

Supplementary appendix

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**DIAGNOSIS AND MANAGEMENT OF PYRUVATE KINASE DEFICIENCY: INTERNATIONAL EXPERT GUIDELINES**

**SUPPLEMENTARY APPENDIX**

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# SYSTEMATIC LITERATURE SEARCH AND IDENTIFICATION OF EVIDENCE

## Overview

To support the development of evidence-based recommendations for the diagnosis and management of pyruvate kinase deficiency, relevant literature was systematically identified, evaluated, and summarized into evidence summaries for use by the key topic groups.

To guide the development of recommendations, key topic groups developed 31 key questions in the following areas:

* 1. Diagnosis and Genetics of PK Deficiency
  2. Monitoring and Management of Chronic Complications in PK Deficiency
  3. Standard Management of Anemia in PK Deficiency
  4. Targeted and Advanced Therapies in PK Deficiency
  5. Special Populations in PK Deficiency

## Systematic Search and Identification of Evidence

Five search strategies were developed and executed in Ovid MEDLINE to identify studies that addressed the key questions relevant to each topic, as developed by the key topic groups. To ensure comprehensiveness, a supplemental search was conducted in Ovid Embase to identify studies on PK deficiency presented at conferences. Search strategies were designed to be very sensitive to ensure all relevant results were identified and included topic-specific controlled subject headings (e.g., MeSH terms), relevant keywords, and free-text search.

Literature searches were developed and conducted in May 2023 (the main searches were conducted on May 8, 2023 and a supplemental search for conference abstracts in Embase was conducted on May 26, 2023) by a medical librarian (KLR), with input from both guideline co- chairs (HA-S and RFG). In total, 1,450 results were identified across the 5 topics. Search strategies for monitoring, standard management, and special populations were limited to results published between 1961-2023. Search strategies for diagnosis and advanced treatments were limited to results published between 2000-2023.

Two reviewers (KLR, and one of ET, CD, HN or JR [full names listed in Acknowledgements section of primary manuscript]) reviewed the titles and abstracts of each record and independently applied the inclusion criteria to all 1,450 results. Based on the titles and abstracts, 368 records that potentially met the inclusion criteria and were indicated by either reviewer were retrieved in full text for further review. Both reviewers then independently reviewed the full text for inclusion. Any disagreements were reviewed and resolved by discussion until concordance was fully satisfied. A total of 50 results met the inclusion criteria and were included in the evidence summaries.

## Inclusion Criteria

While topic-specific inclusion criteria such as population, intervention, comparison, and outcomes of interest (i.e., PICO model) were developed by the Key Topic Groups for each individual key question, additional inclusion criteria for all studies included:

* Patients diagnosed with, or being screened for PK deficiency
* English language publications
* Human subjects
* ≥ 2 subjects
* Original results published in an indexed journal or presented at a conference.

## Data Extraction and Appraisal

Key data from the 50 included studies was extracted and summarized in evidence summaries to facilitate the inclusion of high-quality published evidence in the guideline in a transparent manner.

Due to the lack of randomized trials for most of the key questions in the area, two categories of studies were included in the evidence summaries: randomized control trials, which may be considered ‘high/moderate quality’ evidence if of sufficient size and quality, and other studies without blinding or randomization, considered to be ‘low/very low quality’ evidence according to GRADE. The quality of the included RCT was assessed using the structured framework of the Cochrane Risk of Bias 2 Tool.

## Diagnosis and Genetics of PK Deficiency

Database(s): **Ovid MEDLINE(R) ALL** 1946 to May 04, 2023

Search Strategy:

|  |  |  |
| --- | --- | --- |
| **#** | **Searches** | **Results** |
| 1 | Pyruvate Kinase/df [Deficiency] | 632 |
| 2 | Pyruvate Metabolism, Inborn Errors/ | 144 |
| 3 | (pyruvate kinase adj2 deficien$).mp. | 673 |
| 4 | Anemia, Hemolytic, Congenital Nonspherocytic/ | 856 |
| 5 | ("pk deficien$" or "pyruvate kinase").mp. | 10881 |
| 6 | 1 or 2 or 3 or (4 and 5) | 939 |
| 7 | limit 6 to (english language and yr="2000 - 2023") | 332 |
| 8 | Mice/ or Rabbits/ or Dogs/ or Cats/ | 2428020 |
| 9 | 7 not 8 | 287 |
| 10 | Pyruvate Metabolism, Inborn Errors/di | 39 |
| 11 | exp Genetic Testing/ | 53586 |
| 12 | exp Diagnosis/ | 9330739 |
| 13 | (screen$ or diagnos$ or classif$ or lab$ or test$ or hemoglobin or haemoglobin or reticulocyte or bilirubin or PK enzyme or PK protein or PKLR or genotype or NGS or hexokinase enzyme).mp. | 12071053 |
| 14 | 9 and (10 or 11 or 12 or 13) | 208 |

## Monitoring and Management of Chronic Complications of PK Deficiency

Database(s): **Ovid MEDLINE(R) ALL** 1946 to May 04, 2023

Search Strategy:

|  |  |  |
| --- | --- | --- |
| **#** | **Searches** | **Results** |
| 1 | Pyruvate Kinase/df [Deficiency] | 632 |
| 2 | Pyruvate Metabolism, Inborn Errors/ | 144 |
| 3 | (pyruvate kinase adj2 deficien$).mp. | 673 |
| 4 | Anemia, Hemolytic, Congenital Nonspherocytic/ | 856 |
| 5 | ("pk deficien$" or "pyruvate kinase").mp. | 10881 |
| 6 | 1 or 2 or 3 or (4 and 5) | 939 |
| 7 | limit 6 to (english language and yr="1961 - 2023") | 744 |
| 8 | Mice/ or Rabbits/ or Dogs/ or Cats/ | 2428020 |
| 9 | 7 not 8 | 661 |
| 10 | exp Iron Overload/ | 15099 |
| 11 | Chelation Therapy/ | 1570 |
| 12 | ((iron adj monitor$) or iron overload or chelation or ferritin or Ferriscan or cardiac T2 or MRI).mp. | 373239 |
| 13 | 9 and (10 or 11 or 12) | 48 |
| 14 | exp Hypertension, Pulmonary/ | 41724 |
| 15 | (hypertension or BNP or echocardiogram or ECHO or electrocardiogram or ECG or cardiac).mp. | 1495906 |
| 16 | 9 and (14 or 15) | 13 |
| 17 | (endocrinopath$ or DEXA or vitamin D or thyroid or FSH or LH or testosterone or fructosamine).mp. | 495008 |
| 18 | 9 and 17 | 4 |
| 19 | 13 or 16 or 18 | 57 |

## Standard Management of Anemia in PK Deficiency

Database(s): **Ovid MEDLINE(R) ALL** 1946 to May 04, 2023

Search Strategy:

|  |  |  |
| --- | --- | --- |
| **#** | **Searches** | **Results** |
| 1 | Pyruvate Kinase/df [Deficiency] | 632 |
| 2 | Pyruvate Metabolism, Inborn Errors/ | 144 |
| 3 | (pyruvate kinase adj2 deficien$).mp. | 673 |
| 4 | Anemia, Hemolytic, Congenital Nonspherocytic/ | 856 |
| 5 | ("pk deficien$" or "pyruvate kinase").mp. | 10881 |
| 6 | 1 or 2 or 3 or (4 and 5) | 939 |
| 7 | limit 6 to (english language and yr="1961 - 2023") | 744 |
| 8 | Mice/ or Rabbits/ or Dogs/ or Cats/ | 2428020 |
| 9 | 7 not 8 | 661 |
| 10 | (treat$ or manag$ or therap$ or outcom$ or improv$).mp. | 13532793 |
| 11 | (anemi$ or anaemi$).mp. | 222720 |
| 12 | Blood Transfusion/ | 54258 |
| 13 | (transfus$ or erythrocyte).mp. | 277768 |
| 14 | Iron Overload/ or Iron Chelating Agents/ | 10953 |
| 15 | (iron overload or chelat$).mp. | 96809 |
| 16 | Folic Acid/ | 30029 |
| 17 | folic acid.mp. | 45393 |
| 18 | Splenectomy/ | 22526 |
| 19 | splenectomy.mp. | 31856 |
| 20 | Hematinics/ | 6537 |
| 21 | Erythropoiesis stimulating.mp. | 2788 |
| 22 | Fatigue/ | 34707 |
| 23 | fatigue.mp. | 135898 |
| 24 | cholecystectomy/ or cholecystectomy, laparoscopic/ | 30714 |
| 25 | cholecystectomy.mp. | 42167 |
| 26 | Counseling/ | 39549 |
| 27 | (counsel$ or psych$).mp. | 2389095 |

|  |  |  |
| --- | --- | --- |
| 28 | or/10-27 | 15142183 |
| 29 | 9 and 28 | 565 |

## Targeted and Advanced Therapies for PK Deficiency

Database(s): **Ovid MEDLINE(R) ALL** 1946 to May 04, 2023

Search Strategy:

|  |  |  |
| --- | --- | --- |
| **#** | **Searches** | **Results** |
| 1 | Pyruvate Kinase/df [Deficiency] | 632 |
| 2 | Pyruvate Metabolism, Inborn Errors/ | 144 |
| 3 | (pyruvate kinase adj2 deficien$).mp. | 673 |
| 4 | Anemia, Hemolytic, Congenital Nonspherocytic/ | 856 |
| 5 | ("pk deficien$" or "pyruvate kinase").mp. | 10881 |
| 6 | 1 or 2 or 3 or (4 and 5) | 939 |
| 7 | limit 6 to (english language and yr="2000 - 2023") | 332 |
| 8 | Mice/ or Rabbits/ or Dogs/ or Cats/ | 2428020 |
| 9 | 7 not 8 | 287 |
| 10 | (mitapivat or Pyrukynd or AG-348).mp. | 36 |
| 11 | (allosteric adj3 activat$).mp. | 2662 |
| 12 | pk activat$.mp. | 130 |
| 13 | 9 and (10 or 11 or 12) | 18 |
| 14 | Genetic Therapy/ | 52706 |
| 15 | (gene therapy or genetic therapy).mp. | 78978 |
| 16 | Hematopoietic Stem Cell Transplantation/ | 52828 |
| 17 | (stem cell transpl$ or HSCT).mp. | 118609 |
| 18 | Splenectomy/ | 22526 |
| 19 | splenectomy.mp. | 31856 |
| 20 | 9 and (or/14-19) | 59 |
| 21 | 13 or 20 | 72 |

