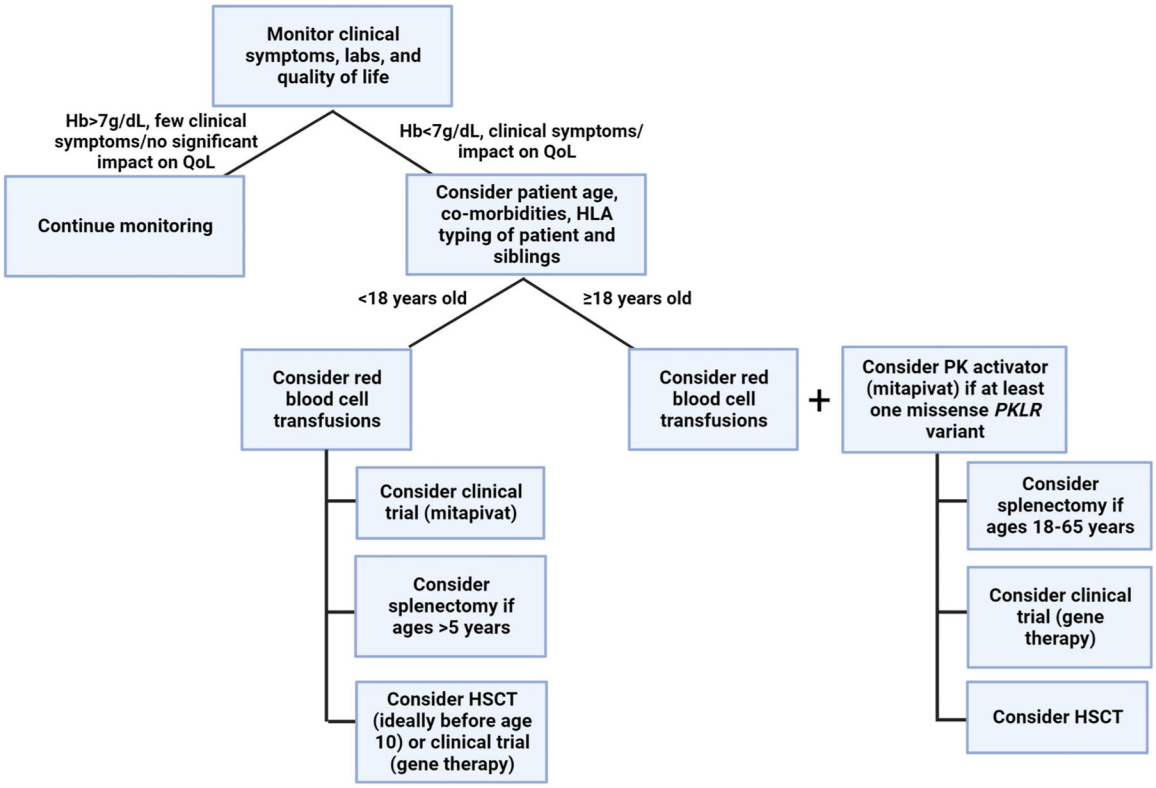

Figure 5.

This algorithm shows an approach to the management of pyruvate kinase (PK) deficiency. Clinical signs and symptoms, in addition to hemoglobin levels, should be considered when deciding to initiate supportive care and/or disease-directed therapy. In patients with signs or symptoms of anemia that impact their quality of life, various treatment options should be considered. Symptomatic patients <18 years old should initiate red blood cell transfusions and then consider clinical trials (mitapivat or gene therapy), splenectomy, and/or a hematopoietic stem cell transplant (HSCT). In symptomatic adults ≥18 years, a treatment trial with a PK activator should be initiated, along with consideration of red cell transfusions. Additional options such as HSCT, clinical trials (gene therapy), and splenectomy can be considered if there is no response to a PK activator.

Concluding remarks

PK deficiency is a rare congenital hemolytic anemia caused by homozygous or compound heterozygous mutations in the *PKLR* gene, leading to decreased production of ATP in the red cells, causing membrane defects and dysfunction. Clinically, patients have chronic hemolysis causing manifestations with variable severity, including jaundice, gallstones, iron overload, extramedullary hematopoiesis, and decreased patient-reported quality of life (see Clinician's corner). Providers must have a high index of suspicion for this disease and send samples for confirmatory diagnostic testing of PK enzyme activity and genetic sequencing, since lab findings, peripheral blood morphology, and clinical manifestations can be nonspecific.

