Hi everyone. I'm Rachael Grace, pediatric hematologist at Boston Children's Hospital and an associate professor at Harvard Medical School in Boston, Massachusetts. And I'm excited today to provide you with highlights with new research developments in pyruvate kinase deficiency that were presented at the 2021 American Society of Hematology annual meeting.

Before I get started, here are my disclosures. Before discussing the recent data presented, I first wanted to just take a few minutes to provide an overview about pyruvate kinase deficiency. PK deficiency is a rare congenital autosomal-recessive hemolytic anemia caused by mutations in the PKLR gene, which encodes a key glycolytic enzyme, pyruvate kinase. As shown here in the figure on the right, which is a simplified version of glycolysis, pyruvate kinase is an enzyme in a key step which converts phosphoenolpyruvate to pyruvate and is an ATP-generating step.

Since red cells lack of nucleus, they lie on glycolysis as their energy source. With a deficiency in pyruvate kinase, there's also a deficiency in ATP and a backlog or an increase in earlier products of glycolysis, including 2,3-DPG. The deficiency in red cell ATP leads to changes within the red cell with lifelong hemolysis and the associated complications. PK deficiency is rare with an unknown true incidence, but it's likely on the order of three to eight per million.

How do you diagnose Pk deficiency? The diagnosis should be suspected in patients with chronic non-immune hemolysis. These are patients who typically have a low hemoglobin, an elevated reticulocyte count, an elevated indirect bilirubin level, and a negative direct antiglobulin test. The purple blood film is usually bland without specific red cell morphology in patients with PK deficiency with an intact spleen. Once a hemoglobinopathy has been excluded, for example, with a hemoglobin electrophoresis, and a membranopathy has been excluded, a red cell enzyme disorder should be considered.

In these patients there is an important role for both PK enzyme activity and genetic analysis. For patients with a low PK enzyme activity, PKLR genetic testing should be sent for confirmation. For patients with genetic testing and a newly described variant, or where no variant is found but PK deficiency is suspected, enzyme activity should be sent.

Chronic hemolysis and ineffective erythropoiesis lead to lifelong anemia and a spectrum of complications, some of which are shown here. These complications occur both in transfused and non-transfused patients and significantly impact patient quality of life. The most underrecognized complication is iron overload, which is common and should be monitored even in patients who were never transfused. Other complications that are common include gallstones and osteopenia, or low bone density.

Increased information about the management and complications of patients with PK deficiency has emerged through natural history studies. And this has helped with establishing recommended monitoring for these patients. And so I point you in the direction of review articles that have been published in the last few years for detailed guidance.

Until February of 2022, there were no disease-modifying treatment strategies for pyruvate kinase deficiency, and supportive treatment has included red cell transfusions, when indicated, and iron chelation. Splenectomy is also a supportive treatment strategy, which ameliorates the anemia in a subset of patients. On February 17th, 2022, the FDA approved Mitapivat, an oral allosteric activator pyruvate kinase as a treatment for PK deficiency in patients 18 years of age and older. Another disease-directed treatment under current clinical investigation includes lentiviral vector gene therapy.

Although PK deficiency is a rare condition, there has been recently a lot of focus and there were a lot of exciting updates at the American Society of Hematology 2021 annual meeting. This meeting was virtual due to the ongoing pandemic. And so a lot of people couldn't attend. And so I will now turn our attention to abstracts that were presented at that meeting.

The first abstract I'll focus on is work from the PK Deficiency Advocacy Advisory Council, which conducted a global survey of 200 adult patients with PK deficiency and 75 caregivers, which had a goal of exploring communication between those affected by PK deficiency and their hematologists. The survey results uncovered unmet needs in the health education, anticipatory guidance, and emotional and psychosocial support provided to patients with PK deficiency. These data showed that only about half of respondents felt that their hematologist was able to or questions about how their PK deficiency is managed and about potential health complications associated with PK deficiency. Less than half of patients felt their hematologists find solutions to optimize their management.

The survey results also uncovered unmet needs in negative communication, most often among non-transfused patients. These figures show non-transfused respondents in yellow and other transfusion groups in blue, a significantly lower proportion of the non-transfusion patients stated their hematologist understands the impact of PK deficiency on their quality of life and reported their hematologist manages their condition well. Based on this survey, there's a clear need to improve understanding of Pk deficiency by hematologists, and in particular, for hematologists to optimize care and support of non-transfused patients with further touchpoints to ensure effective communication and management. Hematologists also need to increase their approach in terms of holistic care of this patient population and consider the emotional and psychosocial health aspects of care.

We'll now turn our attention over to several abstracts with important clinical trial updates about PK activators for treatment of adults with PK deficiency. Mitapivat is an oral, small molecule allosteric activator of pyruvate kinase. A published phase two trial of Mitapivat in adults with PK deficiency has shown that it's generally well-tolerated and raises the hemoglobin in about 40% of patients. The ACTIVATE trial is a phase three double-blind placebo-controlled study in adults with PK deficiency who are not regularly transfused, and the ACTIVATE-T trial is a phase three open-label single arm study in adults with PK deficiency who are regularly transfused. Both studies have been completed and met their primary endpoint, including a rise in hemoglobin and a reduction of transfusions, and met the secondary endpoints, including improvement in markers of hemolysis, hematopoiesis, and improvement in patient-reported outcomes. In addition, the safety profile was consistent with previously reported data at ASH focused on the long-term extension study and the durability of the effects.