## Special Populations in PK Deficiency

Database(s): **Ovid MEDLINE(R) ALL** 1946 to May 04, 2023

Search Strategy:

|  |  |  |
| --- | --- | --- |
| **#** | **Searches** | **Results** |
| 1 | Pyruvate Kinase/df [Deficiency] | 632 |
| 2 | Pyruvate Metabolism, Inborn Errors/ | 144 |
| 3 | (pyruvate kinase adj2 deficien$).mp. | 673 |
| 4 | Anemia, Hemolytic, Congenital Nonspherocytic/ | 856 |
| 5 | ("pk deficien$" or "pyruvate kinase").mp. | 10881 |
| 6 | 1 or 2 or 3 or (4 and 5) | 939 |
| 7 | limit 6 to (english language and yr="1961 - 2023") | 744 |
| 8 | Mice/ or Rabbits/ or Dogs/ or Cats/ | 2428020 |
| 9 | 7 not 8 | 661 |
| 10 | Child/ or Child, Preschool/ or Adolescent/ | 3349969 |
| 11 | Pediatrics/ | 57818 |
| 12 | (child$ or pediatric$ or paediatric$ or adolescent\* or teen$ or youth).mp. | 4022700 |
| 13 | 9 and (10 or 11 or 12) | 205 |
| 14 | Pregnancy/ | 981997 |
| 15 | pregnan$.mp. | 1126041 |
| 16 | 9 and (14 or 15) | 34 |
| 17 | 13 or 16 | 223 |

## Supplemental Search Strategy for Conference Abstracts in Embase

Database(s): Embase Classic+Embase 1947 to 2023 May 25 Search Strategy:

|  |  |  |
| --- | --- | --- |
| **#** | **Searches** | **Results** |
| 1 | (pyruvate kinase adj2 deficien$).mp. | 1155 |
| 2 | pk deficien$.mp. | 604 |
| 3 | \*hereditary hemolytic anemia/ | 1714 |
| 4 | ("PK deficien$" or "pyruvate kinase").mp. | 16042 |
| 5 | 1 or 2 or (3 and 4) | 1501 |
| 6 | limit 5 to english language | 1274 |
| 7 | limit 6 to (cat or dog or mouse or "rabbits and hares") | 136 |
| 8 | 6 not 7 | 1138 |
| 9 | (conference abstract\* or conference review or conference paper or conference proceeding).db,pt,su. | 5558673 |
| 10 | 8 and 9 | 324 |

**Literature Search Results**

|  |  |  |  |
| --- | --- | --- | --- |
| **Topic Group** | **Total Search Results Screened (Title**  **/ Abstract)** | **Results Reviewed in Full Text** | **Total Number of Results Included** |
| Diagnosis and Genetics | 208 | 103 | 17 |
| Monitoring and Management | 57 | 30 | 1 |
| Standard Management of Anemia | 565 | 75 | 9 |
| Special Populations | 223 | 81 | 0 |
| Targeted and Advanced Therapies | 72 | 16 | 3  (Includes 1 RCT) |
| Conference Abstracts (PK Deficiency; Not Limited to Topic Group) | 324 | 63 | Diagnosis: 6  Management: 3  Treatment: 10  Monitoring: 1 |
| **Total** | **1,450** | **368** | **50** |

**PRISMA Flow Diagram**

Studies included (n=50) Journal articles (n=30)

Conference abstracts (n=20)

**Included**

Records excluded (n=318)

Full text review (n=368)

Records excluded (n=1,082)

Title / abstract screening (n=1,450)

Records Identified from: Ovid MEDLINE (n=1,126)

Ovid Embase (n=324)

**Identification**

**Screening**

# STRUCTURED CONSENSUS PROCEDURE

Prior to the guidelines conference and again at the beginning of the conference, the consensus process was reviewed by the guidelines methodologist and GWG co-chairs. For each key topic group, the key topic group leader presented, one at a time, draft recommendations with the certainty of the evidence and strength of recommendation to the full expert panel in attendance. Draft recommendations were presented using a standard format for language and wording and the certainty of the evidence was presented as one of four levels according to GRADE: high, moderate, low, or very low. Strength of recommendation was presented as one of two levels according to GRADE: Strong or conditional (weak). Discussion then ensued about the appropriateness of the recommendation, the wording, and the certainty of the supporting evidence, as well as the strength of recommendation and the expert panel revised these items by consensus. Following discussion and revision, a single anonymous vote was taken on the recommendation wording, certainty of the evidence, and strength of recommendation together. Agreement of ≥67% was required for the recommendation to be included in the final guideline.

Further discussion on recommendations failing to achieve this threshold was deferred until completion of voting on all draft recommendations. The key topic group drafting the failed recommendation convened to discuss the criticisms raised by the full expert panel and addressed edits to language, certainty of the evidence, and/or strength of recommendation to potentially allow the recommendation to achieve consensus on a second round of voting. Key topic groups were instructed to focus on critical factors, such as the balance of benefit and harm, cost, values and preferences, and evidence quality raised during the initial discussion about the recommendation that resulted in disagreement. The revised recommendations were then presented again by the key topic group leader, followed by a period of discussion and revision and a single anonymous vote on all three aspects of the recommendation together.

Agreement of ≥67% was required for the revised recommendation to be included in the final guideline. Recommendations failing to achieve this threshold were not included in the final guideline.

Following completion of voting on recommendations, the co-chairs reviewed next steps in guideline development and implementation. Following this, the co-chairs surveyed the expert panel on future guidelines priorities and future research priorities (the results of which are detailed later in this supplementary appendix).

# COMPLETE KEY EVIDENCE SUMMARY AND CLINICAL CONSIDERATIONS FOR EACH RECOMMENDATION

## Diagnosis and Genetics of Pyruvate Kinase Deficiency

*Recommendation A1: The expert panel recommends testing for pyruvate kinase deficiency in all patients with non-immune hemolytic anemia after exclusion of hemoglobin disorders and*

*erythrocyte membrane disorders.* (Certainty of evidence: moderate; strength of recommendation: strong; agreement, 91%).

*Evidence/clinical considerations:* Given the phenotypic variability, misdiagnosis of patients with PK deficiency is possible.1,2 Studies performed in large cohorts,3-5 smaller case series, and single case reports clearly demonstrate a wide variation in the severity of anemia, ranging from very mild anemia or fully compensated hemolysis to nonimmune hydrops fetalis or life- threatening neonatal anemia; this implies that patients may present any time from very early in life to young adulthood. Given the rarity of the disease and lack of familiarity among providers, even adults reaching middle age or older with PK deficiency may not have been diagnosed and the diagnosis should be considered in a patient with a compatible clinical picture irrespective of age.

Red cell morphology on the peripheral blood film may be relatively bland with polychromasia and few echinocytes in patients with an intact spleen. PK deficiency should be considered in all patients after hemoglobinopathies and membranopathies have been excluded, particularly in those with evidence of dyserythropoiesis (including those originally diagnosed with a congenital dyserythropoietic anemia not confirmed at the molecular level),6,7 all patients with unexplained compensated or transfusion-dependent anemia, and neonates with unexplained hyperbilirubinemia.

PK deficiency may be associated with resistance to malaria.8,9 Given that other red cell defects are common and also associated with malaria resistance (e.g. hereditary stomatocytosis, RBC membrane defects, hemoglobinopathies, glucose-6-phosphate dehydrogenase deficiency), the co-inheritance of *PKLR* mutations with other red cell defects is not infrequent. In the presence of an atypical phenotype or incomplete genotype (e.g., a patient who has a single *PKLR* mutation but a chronic hemolytic phenotype), the possibility of co-inherited conditions should be considered.

*Recommendation A2. The expert panel recommends initial testing for pyruvate kinase deficiency using either* PKLR *gene molecular analysis or pyruvate kinase enzyme activity (when performed following established testing guidance) as both methods currently have similar performance in the diagnosis of pyruvate kinase deficiency.* (Certainty of evidence: moderate; strength of recommendation: strong; agreement, 95%).

*Evidence/clinical considerations:* Establishing the diagnosis of PK deficiency is the final step of a diagnostic workup that begins with the patient’s personal and family medical history and clinical examination. When a red cell enzymopathy is suspected, the initial diagnosis of PK deficiency ultimately depends upon the demonstration of decreased *PKLR* enzyme activity and/or the identification of causative mutations in *PKLR* gene. PK enzyme activity assay and

*PKLR* molecular testing are considered complementary tests and the choice to use molecular testing or biochemical assay as a first step in investigation is dictated by clinical and laboratory findings, by test availability, and national health care system recommendations and coverage.

Where possible, the diagnostic evaluation of PK deficiency should be performed in an expert reference center. For many years, a standardized assay for measuring PK activity has been used, established by the International Committee for Standardization in Haematology (ICSH).10,11 The complete procedure together with the diagnostic approach to the diagnosis of PK deficiency has been previously reviewed by experts in diagnostic testing, offering recommendations in how it is performed and detailing analytical pitfalls.1

The sensitivity of PK enzyme testing (~80-90%) may be improved (>95%) when evaluated against another age-dependent red cell enzyme (PK:hexokinase ratio).12,13 The choice to use molecular testing or enzyme assay as the first step in diagnosis is dictated by clinical circumstances (for example, PK enzyme assays are unreliable if a red cell transfusion has been administered in the preceding 90 days),1 by test availability, and payor/national health system recommendations and coverage. Over 300 known pathogenic mutations in *PKLR* have been identified.14 Single-gene *PKLR* exon sequencing as well as multi-gene next-generation sequencing (NGS) hemolytic anemia panels are both reliable methods for PK deficiency diagnosis15,16 but may result in false negative testing due to pathogenic intronic variants, mutations in regulatory regions, non-canonical splice site mutations, or large deletions.2,16

Care should be taken in interpreting results the results of PK enzyme activity. Falsely normal levels are sometimes encountered due to reticulocytosis, interference from normal donor red cells in recently transfused (<90 days) patients, incomplete platelet and leukocyte removal or mutant PK proteins with unusual kinetics.1

*Recommendation A3. The expert panel recommends confirmation of a diagnosis of pyruvate kinase deficiency initially made with pyruvate kinase enzyme activity measurements with PKLR gene molecular analysis.* (Certainty of evidence: moderate; strength of recommendation: strong; agreement, 91%).