Historically, treatment has focused on supportive care with transfusions, splenectomy, and iron chelation. Recently, treatment has been transformed by disease-directed therapy, including the PK activator, mitapivat, which is now approved for adults with PK deficiency, with studies underway in children. Gene therapy is a promising potentially curative treatment option currently under development, and HSCT is a currently available curative treatment option associated with high morbidity and mortality. PK activation and gene therapy have promise in revolutionizing care for patients by decreasing the rate of both the disease and the treatment-related complications.

Future research should focus on long-term follow-up of disease-directed therapies and the impact on patients' health and quality of life (see Outstanding questions). These therapies may have lifelong benefits when initiated in children, who could potentially avoid transfusion therapy and splenectomy. The role of PK activators in other congenital hemolytic anemias should be explored. There may be unexpected implications of disease-directed therapies which will require close monitoring in the real-world setting. As treatment options for PK deficiency are both expanded and refined, consensus clinical guidelines for providers should be developed to ensure the widespread adoption of evidence-based care for this patient population.

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Glossary

Adenosine triphosphate (ATP)
the source of cellular energy

2,3-Biphosphoglycerate (2,3-BPG), also 2,3-diphosphoglycerate (2,3-DBG)
a glycolytic metabolic intermediate and regulator of hemoglobin–oxygen affinity and assists with off-loading oxygen into tissues

Hemolytic anemia
decreased red cell survival (less than 120 days) leading to premature red cell death

Missense mutation
gene variants that cause an amino acid change

Phosphoenolpyruvate
glycolytic intermediate

PKLR
the gene encoding pyruvate kinase that produces the pyruvate kinase protein of liver and red blood cell

Pyruvate kinase
the key enzyme in an energy-generating step in the glycolytic pathway that leads to the conversion of phosphoenolpyruvate to pyruvate

Pyruvate kinase activator
a drug that stabilizes pyruvate kinase protein and increases its activity (for example, mitapivat and etavopivat)

Pyruvate kinase deficiency
a decrease in the activity or half-life of pyruvate kinase protein, leading to a deficiency in energy in red blood cells

Reticulocyte
the youngest circulating red blood cells, 1–2 days after emerging from the bone marrow

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Highlights

Pyruvate kinase (PK) deficiency is a genetically and clinically heterogeneous congenital hemolytic anemia which manifests with a wide range of symptoms and complications.

Given the clinical heterogeneity, clinicians should have a high suspicion of PK deficiency in patients with congenital hemolytic anemias, and both red cell enzyme and genetic testing should be pursued.

Complications in PK deficiency can be associated with significant morbidity and mortality in transfused and non-transfused patients, including iron overload, osteopenia, and impact on quality of life and mental health, which require regular monitoring and management.

A definitive diagnosis of PK deficiency has become critical with the availability of disease-modifying therapy with mitapivat, an oral PK activator which increases hemoglobin and decreases hemolysis in many patients, as well as the potential future curative option of gene therapy.

Clinician's corner

PK deficiency is a clinically heterogeneous congenital hemolytic anemia with a wide spectrum of manifestations, including pallor, fatigue, and jaundice, and complications, including iron overload, gallstones, osteopenia, and pulmonary hypertension.

The diagnosis of PK deficiency should be suspected in patients with hemolytic anemia once more common causes, such as autoimmune hemolytic anemia, hemoglobinopathies, and membranopathies, have been excluded. The diagnosis should be confirmed through both the enzyme activity of red blood cells and *PKLR* genetic testing (targeted gene testing or congenital hemolytic anemia gene panel).

Supportive care with blood transfusions, splenectomy, and iron chelation have historically been the main management strategies and are also associated with the potential for complications.

Complications in patients with PK deficiency can be associated with significant morbidity, including impacts on their daily quality of life and mental health, which requires regular monitoring and management.