This figure shows the durability of the hemoglobin effect of Mitapivat for up to 19 months. The Y axis on the graph shows the median change in hemoglobin and the X axis shows the number of weeks. The yellow shows the core period of the study with placebo and gray and Mitapivat in blue. 40% of patients had a hemoglobin response in the core phase. The pink shows the placebo group now receiving Mitapivat with a similar response as those who received Mitapivat in the core phase. And then you can see the two arms coincide with a sustained hemoglobin effect.

In the ACTIVATE-T trial of regularly transfuse patients, 33% achieved a reduction in transfusions, which was maintained in the extension study. This figure shows 22% of patients who became transfusion-free with no transfusions once they started on Mitapivat. These patients have been followed with a maintained effect for up to 20 months. Additional data about the effect of Mitapivat in patients with PK deficiency from the ACTIVATE trials and the extension study demonstrate that it both improves ineffective erythropoiesis and reduces iron overload over time.

These data show that the mean erythropoietin, erythroferrone, reticulocyte count and soluble transferrin receptor decreased with Mitapivat treatment in comparison to placebo. In addition, improvements were seen in hepcidin, transferrin saturation, and liver iron concentration with treatment with Mitapivat. This is important because Mitapivat then has the potential not only to increase hemoglobin and decrease hemolytic markers, but also to alter the disease course in terms of preventing iron overload and other complications that develop from ineffective erythropoiesis.

Lastly, this abstract reported on the effect of long-term use of Mitapivat, so over greater than 12 months, on bone mineral density. This data shows that bone mineral density was maintained for up to 65 months. Given that patients with PK deficiency are at risk for declining bone density over time, this may indicate that Mitapivat is protective against worsening bone density.

We'll now turn our attention over to an important update about a lentiviral gene therapy treatment in patients with PK deficiency. An update was provided on two adults who received lentiviral mediated gene therapy. Following treatment, both patients have had normal hemoglobin levels, improved hemolytic markers, and improved report of quality of life and have had no transfusion greater than nine months of follow-up. There were no adverse events attributable to the gene therapy with a favorable safety profile with one year of follow-up. The early data for this study is very encouraging and will be interesting to continue to follow over time, both the safety and the efficacy. There will also be more data to come from this trial with continuing enrollment, including those in the pediatric age range.

The last abstracts I'll be highlighting report on updates in our understanding of the pathophysiology of PK deficiency. These data are from the Peak Registry, an international cohort of children and adults with PK deficiency. And this study describes iron overload by age group. In this cohort, iron overload affected 40% of patients regardless of their age, genotype, transfusion status, or disease severity. Based on this, the co-authors recommended an initial evaluation and regular monitoring of iron overload in all patients with pyruvate kinase deficiency starting early in life and continuing throughout adulthood.

Another study investigating the pathophysiology of iron overload in PK deficiency in comparison to other congenital hemolytic anemias, including congenital dyserythropoietic anemia type II and hereditary spherocytosis. The authors analyzed the levels of erythroferrone, erythropoietin, hepcidin, and soluble transferrin receptor. In PK deficiency, patients had a decreased hepcidin, an increased erythroferrone and erythropoietin most similar to congenital dyserythropoietic anemia, which is known to be associated with dyserythropoiesis and ineffective erythropoiesis in comparison to hereditary spherocytosis. These data provide further evidence of the dyserythropoietic features of PK deficiency.

This last study evaluated the relationship between baseline erythrocyte adhesion and thrombosis potential in 10 patients with PK deficiency in comparison to five healthy controls. The samples from the patients with PK deficiency, many of whom had had a prior splenectomy, had elevated red cell adhesive properties similar to that observed in patients with sickle cell disease. There were no significant differences in other measured parameters, which included thrombin generation, adhesivity, and microparticles. The authors of this study hypothesized that the pathogenic red cell adhesion may be a mechanism that drives hypercoagulability in patients with PK deficiency. This is important because the natural history study data shows that there is an increased risk of about 10% in patients with pyruvate kinase deficiency after splenectomy.

In summary, PK deficiency is a rare congenital hemolytic anemia associated with a wide spectrum of symptoms and complications. There were multiple important updates on the pathophysiology and treatment of PK deficiency, which were presented at the American Society of Hematology 2021 annual meeting. In terms of important clinical updates, we know that there needs to have been improved understanding and communication about the management of PK deficiency by hematologists. We need to change our approach to these patients in terms of having a more holistic approach with a particular focus on patients who are not transfused.

Patients with PK deficiency clearly need to be evaluated and monitored for iron overload at all ages, independent of transfusion status. We're now having growing evidence that non-transfused iron loading may be due to dyserythropoietic and ineffective erythropoiesis in these patients. Disease-directed treatments are currently in clinical development and very excitingly demonstrated in improvement in hemoglobin, improvement in hemolytic markers, decrease in transfusion burden, and improvement in

quality of life. Mitapivat, an oral PK activator, has also been shown to improve ineffective erythropoiesis, to reduce iron overload, and to maintain stable bone mineral density in patients with PK deficiency. I thank you very much for your attention.