*Evidence/clinical considerations:* Upon identification of decreased PK enzyme activity, *PKLR* genotyping should be performed where possible as enzyme activity alone cannot reliably discriminate between the homozygote/compound heterozygote (disease) state and the heterozygote (carrier) state, cannot distinguish primary (congenital) from secondary (acquired) PK deficiency (which may occur in patients with clonal myeloid disorders such as myelodysplastic syndrome, acute myeloid leukemia, and others), and may be reduced due to mutations in genes other than *PKLR* (e.g. *KLF1* or *GATA1*).1,17,18 The expert panel discussed the role of *PKLR* genetic testing results for prognosticating the likelihood of future disease findings and complications and predicting response to specific treatments. Those individuals with two drastic *PKLR* mutations have been shown to have a lower hemoglobin, higher hemolytic rate, and highest frequency of complications and to be unlikely to have a hemoglobin response to splenectomy or PK activators.2,19-21 At the time of this guideline, however, there was not sufficient agreement among the expert panel regarding a predictive role of genetic testing for disease course.

*Recommendation A4. The expert panel recommends confirmation of a diagnosis of pyruvate kinase deficiency initially made with PKLR gene molecular analysis with pyruvate kinase enzyme activity measurement in patients without two known pathogenic mutations in PKLR.* (Certainty of evidence: moderate; strength of recommendation: strong; agreement, 100%).

*Evidence/clinical considerations:* Confirmatory reduced PK enzyme activity should be obtained where possible to confirm pathogenicity of novel *PKLR* variants or variants of unknown significance detected by molecular testing.1,2 As many as 20% of patients recently diagnosed with PK deficiency had a previously unreported *PKLR* genetic variant2. False positive testing can occur when variants of unknown significance are considered pathogenic in the absence of functional characterization/evidence, consequently, a decreased PK activity is considered functional evidence to confirm pathogenicity of these variants.

*Recommendation A5. The expert panel recommends against the use of pyruvate kinase enzyme activity predicting disease severity or disease course.* (Certainty of evidence: moderate; strength of recommendation: strong; agreement, 96%).

*Evidence/clinical considerations:* No relationship exists between PK enzyme activity and either genotype or clinical phenotype.1,2 Therefore, the utility of PK enzyme testing is limited to diagnosis only.

## Monitoring and Management of Chronic Complications of Pyruvate Kinase Deficiency

*Recommendation B1. The expert panel recommends screening for iron overload with serum ferritin in children and adults with pyruvate kinase deficiency to detect and avoid complications of iron overload, irrespective of transfusion status, beginning at 3 years of age or after 12 transfusion episodes, whichever occurs first.* (Certainty of evidence: low; strength of recommendation: strong; agreement, 100%).

*Evidence/clinical considerations:* While evidence highlighting the risk, consequences, and management of iron overload in PK deficiency is limited,3,22 existing data are concordant with more exhaustive studies undertaken in patients with non-transfusion dependent thalassemia and transfusion-dependent thalassemia.23,24 This concordance underscores the robustness of the recommendations.

From the thalassemia literature, it has been demonstrated that the liver iron concentration (LIC) is a reliable indicator of the total body iron burden.23 Furthermore, it can be predicted that iron overload necessitating chelation would develop after 12 transfusion episodes (based on iron balance studies reported in thalassemia patients having undergone successful allogeneic stem cell transplantation).25 Further, it is clear that the correlation between serum ferritin levels and LIC is not optimal.26 The expert group discussed the clinical utility of screening for iron overload with both ferritin and transferrin saturation but concluded that the transferrin saturation would increase the sensitivity of screening but decrease specificity, particularly in infants and children and in those with hereditary hemochromatosis. For these reasons, the GWG did not include transferrin saturation in the recommendation.

Most of the data specific to PK deficiency are from the PK deficiency Natural History Study,3,22 which demonstrated that even patients with PK deficiency not receiving regular transfusions developed iron overload over time:

* Patients were considered to have iron overload if: i) their highest ferritin was over 1000 ng/mL; or ii) they received chelation therapy in the 12 months prior to enrollment; or iii) their highest liver iron concentration (LIC) was >3 mg/g dry weight liver (DW) on T2\* MRI; or iv) they had cardiac iron overload as defined by a cardiac T2\* ≤20ms at any time in their history.
* Patients were defined as regularly transfused if they had received ≥6 transfusions in the 12 months prior to enrollment. At enrollment, 82% (198/242) of patients were not receiving regular transfusions; 38% (53/138) of these patients had iron overload as defined by ferritin.
* Of the 242 patients, 175 (72%) had ferritin levels measured within the prior 12 months. The median ferritin was 583 ng/mL (range, 17-5630 ng/mL). The overall prevalence of iron overload as defined by ferritin or chelation was 45% (82/181). Patients without ferritin monitoring had fewer transfusions (1% vs. 25% regularly transfused; P<0.0001) and a higher hemoglobin (Hb) level (median Hb 9.6 vs. 8.8 g/dL; p=0.01)

Iron overload results in several complications, all of which can potentially be avoided with effective iron chelation therapy.23,24 Hence early assessment and initiation of chelation when appropriate could prevent complications. Ferritin-based monitoring frequency should be

customized to each patient, although it must be conducted at least once a year. For patients receiving regular red cell transfusions, ferritin should be assessed every 1 to 3 months. Given that ferritin is an acute phase reactant, elevated levels in the context of inflammation should be cautiously interpreted, with a repeat assessment advised before proceeding with MRI. For example, it is unlikely that a child who has not received transfusions would develop iron overload by the age of 3 years regardless of the ferritin level.

*Recommendation B2*. *The expert panel recommends measurement of liver iron concentration using magnetic resonance imaging (MRI) in children and adults with pyruvate kinase deficiency with consistent serum ferritin measurements >500 ng/mL to detect and avoid complications of hepatic iron overload, irrespective of transfusion status.* (Certainty of evidence: low; strength of recommendation: strong; agreement, 100%).

*Evidence/clinical considerations:* Liver iron overload may result in complications including cirrhosis and hepatocellular carcinoma, with significant morbidity and mortality.26 Where possible, MRI assessment of liver iron concentration (LIC) is strongly recommended. It is important to balance the risks associated with sedation for MRI in children with the benefits of knowing the precise LIC. For patients receiving regular red cell transfusions or undergoing iron chelation therapy, annual liver T2, T2\*, R227 or R2\* MRI28 should be performed. Patients who are not regularly transfused may have LIC measurements less frequently, based on trends in serum ferritin, but at least once every 5 years. For non-transfused young children, the initial liver MRI may be deferred until after age 5 years to balance the risks of sedation with the utility of the information to be gained from the MRI. It is unlikely that a child not receiving transfusions would develop iron overload by the age of 3-5 years. If the serum ferritin is consistently elevated at a young age, other causes (infection, inflammation) should be considered before an MRI with sedation is performed.

Regularly transfused children who have received more than 12 transfusions may begin chelation therapy without an MRI, but MRI monitoring is recommended to ensure that effective chelation is occurring.

The ideal range for the LIC is 2-5 mg/g DW, achieved by intermittent chelation in non-transfused individuals, and regular chelation in transfusion dependent individuals.29

When MRI is not available, serum ferritin should be monitored, and though not optimal, trends can be used to guide chelation. In such situations, also tracking the number of transfusions and chelation compliance may provide some assessment of chelator efficacy. Liver biopsy for quantitative estimation of LIC has been used in the past but is invasive and has associated risks.

*Recommendation B3. The expert panel recommends cardiac iron measurement using magnetic resonance imaging in all patients with pyruvate kinase deficiency with liver iron concentration*

*greater than 7 mg/g dry weight to detect and avoid complications of cardiac iron overload, irrespective of transfusion status.* (Certainty of evidence: low; strength of recommendation: strong; agreement, 92%).

*Evidence/clinical considerations:* Iron loading is slower in patients who are not transfused or episodically transfused than in regularly transfused individuals. Iron initially accumulates in the liver since it is a normal storage organ.26 Once the liver is saturated, iron may deposit in other organs such as the heart and endocrine organs. Thus, the potential for cardiac iron loading is low in children and those who are not regularly transfused. It is rare for these organs to be affected if the LIC has always been under 7 mg/g DW by MRI.26 Generally, experts agree that the risk of cardiac iron overload increases if the LIC exceeds 7 mg/g dry weight. In all patients with an LIC above 7 mg/g dry weight and in patients with a lower LIC, but who likely had periods with LIC greater than 7 mg/g dry weight in the past, cardiac iron should be assessed using MRI. Therefore, the expert panel recommends cardiac MRI for patients who have an LIC >7 mg/g dry weight or have been above this threshold in the past. Notably, no study has identified the optimal LIC threshold for initiation of cardiac MRI monitoring in PK deficiency (or other congenital hemolytic anemias). A more conservative threshold of 5 mg/g dry weight, which is the same threshold recommended for initiation of iron chelation therapy (Recommendation B4), is also reasonable.

Cardiac iron overload may result in complications including heart failure and arrhythmias, with significant morbidity and mortality.30 Where possible, cardiac T2\* MRI assessment of myocardial iron may identify patients at risk for these complications and guide chelation therapy. The cardiac T2\* should be maintained above 20 milliseconds which correlates with minimal or no iron deposition.31 Cardiac T2\* and LIC do not correlate well since cardiac loading and unloading are both slower than in the liver.26 Children should have their first cardiac T2\* at age 10 years if they are not regularly transfused or are transfused but well chelated. In regularly transfused patients, monitoring is recommended every year (if high LIC, or ineffective chelation) or 2 years (if LIC in target range and effectively chelated). In patients not on regular transfusions, cardiac T2\* may be done less frequently depending on the LIC.

It is preferred that liver and cardiac MRI assessments are done during the same scan, when possible, especially in regularly transfused patients. Cardiac T2\* assessment requires a long breath hold, which is often feasible after age 8-10 years.

There is no other method for assessing the cardiac iron burden, but functional assessment by cardiac echocardiogram may be performed to assess cardiac function and look for evidence of cardiomyopathy. However, the cardiac ejection fraction is preserved until there is severe iron- induced cardiomyopathy.30

*Recommendation B4. The expert panel recommends iron chelation therapy in patients with*

*pyruvate kinase deficiency aged 2 years or older who have a liver iron concentration exceeding 5 mg/g dry weight, irrespective of transfusion status, to reduce the risk of complications from iron overload.* (Certainty of evidence: low; strength of recommendation: strong; agreement, 100%).