Disease-targeted therapy with a recently approved PK activator, mitapivat, offers an innovative disease-directed approach that, in addition to other therapies undergoing trials (gene therapy), may transform the manifestations of the disease and the quality of life of many patients with PK deficiency.

Outstanding questions

Will mitapivat allow children with PK deficiency to avoid blood transfusions and splenectomy and their associated complications, as has been demonstrated in adults?

If disease-targeted therapies, including mitapivat and gene therapy, are safe and have short-term efficacy in children, will they have life-long efficacy and transform the natural history of the disease in terms of lifelong symptoms and complications?

Will PK activators be efficacious in raising hemoglobin levels, reducing transfusions, and improving the patients' quality of life in other congenital and acquired hemolytic anemias?

Which individuals with PK deficiency should consider specific treatments, including hematopoietic stem cell transplant, mitapivat (or other PK activators), splenectomy, and/or transfusions? When is the optimal time to initiate such treatments?

What guidelines should providers follow with regard to monitoring and managing patients with PK deficiency?

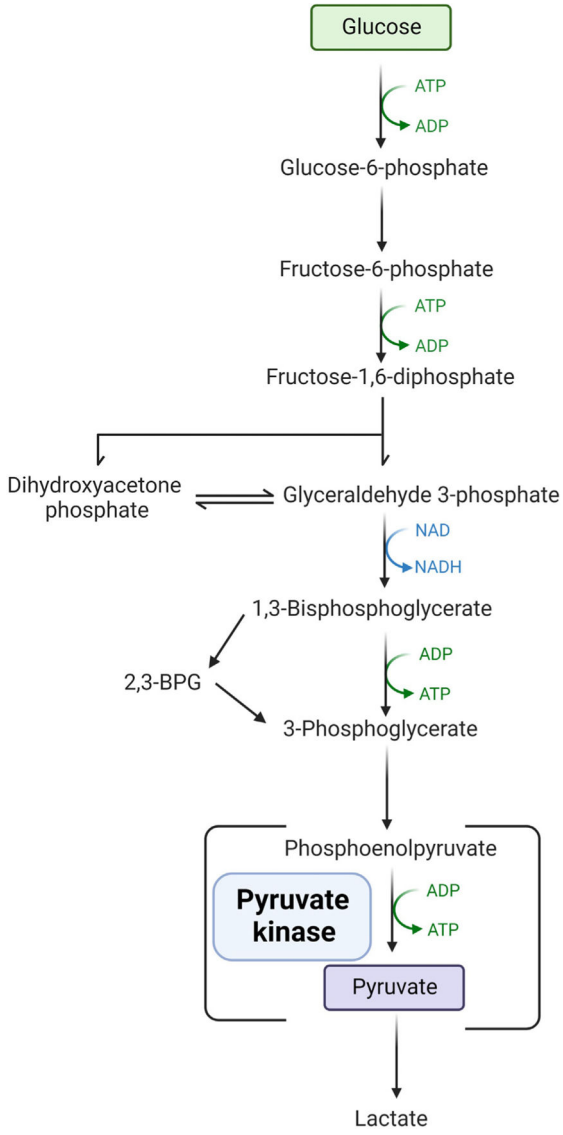

Figure 1. Glycolytic pathway.

Mature red blood cells lack a nucleus and mitochondria and rely on glycolysis for the production of adenosine triphosphate (ATP). Pyruvate kinase is an enzyme in the terminal part of the pathway converting phosphoenolpyruvate to pyruvate with the production of ATP. With a deficiency of pyruvate kinase, the production of proximal byproducts, such as 2–3-biphosphoglycerate (2,3-BPG), is increased. Abbreviations: ADP: adenosine diphosphate; NAD: nicotinamide adenine dinucleotide; NADPH: nicotinamide adenine dinucleotide phosphate.