*Evidence/clinical considerations:* Chelator agent selection, dosing, and monitoring is described in detail elsewhere.29 Where possible, continued MRI LIC monitoring is important to determine the effectiveness of chelation and tailoring of the regimen. In individuals not receiving regular transfusions who have slower iron loading, LIC monitoring is important to determine when to stop chelation so as not to over-chelate or cause chelator toxicity (possible when LIC is <2 mg/g

dry weight).29 In regularly transfused individuals with continued more rapid loading, annual LIC assessment will guide tailoring of the chelation regimen.

*Recommendation B5. The expert panel recommends iron chelation therapy in patients with*

*pyruvate kinase deficiency aged 2 years or older who have received >12 transfusions or serum ferritin >1000 ng/ml, to reduce the risk of complications from iron overload.* (Certainty of evidence: low; strength of recommendation: strong; agreement, 100%).

*Evidence/clinical considerations:* Where LIC is not available, serum ferritin and/or red cell transfusion thresholds can be used to trigger initiation of iron chelation. While the correlation with LIC is not optimal, most individuals with ferritin levels above 1000 ng/mL would be considered to have iron overload and would likely benefit from chelation.32 In regularly transfused individuals, iron overload can be predicted to have developed after 12 transfusions, necessitating the initiation of chelation. Continued serum ferritin monitoring is important in these patients to determine chelation effectiveness and tailor the regimen.

*Recommendation B6. The expert panel suggests echocardiography in all patients with pyruvate kinase deficiency 18 years of age or older to screen for pulmonary hypertension.* (Certainty of evidence: very low; strength of recommendation: conditional; agreement, 100%).

*Evidence/clinical considerations:* Pulmonary hypertension (PH), while uncommon in PK deficiency,3 is a morbid complication severely impacting health-related quality of life (HRQoL).33 The recognition that PH is a known and morbid complication of PK deficiency led to this conditional recommendation by the expert panel. Most of the data for screening for PH in patients with red cell disorders is from the literature in sickle cell disease, and there are limitations of this data in this population. There are no evidence-based recommendations for screening for PH in the thalassemia population, but, in this population, PH appears to be more common in splenectomized patients who are non-transfusion dependent.

Where possible, screening with echocardiography utilizing the tricuspid regurgitation jet method may promote early detection of PH and thus timely intervention,34 thereby potentially improving patient outcomes. Upon reaching adulthood, the tricuspid regurgitation jet method should ideally be incorporated into all cardiac echocardiography tests performed on a patient with PK deficiency, even if the study is not specifically being performed to screen for PH. The frequency of echocardiography screenings may range between 1 and 5 years and should be tailored based on individual risk factors (e.g., dyspnea, syncope, edema), prior elevation in tricuspid regurgitation jet by echocardiography, a history of splenectomy, and severity of hemolysis as judged by severity of anemia and degree of elevation of hemolytic markers.

It is important to note that neither brain natriuretic peptide nor electrocardiogram are specific for PH, which limits their utility as generalized screening measures.

*Recommendation* B7. *The expert panel recommends annual 25-hydroxy vitamin D measurement beginning at one year of age in all patients with pyruvate kinase deficiency not on*

*regular vitamin D supplementation to detect and treat vitamin D deficiency and reduce the risk of bone density loss.* (Certainty of evidence: low; strength of recommendation: strong; agreement, 100%).

*Evidence/clinical considerations:* Early-onset reduced bone mineral density, resulting in heightened fracture risk, is common in patients with PK deficiency irrespective of their transfusion status,3,35 underscoring the importance of screening and treating all patients for vitamin D deficiency. In a study of 159 adults with PK deficiency, over half had low bone density, osteopenia or osteoporosis, with an early onset of bone disease (young adulthood).35 Repletion of vitamin D in patients with PK deficiency should be performed as per best practice in other patients (the general population) without PK deficiency.

*Recommendation B8. The expert panel recommends screening for reduced bone mineral density using dual-energy x-ray absorptiometry (DEXA) scanning in all patients with pyruvate kinase deficiency beginning at 18 years of age to diagnose and manage low bone mineral density, osteopenia, and osteoporosis.* (Certainty of evidence: low; strength of recommendation: strong; agreement, 92%).

*Evidence/clinical considerations:* Early-onset reduced bone mineral density, resulting in heightened fracture risk, is common in patients with PK deficiency irrespective of their transfusion status,3,35 For patients with reproductive potential, treatment options primarily include vitamin D supplementation and optimizing calcium intake, complemented with physical activity. While DEXA scans are straightforward procedures with minimal radiation exposure, the frequency should be adjusted based on the potential implications of monitoring.

The frequency of monitoring, suggested to be every 1-3 years in this population, should be tailored according to the results of prior DEXA scans and individual risk factors, such as fracture history, vitamin D status, and low levels of physical activity. Antiresorptive and osteoanabolic therapies can be initiated when appropriate to maintain and improve bone density. Young patients diagnosed with low bone density (Z-score ≤ −2.0 in women of childbearing potential and men <50 years of age) and older patients diagnosed with osteoporosis (T-score ≤2.5 in women of nonchildbearing potential and men ≥50 years of age) should be referred to an endocrinologist where possible to manage the treatment and oversee further DEXA monitoring.

Screening with DEXA scans is recommended beginning in adulthood because of the availability of supporting data, and because interventions other than optimizing nutrition and vitamin D and calcium supplementation (such as bisphosphonates) are usually not recommended before completion of puberty. While the recommended age for starting DEXA screening is 18 years, special situations (e.g., recurrent fractures, evidence of massive extramedullary hematopoiesis which could predict marrow hyperplasia, other endocrinopathies, history of vitamin D deficiency) may necessitate DEXA screening prior to 18 years of age.

*Recommendation B9*. *The expert panel recommends age-appropriate laboratory endocrine monitoring in patients with pyruvate kinase deficiency receiving regular transfusions and non- transfused patients who have iron overload, defined as serum ferritin >1000 ng/mL or liver iron*

*concentration >5 mg/g dry weight, to identify and treat endocrinologic complications of iron overload.* (Certainty of evidence: very low; strength of recommendation: strong; agreement, 100%).

*Evidence/clinical considerations:* The heightened risk of thyroid, pancreatic, or pituitary dysfunction in patients with PK deficiency primarily pertains to those with iron overload.3,22,26 Therefore, enhanced screening can be limited to these individuals and directed by an endocrinologist. Because hemoglobin A1c measurements are unreliable in hemolytic anemia,36 annual diabetes screenings utilizing alternative measures (fasting glucose, oral glucose tolerance testing, or fructosamine measurement) are recommended.

Where possible, consider MRI of the pituitary and pancreas, particularly in patients with known endocrinopathy or severe iron overload.

In children, monitoring growth and achievement of puberty are critical to achieve full adult potential. In those with severe iron overload, levels of growth hormone and gonadotropins should be monitored with endocrinologist input.

*Recommendation B10. The expert panel suggests monitoring of renal function in children and adults with pyruvate kinase deficiency, irrespective of transfusion status for early detection of renal dysfunction.* Certainty of evidence: low; strength of recommendation: conditional; agreement, 68%).

*Evidence/clinical considerations:* Kidney disease in hemolytic anemias, often characterized by hyperfiltration, hypercalciuria and albuminuria, is believed to occur from chronic anemia, ongoing hemolysis, and free heme-mediated renal damage, as well as iron-mediated glomerular injury.37 Hemolysis-related renal injury can result from reactive oxygen species production and activation of inflammatory pathways.38 Iron chelators, such as deferasirox, may additionally lead to renal tubular abnormalities including renal tubular acidosis.39 Based on the available data for other congenital hemolytic anemias, the expert panel suggests monitoring renal parameters including creatinine, phosphorus, magnesium, albuminuria and urine protein:creatinine ratio based on individual risk factors (underlying kidney dysfunction, chelation drugs).

## Standard Management of Anemia in Pyruvate Kinase Deficiency

*Recommendation C1*. *The expert panel recommends discussion of the individualized risks and benefits of splenectomy to treat anemia in children greater than 5 years old and adults who require regular or frequent red cell transfusions or who have symptomatic anemia, to reduce transfusion burden and alleviate symptoms.* (Certainty of evidence: moderate; strength of recommendation: strong; agreement, 96%).

*Evidence/clinical considerations:* Observational studies demonstrate that splenectomy is associated with increased hemoglobin in some adults and children with PK deficiency. In the PK Deficiency Natural History Study of 254 patients, 150 had undergone splenectomy, with a mean increase in hemoglobin of 1.6 g/dL, and reduced transfusion burden in 90%, although 20% had no response to splenectomy.3 Response to splenectomy was predicted by higher pre- splenectomy hemoglobin levels, lower bilirubin levels and missense *PKLR* variants.

Splenectomy is not curative, and hemolytic anemia and the associated complications of chronic hemolysis continue in the absence of a spleen. The potential benefits need to be balanced against the risks, including the increased risks of serious infections, particularly with encapsulated bacteria (*Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae*),40 malaria, *Babesia* and *Bordetella holmesii*.41 To some extent these risks can be reduced by vaccinations, prophylactic antibiotics and patient education, although the risks are highest in young children, and splenectomy is not recommended in children under 5 years of age. Splenectomy is also associated with a less-well-defined increased risk of thromboembolic complications and late cardiovascular complications, including pulmonary hypertension and ischemic heart disease.42 Every patient with PK deficiency and a venous thromboembolic event in the PK Deficiency Natural History Study had been splenectomized.3

Overall, there is insufficient evidence to give strong recommendations either for or against splenectomy in PK deficiency, and it is not possible to identify reliably those who are likely to benefit or those who are especially likely to be at increased risk of post-splenectomy complications. It is therefore recommended that risks and benefits are discussed with families, adults and children with PK deficiency, and a decision regarding splenectomy made based on individual preferences, availability of safe blood transfusions and iron chelation, and access to newer therapies.

In adults and children undergoing splenectomy, additional vaccinations prior to splenectomy and regular booster vaccine doses are recommended to reduce the risk of infection. Specific vaccines and vaccination schedules vary by country and are updated annually, but available vaccines against encapsulated organisms, including *Streptococcus pneumoniae*, *Haemophilus influenzae* type b, and *Neisseria meningitidis,* are strongly recommended. As an example of updated recommendations, please see the Centers for Disease Control and Prevention vaccine recommendations for adults with asplenia (<https://www.cdc.gov/vaccines/schedules/hcp/imz/adult-conditions.html>) and children with asplenia (https://[www.cdc.gov/vaccines/schedules/hcp/imz/child-indications-compliant.html).](http://www.cdc.gov/vaccines/schedules/hcp/imz/child-indications-compliant.html))

The length of antibiotic prophylaxis following splenectomy is more controversial than vaccination and practices vary widely. In general, splenectomy is delayed until after the age of 5 years. For children undergoing splenectomy at a younger age, antibiotic prophylaxis is recommended until at least age 5 years. In children age >5 years and adults, antibiotics are recommended for at

least year one following splenectomy. Many hematologists recommend indefinite antibiotic prophylaxis after splenectomy. Daily penicillin or amoxicillin are the usual antibiotics of choice, but alternatives can be used in patients with a penicillin allergy.

*Recommendation C2. The expert panel recommends initiation of regular red cell transfusions in children <5 years of age with pyruvate kinase deficiency who have symptomatic anemia or anemia that has an impact on growth and development, to improve anemia symptoms and growth.* (Certainty of evidence: low; strength of recommendation: strong; agreement, 91%).

*Evidence/clinical considerations:* There are no randomized trials or large case series evaluating the use of red cell transfusion in patients with PK deficiency. Most evidence is inferred from studies and guidelines on the management of other transfusion-dependent conditions, such as thalassemia and Diamond-Blackfan anemia. Regular transfusions typically involve administering planned red cell transfusions every 3 to 12 weeks. The aim is to optimize the child’s growth and development and to avoid a marked increase in symptoms in the days leading up to the next scheduled transfusion. In patients with transfusion-dependent thalassemia, recommendations involve maintaining a specific pre-transfusion hemoglobin threshold, based partly on the need to suppress ineffective erythropoiesis. In PK deficiency, lower pre-transfusion hemoglobin levels are typically accepted, because of the lower affinity of hemoglobin for oxygen in PK deficiency and less concern about ineffective erythropoiesis.

In children it is particularly important that growth and development are optimized, and it is recommended that regular transfusions are started if there is any suggestion that anemia is causing impairment. Relevant indications for transfusion in children include the need for frequent unplanned transfusions (>3-4 times per year), reduced growth with failure to increase height and/or weight along the expected centiles, dyspnea, fatigue, and reduced capacity for normal activities including school, sports, and socializing. The decision to start regular transfusions does not depend on a particular hemoglobin level or other laboratory parameters, although regular transfusions will likely be beneficial if the steady-state hemoglobin is less than 6 g/dL. Mitapivat is not presently approved for treatment in children, and splenectomy is not recommended in children under 5 years of age because of the increased risk of infection.

Therefore, regular transfusions are the only available option to reduce symptoms in children under 5 years of age.

The expert panel recommends that the decision about starting regular transfusions is taken jointly with the family and children, and that transfusion regimes are individualized and according to the specific circumstances and symptoms of each patient.

The expert panel was also not able to make evidence-based recommendations regarding the use of folic acid in PK deficiency, due to lack of evidence and diversity of clinical practice. Folic acid has traditionally been given to all people with chronic hemolytic anemia, because of the increased need for folic acid with increased rates of erythropoiesis, and anecdotal reports of folic acid deficiency occurring. Conversely, in high income countries, most diets are enriched in folic acid and deficiency is rare, including those with hemolytic anemia. In practice, many people with PK deficiency take folic acid as it has almost no side effects and very few short- or long- term risks.

*Recommendation C3. The expert panel recommends treatment with regular red cell transfusions in children 5 years of age and older and adults with pyruvate kinase deficiency who have symptomatic anemia despite splenectomy or are unsuitable for or unwilling to undergo splenectomy to improve symptoms of anemia.* (Certainty of evidence: low; strength of recommendation: strong; agreement, 92%).

*Evidence/clinical considerations*: Supportive management of symptomatic patients with PK deficiency older than 5 years of age includes splenectomy and regular red cell transfusions. Splenectomy offers the possibility of symptomatic improvement, without the need for regular hospital attendance for transfusions, but may not be acceptable to some individuals because of the surgical risk, the long-term complications associated with immunodeficiency and cardiovascular disease and the irreversibility of the procedure if there is no clinical benefit.

Regular blood transfusions offer the potential advantages of providing a more predictable increase in hemoglobin level, and can be modified or stopped as circumstances change, but involve much more time in hospitals and long-term iron chelation. Blood transfusions are also not readily available in many countries and may carry significant risks, which vary with country and are typically much greater in low- and middle-income countries; these risks include infections, alloimmunization and iron overload.

The degree of anemia may vary over the lifespan in patients with PK deficiency. Typically, anemia is more severe in infancy and tends to improve with adolescence, which may coincide with a decreased frequency of viral infections and relative improvement post-splenectomy in patients undergoing splenectomy prior to adolescence. However, worsening of hemoglobin levels and fatigue may reoccur in adults as patients age.3-5 Additionally, tolerance for an unchanged chronic anemia may worsen due to age-related cardiopulmonary decline.43

The decision to start long-term regular blood transfusions should be made jointly with patients and families and is an alternative to splenectomy.

*Recommendation C4. The expert panel recommends that red cell transfusions be administered to children and adults with pyruvate kinase deficiency on the basis of anemia symptoms and complications rather than a universal hemoglobin transfusion threshold.* (Certainty of evidence: low; strength of recommendation: strong; agreement, 87%).

*Evidence/clinical considerations:* Hemoglobin levels vary in people with PK deficiency, and typically are lower during infections or other concomitant illnesses. The symptoms associated with a certain degree of anemia also vary between individuals and in the same person at different times; relevant factors include co-existent disease, particularly cardiovascular and respiratory problems, levels of fitness, and environmental factors such as physical and emotional demands, altitude, and planned activities. This variation occurs in both non- transfused and regularly transfused individuals, although is more marked in the former.

Unplanned/episodic transfusions are frequently necessary in PK deficiency, typically when increased anemia is associated with related symptoms, such as increased fatigue, dyspnea, headaches or chest pain. The need for transfusions may also be supported by signs such as tachypnea, tachycardia, and increased pallor. The decision to give an unplanned/episodic transfusion is primarily based on symptoms rather than an arbitrary hemoglobin threshold often

used to trigger transfusions in the general population (such as 7 or 8 g/dL that are commonly employed), and the hemoglobin level should be interpreted relative to the patient’s known steady-state level; transfusion will almost always be needed if the hemoglobin is below 5 g/dL. The decision to transfuse is more difficult in infants, who are typically unable to describe symptoms and so typically may require transfusion based on hemoglobin levels and signs.

Older patients with cardiovascular impairment may require the hemoglobin to be kept at higher levels. The use of transfusions may also be influenced by planned activities, such as travel, school trips and certain activities, such as skiing or unaccustomed exercise. Overall, the giving of unplanned transfusions is a complicated decision based on a combination of symptoms, laboratory results, circumstances and the wishes of patients and families.

*Recommendation C5. The expert panel suggests that if a splenectomy is planned in a patient with pyruvate kinase deficiency, cholecystectomy is also considered and discussed with the patient, family, and/or caregiver.* (Certainty of evidence: low; strength of recommendation: conditional; agreement, 96%).

*Evidence/clinical considerations*: The incidence of gallstones and gallstone complications are increased in PK deficiency, predominantly due to the development of pigmented gallstones associated with chronic hemolysis.3 In the PK Deficiency Natural History Study, 40% of the 254 patients had undergone cholecystectomy, and 21% cholecystectomies were performed simultaneously with splenectomy.3 However, of 121 patients with splenectomy without prior or simultaneous cholecystectomy, 48% later required cholecystectomy.

Based on the high risk of cholecystectomy at some point in people with PK deficiency, it is recommended that cholecystectomy is considered if a splenectomy is being performed. This is likely to be particularly beneficial if gallstones, or possibly biliary sludge, are already present, and potentially avoids the need for a second operation and reduces the risk of the serious complications associated with gallstones, including cholecystitis, pancreatitis, biliary sepsis and obstructive jaundice. It is less clear whether a healthy gallbladder should be removed at the time of splenectomy, although some experts also recommend this.

*Recommendation C6. The expert panel recommends that appropriate psychological support be offered to children and adults with pyruvate kinase deficiency and their families and/or caregivers.* (Certainty of evidence: low; strength of recommendation: strong; agreement, 100%).

*Evidence/clinical considerations:* As with many chronic medical conditions, comorbid psychiatric complications including anxiety and depression are common in patients with PK deficiency and their caregivers.44 A survey of 200 adults with PK deficiency and 75 adult caregivers found that 21% were worried by their condition, 17% suffered from anxiety and 17% were depressed.44 There is little or no evidence on which specific psychological therapies are most beneficial for people with PK deficiency, although given the data on psychiatric comorbidity, mental health should be discussed with patients and caregivers and services should be made available where appropriate.

## Targeted and Advanced Therapies in Pyruvate Kinase Deficiency

*Recommendation D1. The expert panel recommends initiation of mitapivat therapy in adult patients with pyruvate kinase deficiency who are anemic, who are not regularly transfused, and who do not have two non-missense mutations, irrespective of splenectomy status, to improve hemoglobin and health-related quality of life.* (Certainty of evidence: high; strength of recommendation: strong; agreement, 100%).

*Evidence/clinical considerations:* This recommendation is based on the results of the ACTIVATE phase 3 randomized controlled trial (RCT) for mitapivat.45 The qualifications for age, transfusion status, and mutation status in the recommendation were eligibility criteria for enrollment on the ACTIVATE trial. The observation that the drug could be effective, regardless of splenectomy status, was made in the phase 2 and 3 trials. In addition to improvement of anemia manifestations and HRQoL, data are emerging suggesting that long-term mitapivat therapy may ameliorate certain chronic complications of hemolysis, notably iron overload.46 Along with monitoring for potential adverse effects of treatment, standard monitoring for complications of PK deficiency should be continued in patients receiving mitapivat.

The expert panel recognized that standard nomenclature for description of genetic changes has moved away from “mutations” to “variants.” However, the ACTIVATE clinical trial exclusion criteria referenced that the patient could not have two ‘non-missense mutations’. Because mitapivat is an allosteric activator of the PK enzyme itself, it lacks activity if the protein is absent.47 This would include patients in which both *PKLR* mutations are nonsense mutations (early stop codons), large deletions, or insertions or deletions that cause frameshifts.