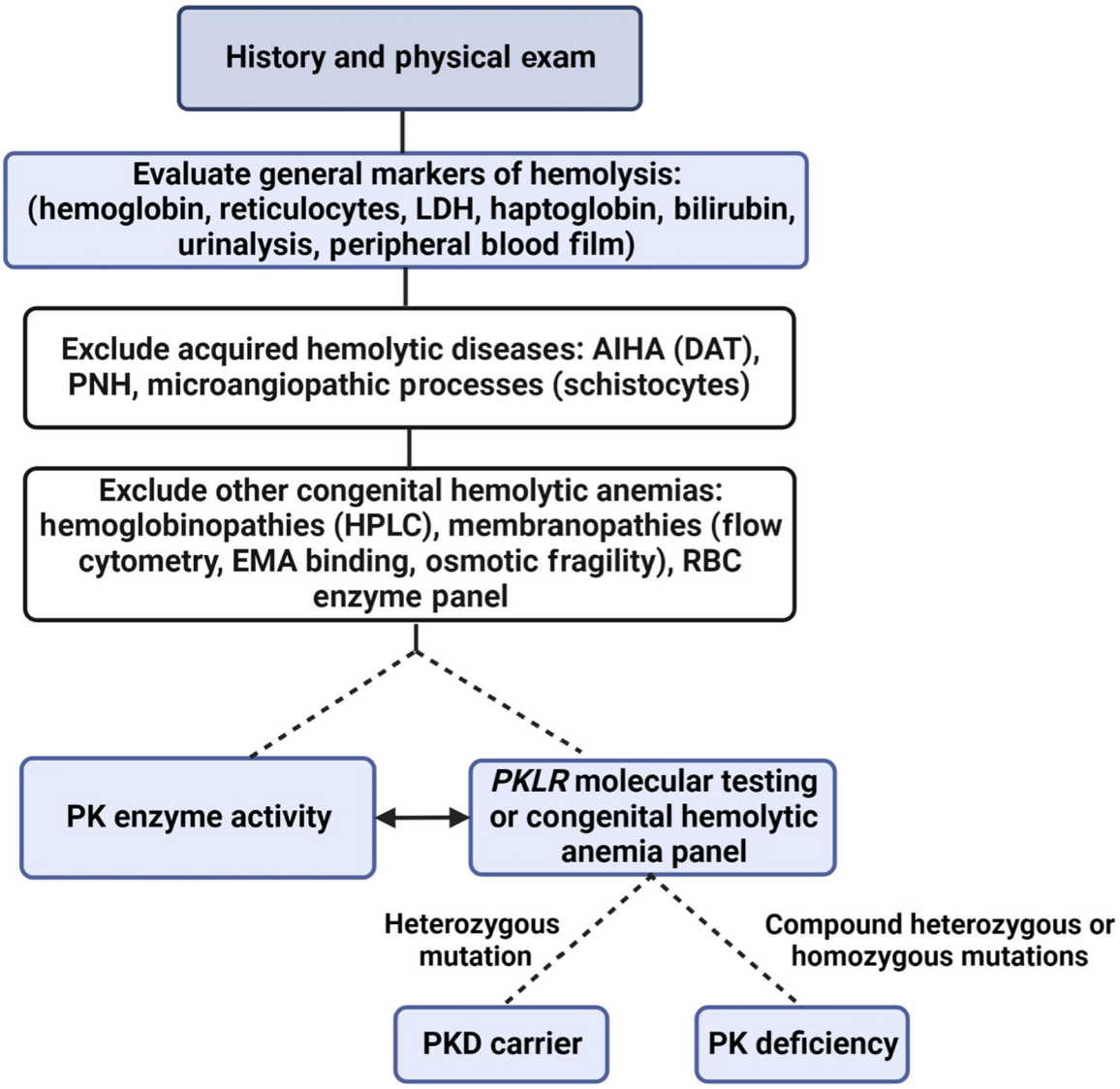

Figure 2. Diagnostic algorithm for the work-up of suspected congenital hemolytic anemia.

This algorithm shows an approach to the evaluation of a suspected congenital hemolytic anemia. In general, a thorough history and physical exam should be completed. Laboratory testing should evaluate for evidence of hemolysis and include an evaluation of the red cells' morphology on the peripheral blood film. Acquired causes of hemolytic diseases should be excluded, and a full differential diagnosis for congenital hemolytic anemias should be considered. Pyruvate kinase deficiency should be suspected as a cause of congenital hemolytic anemia after more common causes are excluded, and then testing should include pyruvate kinase activity levels and *PKLR* genetic testing. Abbreviations: LDH: lactate dehydrogenase; AIHA: autoimmune hemolytic anemia; DAT: direct antiglobulin test; PNH: paroxysmal nocturnal hemoglobinuria; HPLC: high-performance liquid chromatography; EMA: eosin-5′-maleimide; PK: pyruvate kinase; PKD: pyruvate kinase deficiency.