Alternatively, some small, in-frame indels, despite being “non-missense mutations,” may yield partially functional protein amenable to mitapivat activation and/or stabilization (**Supplementary Table**), and a trial of the drug should be considered in such patients. In addition, some true missense (amino acid substitution) variants fail to respond and therefore some patients with homozygous or compound heterozygous missense mutations will be insensitive to mitapivat.

This is true of the R479H missense mutation common among patients with PK deficiency in the Pennsylvania Amish community in the United States.47 The relationship between genotype and hemoglobin response to mitapivat has been explored based on available clinical trial data.48 As a general principle, for variants of unknown responsiveness to mitapivat, which could include novel variants, rare variants not yet tested, or the non-missense variants noted above, a trial of mitapivat is reasonable and recommended.

At the time of the expert panel review, no PK activators other than mitapivat had been studied or approved for PK deficiency. It is possible that a future PK activator would bind the enzyme at a different site from mitapivat, and so a different subset of missense mutations might be activated by a novel agent.

## Supplementary Table. Classes of *PKLR* gene variants and predicted response to a trial of mitapivat.

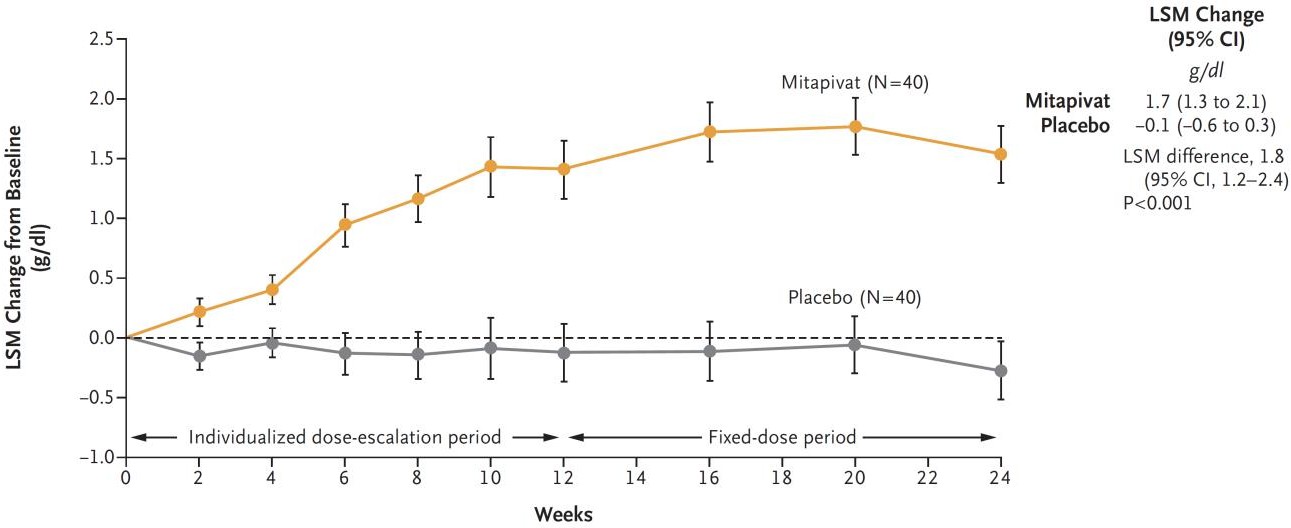
|  |  |
| --- | --- |
| **Class of variant** | **Genetic mechanisms** |
| **Variants associated with low likelihood of hemoglobin response to mitapivat** | |
| ‘Non-missense’ variants: Known or predicted “null” allele | Large deletions, nonsense mutations (early termination codon), indels out of frame across functional domains, select promoter mutations, select splice site mutations |
| Missense (amino acid substitution) variants with low possibility of mitapivat  activation | Active site mutations which render enzyme active site nonfunctional; mutations which abrogate mitapivat binding |
| **Variants associated with a high likelihood of hemoglobin response to mitapivat** | |
| Missense variants (amino acid substitution) | Amino acid change that leaves the enzyme active site functional |
| Non-missense variants which may be amenable to mitapivat therapy | Small, in-frame insertions or deletions; some intronic splice variants with reduced splicing efficiency; some promoter mutations |

*Recommendation D2. The expert panel recommends that “failure to respond” to mitapivat in patients with pyruvate kinase deficiency who are not regularly transfused should be declared only after at least 3 months of treatment with mitapivat at an optimal or maximum dose.* (Certainty of evidence: high; strength of recommendation: strong; agreement, 100%).

*Evidence/clinical considerations:* The expert panel recommends dose optimization of mitapivat according to the drug prescribing information. For many subjects in the mitapivat arm of the ACTIVATE trial,45 evidence of efficacy occurred over less than a month at an optimal dose

(**Supplementary Figure**). However, if a potential delay might arise in observing effect (for example, due to an intercurrent illness), three months should be sufficient to assess hemoglobin response in most patients. Trialing at least 3 months of treatment at optimal dosing before declaring a failure to respond may not apply to other PK activators.

**Supplementary Figure. Average Change from Baseline in the Hemoglobin Level.** Baseline was defined as the average of all screening assessments within 45 days before randomization in patients who underwent randomization and did not receive mitapivat or placebo, or before the start of the trial treatment period in patients who underwent randomization and received mitapivat or placebo. The figure shows the least-squares mean (LSM) change from baseline in the hemoglobin level in the two groups during the trial period. The 𝙸 bars indicate the standard error.



From Al-Samkari et al., Mitapivat versus Placebo for Pyruvate Kinase Deficiency, *New England Journal of Medicine* 2022; 386(15): 1432-42, copyright © 2022 Massachusetts Medical Society. Reprinted with permission.45

*Recommendation D3. The expert panel recommends initiation of mitapivat therapy in adult patients with pyruvate kinase deficiency who are regularly transfused and who do not have two non-missense mutations, irrespective of splenectomy status, to reduce transfusion burden.* (Certainty of evidence: moderate; strength of recommendation: strong; agreement, 92%).

*Evidence/clinical considerations:* This recommendation is based directly upon results of the prospective, single-arm phase 3 ACTIVATE-T trial of adults with PK deficiency receiving regular transfusions.49 This trial demonstrated ≥33% reduction in transfusion burden in 37% of patients, including 22% who achieved complete transfusion independence.

As described in Recommendation D1, and categorized in the **Supplementary Table**, some missense (amino acid substitutions) *PKLR* variants are non-responsive to mitapivat (such as R479H). On the other hand, some non-missense variants may be responsive, including small in- frame indels, promoter variants, splicing variants. Similar to non-regularly transfused patients, for patients with variants of unknown responsiveness to mitapivat, including novel variants, rare variants not yet tested, or the non-missense variants noted above, a trial of mitapivat is recommended.

The expert panel noted that in some patients with congenital hemolytic anemia, only one *PKLR* gene mutation is detected. Therapy of such patients with mitapivat is not recommended unless biochemical assays demonstrate deficient PK activity AND the sole variant detected is predicted to be amenable to therapy. Referral to an expert center for consultation is recommended in this circumstance.

*Recommendation D4. The expert panel recommends discontinuation of mitapivat therapy, and return to best supportive care, in patients with pyruvate kinase deficiency who are non- responders to mitapivat, irrespective of transfusion status. (Certainty of evidence: moderate;*

*strength of recommendation: strong; agreement, 92%).*

*Evidence/clinical considerations:* Neither ACTIVATE nor ACTIVATE-T directly address management strategies in mitapivat non-responders as a trial outcome, but all of the alternative treatment strategies discussed herein are available for mitapivat non-responders. The expert panel acknowledges the fact that both splenectomy status and transfusion status are related to patient/physician decision making, not necessarily to underlying biology. Therefore, in a mitapivat non-responding patient, splenectomy may be a subsequent option, and if the patient was not previously transfused, a strategy of chronic transfusions plus iron chelation is an option.

The panel concluded that mitapivat should be discontinued in the absence of a response, although the supporting evidence is not strong, but the expert panel considered discontinuation consistent with best practice. As is described in Recommendation D6 and Recommendation D8, the definition of “response” should not be limited to a specific hemoglobin threshold as was the case in the clinical trials but rather should include clinical improvements relevant to a patient’s wellbeing, including improvement in hemoglobin and also reduction in jaundice, improved control of iron overload, improved HRQoL, or other important disease parameters.

The question of clinical trials or measures outside of standard supportive care are not as well supported by the literature, and so are addressed in a separate recommendation (Recommendation D5).

*Recommendation D5. The expert panel recommends consideration of alternative approaches, including clinical trials, in patients with pyruvate kinase deficiency who are non-responders to mitapivat, irrespective of transfusion status. (Certainty of evidence: very low; strength of recommendation: strong; agreement, 100%).*

*Evidence/clinical considerations:* Despite a paucity of high-quality evidence, this recommendation is included by the panel to acknowledge the importance of novel therapeutic approaches in non-responders to mitapivat. Trials of alternative approaches are now available or may become available in the future.

Hematopoietic stem cell transplantation (HSCT) deserves special mention. The expert panel found the available data to be insufficient to make specific recommendations broadly in favor of HSCT. The largest published case series is that of van Straaten and colleagues in 2018 describing 16 patients with PK deficiency undergoing HSCT,50 and the cases lack enough

common elements to make any form conclusions. The outcomes were generally not favorable in this compilation.

This is a key issue that should be discussed on an individualized basis between patient/family members and the medical care team. Several considerations arise in considering risk vs benefit in HSCT for PK deficiency in patients unresponsive to mitapivat. Are there factors present that suggest higher or lower risk of the intervention? If a patient is doing well with supportive care, then the risks of transplant need to be especially carefully weighed; among these, risk of acute and chronic graft-versus-host disease (GVHD), hepatic vaso-occlusive disease, graft failure, and complications of conditioning.

It is not possible to glean from the literature the optimal age for a potential HSCT, the optimal preparative regimen, or the best GVHD prophylaxis strategy. Ideally, therapies such as HSCT would be pursued in an organized setting (observational if not interventional trials), with plans to disseminate the results. The best examples of proper such models from other disorders may be the literature for HSCT in transfusion-dependent thalassemic disorders.51

Lentiviral-mediated gene therapy has been studied in a phase 1 trial of patients with PK deficiency,52 but the data are too preliminary for recommendation development at this time.

*Recommendation D6. The expert panel recommends discontinuation of mitapivat therapy in patients with pyruvate kinase deficiency receiving regular transfusions who do not achieve at least a 33% reduction in transfusion requirement, with the exception of patients who achieve marked improvement in iron status, patient-reported health outcomes, jaundice, or other key disease parameters.* (Certainty of evidence: low; strength of recommendation: strong; agreement, 88%).

*Evidence/clinical considerations:* In the ACTIVATE-T trial, the proportion of patients with a transfusion reduction of ≥33% was 37% over a duration of 24 weeks.49 The review panel found it plausible that outcomes other than transfusion reduction success might be clinically meaningful and merit continued mitapivat treatment. This includes the ability to be more effectively chelated,46 improved patient-reported outcome (PRO) measures, or significantly improved jaundice. The trial was not designed for these outcomes to be declared “successes,” but improvement in these outcomes might be reasonable cause to continue mitapivat in some selected transfused patients, with the caveat that mitapivat should not be considered as only an adjunctive iron chelator without other evidence of improvements.

*Recommendation D7. The expert panel recommends that adults with pyruvate kinase deficiency who are regularly transfused and who have not undergone splenectomy receive a trial of mitapivat therapy prior to consideration of splenectomy.* (Certainty of evidence: low; strength of recommendation: strong; agreement, 100%).

*Evidence/clinical considerations: S*plenectomy is irreversible and comes with potential irreversible harms and heightened lifelong risks,3,53 while mitapivat can be easily discontinued and has demonstrated a favorable safety profile in clinical trials.45,47,49

The expert panel recommendation is presently limited to adult patients. Pediatric trials of mitapivat to evaluate safety and efficacy are presently underway.54,55 If the trials are successful, with efficacy and safety in children similar to that demonstrated in adults, and mitapivat becomes approved in children, the expert panel felt that this recommendation would then also apply in children, who have even more to gain from avoidance of early splenectomy if a pharmacologic therapy were available for their disease. This issue will be settled in the coming years. Considerations for timing of splenectomy in children with PK deficiency today are listed in Section C (Standard Management of Anemia in Pyruvate Kinase Deficiency).

*Recommendation D8. The expert panel suggests that pyruvate kinase deficiency-specific measures of health-related quality of life (patient-reported outcomes) can be a determining factor of success in an individual trial of mitapivat in cases where the reduction in transfusion burden or increase in hemoglobin falls short of an arbitrary numerical cutoff.* (Certainty of evidence: moderate; strength of recommendation: conditional; agreement, 82%).

*Evidence/clinical considerations:* In both ACTIVATE and ACTIVATE-T, responders had improvement in disease-specific PRO measures as secondary endpoints.45,49 The expert panel recognizes that PRO measures are therefore important in judging success or failure of treatment.56,57 Taking PRO measures into account may be an ideal demonstration of value to heath authorities and payers when therapeutic response falls short of a particular hemoglobin or transfusion reduction target. PROs in this population have been validated in the clinical trial setting but have not been previously used in the clinical setting. These include the PK Deficiency Daily Diary (PKDD) and PK Deficiency Impact Assessment (PKDIA).

## Special Populations in Pyruvate Kinase Deficiency

*Recommendation E1. The expert panel recommends regular monitoring of children and adults with pyruvate kinase deficiency by a hematologist, irrespective of transfusion status.* (Good Practice statement; agreement, 96%)

*Evidence/clinical considerations:* While specific studies evaluating the effectiveness of regular monitoring in PK deficiency do not exist, other international guidelines for non-transfusion dependent congenital anemias suggest routine monitoring to assess risk for complications including hypersplenism and splenomegaly, extramedullary hematopoiesis, iron overload, delayed puberty and growth delay. Interventions for anemia need to be made on an individual basis based on the overall growth, health, and quality of life of the individual. Prevention of complications such as iron overload, PH and osteopenia will reduce the morbidity of the disease and may impact overall mortality. Due to the complexity of the disorder, the expert panel recommends that all children and adults with pyruvate kinase deficiency be regularly monitored by a hematologist with experience in managing red cell disorders irrespective of transfusion status.

Recommendations for frequency of monitoring will vary by age and underlying disease complications. Perinatal complications are common with approximately 30% of infants with PK deficiency having severe manifestations such as preterm birth, intrauterine growth restriction, transfusion dependent anemia or severe neonatal jaundice requiring exchange transfusion.58 Infants should be monitored closely in partnership with the general pediatrician to manage hyperbilirubinemia and evaluate growth and impact of anemia. Signs of clinically relevant anemia in the newborn period may be poor feeding and poor weight gain. For infants diagnosed at birth, it is reasonable to monitor growth at 2, 4 and 12 weeks of age and then transition to monitoring visits every 3 months with interval primary care visits at 2 and 4 months of age.

Transfusions may be required more frequently to maintain appropriate weight gain with <50% of children < 5 years of age requiring regular transfusions. The interval of visits with the hematologist will vary depending on transfusion needs.

The expert panel recommends that young children <5 years of age continue to be monitored by a hematologist every 3 months to assess growth, development, hypersplenism and signs of extramedullary hematopoiesis. Symptoms of clinically relevant anemia in this age group may be poor growth, fatigue (frequent napping), and irritability. Irritability and poor concentration at this age may manifest as hyperactivity. Children may additionally develop splenomegaly or frontal bossing associated with extramedullary hematopoiesis. As children age, they are less likely to require regular transfusions frequently due to the fact that many children over 5 may progress to splenectomy and the fact that viral and other infectious triggers are less common.59,60 Specific recommendations regarding splenectomy are discussed in Section C, Standard Management of Anemia in PK Deficiency.

For children aged 5 years and older who are not on regular transfusions, the expert panel recommends visits with a hematologist every 6-12 months to continue to evaluate growth, physical development, and pubertal development. The decision to transfuse at this age again should consider growth, development, physical functioning, and quality of life. Signs of clinically relevant anemia may be poor concentration and school performance or an inability to keep up with peers in activities. Patients should be educated and screened for common complications

such as aplastic crisis, iron overload, bone health, and gallbladder disease. Specific screening recommendations are discussed elsewhere in these guidelines.

Though they are not a special population, the expert panel felt it important to include in the guideline that adults with PK deficiency should be monitored at least annually, and usually more frequently, by a hematologist for ongoing education, discussion of therapeutic interventions and screening for PK deficiency related complications. It is important to note that as people age or develop other medical comorbidities, the level of anemia at which symptoms emerge may change. Therefore, physical functioning and quality of life needs to be continually assessed.

Women of childbearing potential should be counseled and educated on the potential complications from PK deficiency during pregnancy. Iron management should be optimized prior to pregnancy to prevent liver and cardiac complications.61

The decision to transfuse on a regular schedule at any age should be based on individual patient needs, not hemoglobin alone, as patients with PK deficiency may be symptomatic at varying levels of anemia even within an individual. This is discussed further in Section C, Standard Management of Anemia in PK Deficiency. Growth, level of activity and functioning should be considered and balanced against risk burden of transfusion therapy and iron overload.62

*Recommendation E2. The expert panel recommends that women with pyruvate kinase deficiency irrespective of transfusion status who are pregnant or are planning pregnancy be referred to a multidisciplinary feto-maternal team (including a hematologist, obstetrician, neonatologist, and other specialists as appropriate) to reduce maternal and fetal complications.* (Good Practice statement; agreement, 100%)

*Evidence/clinical considerations:* The PK Deficiency Natural History Study found that approximately 50% of women with PK deficiency received a red cell transfusion during pregnancy.58 Based on the frequency of transfusions reported in this study and the potential benefit to pregnancy outcomes by avoiding severe anemia, the expert panel recommend routine screening for anemia and its complications during pregnancy in addition to standard (or high- risk) maternal care. Pregnancy is a potential trigger for increased rate of hemolysis and increased physiologic stress. Multiple international guidelines for congenital hemolytic anemias such as thalassemia and sickle cell disease recommend maintaining maternal hemoglobin >10 g/dL to reduce maternal complications, prematurity and fetal growth restrictions.63,64 In a large population based study, anemia defined as hemoglobin <10.0 g/dL was associated with an increased risk of preterm delivery, low birthweight, cesarean delivery, and placental complications.65 This risk was further increased when hemoglobin was <8.0 g/dL.65

Pregnancy outcomes in the PK Deficiency Natural History Study included 82 live births (70 full- term and 12 preterm). Rates for preterm birth or miscarriages were not different from the healthy female population. The risk for perinatal complications was elevated.58,66 The expert panel recommends that all patients with PK deficiency planning pregnancy be referred by their primary hematologist to a multidisciplinary feto-maternal team (consisting of a hematologist, obstetrician, neonatologist, and other appropriate specialists) for comprehensive counseling and to discuss the management of PKD during pregnancy. To monitor growth restriction, fetal distress, or hydrops fetalis, the expert panel recommends a comprehensive risk assessment to determine

the frequency of monitoring at a minimum of monthly visits and serial fetal ultrasounds beginning at week 20 of gestation.

The expert panel recommends routine screening for anemia during pregnancy based on the frequency of transfusions reported by the NHS and the potential benefits of avoiding severe anemia on the outcome of the pregnancy. During pregnancy, the patient, hematologist, and obstetrician should determine the risks and benefits of transfusions to maintain an adequate hemoglobin level for fetal growth.

The PK Deficiency Natural History Study additionally found the overall prevalence of iron overload as defined by ferritin or chelation was up to 57%.22 In those patients with known poorly controlled iron overload, a thorough assessment for cardiac abnormalities due to cardiac iron overload using electrocardiogram, echocardiogram, and, if available, T2\* MRI should be performed to optimize iron status through intensified iron chelation prior to pregnancy. Chelation is often withheld until at least the second trimester of pregnancy due to potential risk of teratogenicity in the first trimester, therefore monitoring of iron overload is crucial and important part of pregnancy care.66

The expert panel also recommends consideration of other contributing maternal and fetal risk factors during counseling. This should include risk assessment for thrombosis and preeclampsia, as there is an increased risk of thrombosis during pregnancy. This risk is increased in those who are splenectomized and if the women have additional risk factors (such as obesity).63,64,66,67

# GUIDELINE DISSEMINATION AND IMPLEMENTATION AND FUTURE PRIORITIES FOR RESEARCH AND GUIDELINE DEVELOPMENT IN PK DEFICIENCY

*Dissemination and Implementation*

These evidence-based guidelines will be freely and globally available to patients and clinicians, including adult and pediatric hematologists, general practitioners, other specialists, patients, and caregivers. The guidelines will be openly available on the website pkdguidelines.org. The recommendations for individual topic areas will also be summarized in one-page fact sheets and a teaching slide set available to view and download on the website. Two patient advocacy organizations, the Thrive with Pyruvate Kinase Deficiency Organization and the Pyruvate Kinase Deficiency Foundation, will post the recommendations from the guidelines in a patient- friendly downloadable brochure format on their websites. A patient-oriented webinar will be hosted to review sections from the guidelines. Videos from the webinar will also be posted on the patient advocacy organization websites. The guidelines will be promoted through a PK deficiency podcast and social media accounts of the global PK deficiency patient organizations in multiple languages. Further promotion will occur in the medical community with endorsements and affirmations of value from national and international societies, reference networks, and foundations.