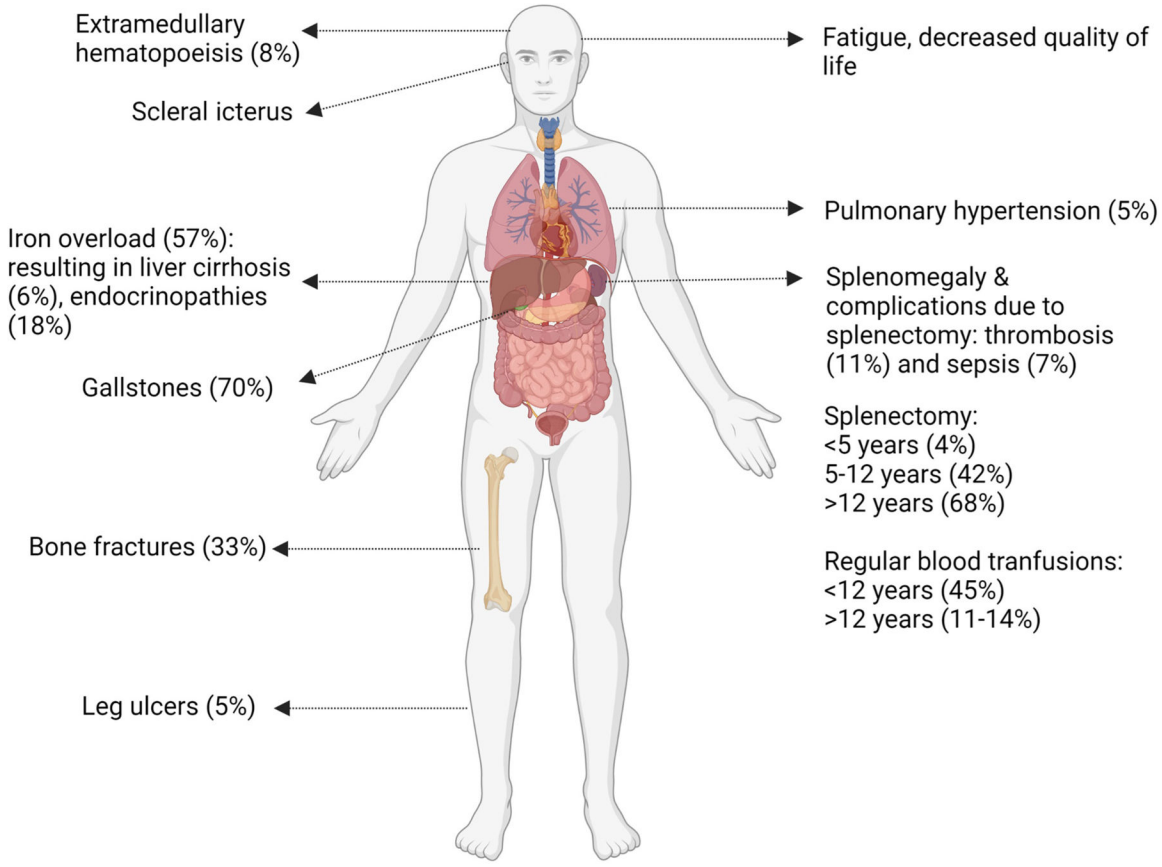

Figure 3. Symptoms and complications of pyruvate kinase deficiency in adults.

Pyruvate kinase deficiency is clinically heterogeneous, with both common and rare complications arising from both the disease and its historic treatment with blood transfusions and consideration of splenectomy.