The included recommendations are primarily focused on patients who have access to safe and affordable blood transfusions, medications and surgery. Although epidemiologic data are scarce for PK deficiency, many patients likely live in countries in which complete implementation of these recommendations may be challenging (particularly access to advanced therapies).

Wherever possible, the clinical considerations sections that follow each recommendation have highlighted potential alternative management strategies if the recommended strategy is not possible (for example, monitoring liver iron overload with serum ferritin rather than MRI with iron quantitation).

*Future Priorities for Research and Guideline Development*

The expert panel highlighted certain key areas for future research and guideline development in PK deficiency. These are listed below, in no particular order.

## Future Priorities for Research in PK Deficiency:

* Use of mitapivat in children
* Safety and efficacy of gene therapy in PK deficiency
* Optimal treatment for mitapivat-nonresponsive patients
* Outcomes of novel therapeutics compared to splenectomy in children
* Long-term outcomes of mitapivat treatment in children and adults, including quality of life
* Evaluation of alternative PK activators in patients with PK deficiency
* Relationship between *PKLR* genotype and clinical course
* The prevalence and phenotype of acquired PK deficiency, and in which disorders it can be observed
* Further research into non-exon mutations that result in PK deficiency
* Implications of early identification and treatment of complications including pulmonary hypertension and renal disease
* The relationship between *PKLR* mutations and PK activator response
* Long-term safety and efficacy outcomes of splenectomy, HSCT and gene therapy
* Safety and utitlity of erythrocytapheresis (red cell exchange transfusion)
* Further research into the mental and physical wellbeing of patients with PK deficiency
* Neuropsychiatric outcomes of patients compared with degree of anemia
* Prospective registries/cohort studies of pregnant patients with PK deficiency; additional research on pregnancy in PK deficiency in general
* Clinical trials directly comparing transfusions, splenectomy, and PK activators
* Role of folic acid supplementation
* Exploration of therapeutics with alternative mechanisms of action outside of PK activation and gene therapy
* Further research into hemolytic crises in PK deficiency and their symptoms and optimal management
* PK deficiency impacts on physiologic aging
* Vascular complications of PK deficiency
* Management of PK deficiency in low-resource areas

## Future Priorities for Guideline Development in PK Deficiency

* Guidance on the potential use of mitapivat in children
* Guidance on the potential use of gene therapy in children and adults
* Monitoring of iron status (e.g., optimal frequency of MRIs), chelator use, and chelator toxicity based on studies of patients with PK deficiency specifically (rather than extrapolation from thalassemia)
* Guidance on optimal sequencing of various therapeutic options
* Detailed guidance on management of pregnancy in PK deficiency, should data specific to these patients become available

# EXTERNAL REVIEW OF GUIDELINES: PROCESS AND RESULTS

The draft guideline manuscript and supplement were reviewed by topic experts and non-experts not involved in the development of the guidelines representing a variety of different specialties and subspecialties, as well as experts in clinical guidelines. Reviewers were asked to read the manuscript and supplement and then to complete an online, fully anonymous external reviewer survey instrument. This instrument, hosted by Survey Monkey, was used to collect and tabulate responses from external reviewers. Invitations were sent out to 44 potential external reviewers from 11 different countries, of whom 27 accepted the invitation, 5 declined the invitation, and 12 did not respond to the invitation. Of the 27 who accepted the invitation, the review was completed and responses to the anonymous external reviewer questionnaire were received by 22 reviewers.

## Responses to Required Yes/No Questions from External Reviewers:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Question | Total  responses, n (%) | Yes, n (%) | No, n (%) | No  opinion, n (%) |
| Would you make use of this guideline in your professional decision making? | 22 (100%) | 21 (95%) | 0 (0%) | 1 (5%) |
| Are these guidelines flexible enough to allow for clinical judgement? | 22 (100%) | 21 (95%) | 1 (5%) | 0 (0%) |
| Do you believe the systematic literature review was adequately comprehensive and robust? | 22 (100%) | 22 (100%) | 0 (0%) | 0 (0%) |
| **I would recommend these guidelines for use in clinical practice.** | **22 (100%)** | **22 (100%)** | **0 (0%)** | **0 (0%)** |

**Required Rating of Overall Guideline Quality:** Reviewers were asked to rate the overall quality of the guidelines on a scale of 1 to 5, in which 5 was high quality (defined as impactful, clear, clinically relevant, and rigorous methodology) and 1 was low quality (defined as low impact, unclear, lack of clinical relevance and weak methodology).

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Question** | **1 (low), n (%)** | **2, n (%)** | **3, n (%)** | **4, n (%)** | **5 (high), n (%)** | **Weighted average** | **Total**  **responses, n (%)** |
| Rate the overall quality of these  guidelines. | 0 (0%) | 0 (0%) | 0 (0%) | 7 (32%) | 15  (68%) | 4.68 | 22 (100%) |

**Dissemination and Implementation Input:** External reviewers were asked to suggest strategies for successful dissemination and implementation. 16 external reviewers answered this open-ended free text response question. A majority of responses recommended sessions/presentations of the guidelines at the annual meetings of professional societies in hematology as well as formal endorsements/affirmations of value by these societies, such as the American Society of Hematology, the American Society of Pediatric Hematology and Oncology, the European Hematologic Association, and others. A few respondents suggested

presentations at other society meetings, including societies in pediatrics, internal medicine, and oncology. Multiple respondents suggested dissemination via patient advocacy groups and social media, as well as a guidelines webpage. All of these recommendations were part of the GWG’s initial dissemination and implementation plan and are represented in the final plan.

External reviewers were additionally asked to describe the barriers they saw to successful dissemination and implementation of the clinical guideline. 11 external reviewers answered this open-ended free text response question. The most common response was an inadequate awareness of PK deficiency among healthcare professionals. An inadequate number of practicing classical hematologists was also mentioned by multiple respondents.

**Input Regarding Literature Review and Essential References:** External reviewers were additionally asked if they believed any pertinent references were not included in the manuscript or supplementary appendix, to enter the pertinent missing references along with the topic/specific evidence covered by the reference. No reviewer entered any references, and several reviewers affirmatively answered that no pertinent references were missed.

## Input Regarding Future Directions for Research or Guideline Development in PK

**Deficiency:** External reviewers were asked to suggest strategies for successful dissemination and implementation, if they had any recommendations. Reviewers suggested future recommendations regarding the use of mitapivat in pediatric patients and gene therapy once evidence has matured for these treatment options. Regarding future directions for research, reviewers suggested real-world evidence of mitapivat, evaluation of etavopivat (another PK activator not currently in development for PK deficiency), more research in hematopoietic stem cell transplant for PK deficiency, and more research in the management of PK deficiency in general.

**Input Regarding Additional Comments or Concerns Regarding the Guideline or Any of the Recommendations:** External reviewers were asked to state any additional comments or concerns regarding the guideline, if they had any. Several reviewers recommended some corrections to the phrasing of words in several of the clinical considerations sections, which were made. Multiple reviewers lauded the quality, balance, and/or methodological rigor of the guidelines. Several reviewers suggested adding tables to summarize recommendations (to respond to this request, the Table in the final primary manuscript is a full summary of the recommendations). Two reviewers contributed “minor” comments regarding the phrasing of MRI monitoring for iron quantitation recommendations but did not raise significant concerns. All other comment topics and suggestions were made by a single external reviewer, and generally highlighted clarity issues in various sections of the guidelines, which were corrected wherever possible.

# DRAFT RECOMMENDATIONS NOT ACHIEVING ≥67% AGREEMENT THRESHOLD

One draft recommendation did not achieve the prespecified agreement threshold of 67% upon the initial vote and then again following revision and second vote. It was therefore not included in the final guideline.

*Draft Recommendation A6*. *The expert panel suggests the use of PKLR gene molecular analysis results for prediction of overall disease severity.* (Certainty of evidence: moderate; strength of recommendation: conditional; agreement, 63%).

*Evidence/clinical considerations/expert panel discussion:* Clinical studies have demonstrated an association between genotype and phenotype in PK deficiency.2,3,19 Specifically, patients with homozygous or compound heterozygous non-missense mutations display typically a more severe phenotype with more pronounced anemia, higher ferritin levels, higher number of lifetime transfusions, higher rate of splenectomy, and reduced response to treatments, such as mitapivat or splenectomy. However, genotype is unable to predict other complications such as gallstones, leg ulcers, bone fractures, and endocrinopathies, as well as the in utero and perinatal course of patients. Iron overload may be present in all patients regardless of the genotype and requires monitoring. Additionally, since most patients have a unique combination of PKLR mutations, each combination likely has unique effects on catalysis, stability, and tetramer composition, making it difficult to predict clinical outcomes.68

The expert panel did not achieve the prespecified agreement threshold of 67% on either the initial vote (agreement, 63%) or second vote (agreement, 63%). There was extensive discussion regarding the draft recommendation prior to the votes. Some members of the expert panel noted that genotype does not appear to be more predictive of clinical course than hemoglobin or markers of hemolysis in a given patient and worried that the recommendation would not be practical in some clinical practice settings. Some members of the expert panel argued that at present, genotype results are not practically useful to patients outside of predicting mitapivat response. To the contrary, others felt it is helpful for planning the general management of patients with two non-missense mutations.

Ultimately, after failing to achieve the threshold for agreement following the second vote, the expert panel agreed to incorporate some of the information relevant to this failed draft recommendation to the evidence/clinical considerations sections of other recommendations where relevant.

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