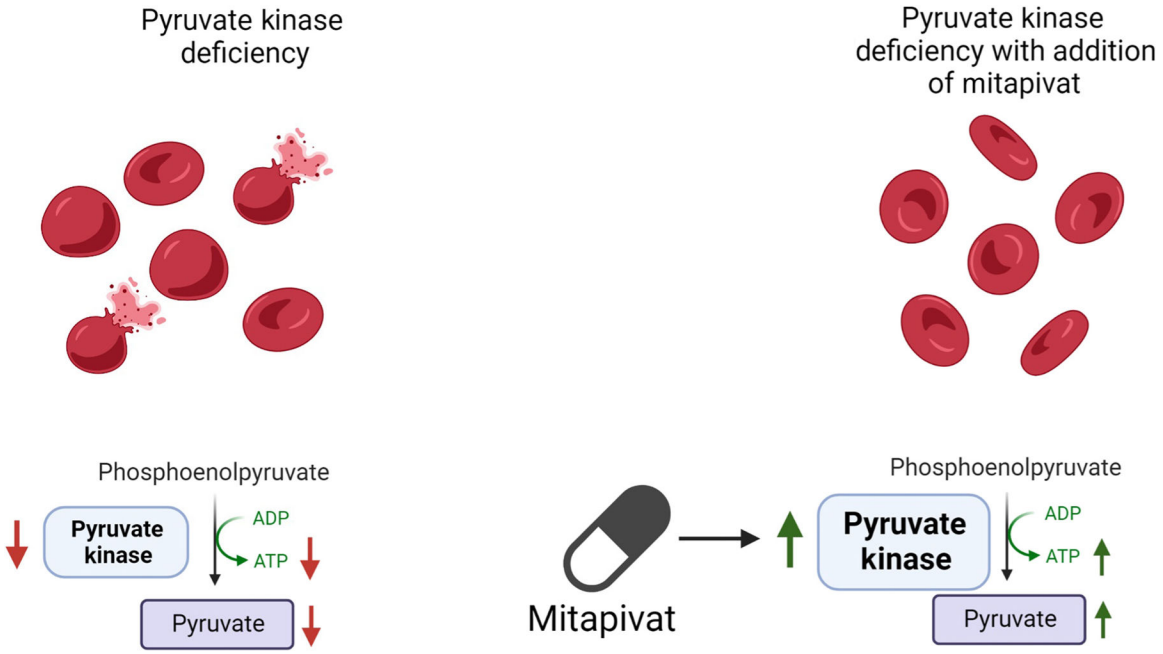

Figure 4. Mechanism of mitapivat.

Mitapivat, an oral allosteric pyruvate kinase activator, stabilizes and increases the activity of pyruvate kinase, thereby leading to increased intracellular red cell adenosine triphosphate (ATP), resulting in an improvement in anemia and patient-reported outcomes and a reduction in hemolysis. Abbreviation: ADP, adenosine diphosphate.

Table 1.

Clinical monitoring in patients with pyruvate kinase deficiency

Complication	Recommended test(s)	Frequency of monitoring (age <18 years)	Frequency of monitoring (Age ≥18 years)
Hemolytic anemia	Complete blood count, reticulocyte count, bilirubin	Annually or more frequently based on hemolytic parameters and transfusions	Annually or more frequently based on hemolytic parameters and transfusions
Iron overload	MRI for liver iron concentration	If regular transfusions: MRI after first 10–14 transfusions, then annually If no regular transfusions: complete first MRI when the patient is able to complete an unsedated study; follow up patients annually if >5 mg/g or every 5 years if <5 mg/g	If regular transfusions: annually If no regular transfusions: annually if >5 mg/g or every 5 years if <5 mg/g
	Serum ferritin and transferrin saturation	If regular transfusions: every 3–6 months If no regular transfusions: annually On chelation: every 1 −3 months	If regular transfusions: every 3–6 months If no regular transfusions: annually On chelation: every 1 −3 months
Cholestasis	Abdominal ultrasound	Age 2 years then every 2–3 years or until cholecystectomy	Every 2–3 years or until cholecystectomy
Osteopenia	DEXA scan, vitamin D levels	First at age 16–18 years and then annually if low	Annually if low
Endocrinopathies	Thyroid hormone, sex hormones, fructosamine	-	If regular transfusions (or significant iron overload): annually
Pulmonary hypertension, cardiac complications	Echocardiogram	-	Consider after 30 years, before pregnancy, or any time if concerns arise
Viral infections	Viral hepatitis serology	If regular transfusions: annually	If regular transfusions: annually