Beyond adenosine triphosphate: unveiling the pleiotropic effects of pyruvate kinase activation in sickle cell anemia

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In this issue of *Haematologica*, D'Alessandro *et al.*[1] describe detailed multi-omics data on 15 individuals with sickle cell anemia (SCA) treated with mitapivat, a novel oral activator of pyruvate kinase (PK).

SCA, being the most prevalent genetic hematologic disease worldwide, inflicts a devastating toll on global health, particularly affecting childhood survival rates in low-income countries. The unmet need for improved care is evident. Despite recent advancements, current treatment remains mostly limited to infectious prophylaxis, hydroxyurea, and transfusion therapy. The main global challenge lies in ensuring early diagnosis and widespread access to these critical treatments. For the foreseeable future, access to curative treatments, such as hematopoietic stem cell transplantation and gene therapy, is confined to a small fraction of young patients in high-income countries. Meanwhile, there is a critical need to develop accessible and scalable pharmacological treatment options.

PK is the rate-limiting step of glycolysis and inhibition of PK has been extensively studied as an anti-neoplastic strategy through counteracting the Warburg effect.[2] Contrarily, mitapivat activates all isotypes of PK.[3] This is particularly relevant for mature red blood cells (RBC), which rely solely on glycolysis for ATP generation. Patients with PK deficiency lack the RBC specific isoform of PK (PKR) and are unable to produce sufficient ATP in RBC, resulting in lifelong hemolytic anemia. The RBC metabolome shows a buildup of glycolytic intermediates upstream of PK,[4] most notably increasing 2,3-diphosphoglycerate (2,3-DPG) levels, which effectively decrease hemoglobin oxygen affinity and thereby ameliorate symptoms of anemia.[5] Unsurprisingly, mitapivat can effectively treat patients with PK deficiency, although the response is heavily dependent on *PKLR* genotype and residual PK protein.[6]

Benefits of PK activation in anemias beyond PK deficiency may seem less evident. However, numerous studies have found insufficient glycolytic capacity and ATP generation in a range of hereditary anemias.[7] In SCA, activating PK exerts multifaceted effects (Figure 1). It not only enhances ATP production in RBC but also reduces 2,3-DPG levels, which in turn increases the oxygen affinity of hemoglobin. As deoxygenation is a key trigger of sickling, this could also be a contributing mechanism of the action of mitapivat. Additionally, PK activation has been suggested to improve the glutathione pool and thereby have an antioxidant effect, although until now this has only been shown in a mouse model of β-thalassemia.[7] Overall, PK activators constitute a promising class of drugs for the treatment of SCA. Currently, three PK activators - mitapivat, etovapivat, and AG-946 - are being used in clinical trials for SCA.

The study by D'Alessandro *et al.* examines mitapivat's extensive molecular effects on SCA patients over a period of up to 2 years, utilizing metabolomics, lipidomics, and proteomics within the framework of a long-term extension phase I study (NCT04610866).

Unsurprisingly, a decrease in 2,3-DPG combined with increased ATP levels were confirmed along with improved hematologic and sickling parameters. Notably, a rise in reduced glutathione and activation of Lands cycle point to an improvement in oxidative stress, which provides some evidence to a central proposed benefit of PK activators. Less intuitive is the reported decrease in mitochondrial proteins as RBC are, at least in healthy individuals, mostly known for their absence of mitochondria. However, various recent studies have highlighted the frequent occurrence and potential negative clinical impact of mitochondrial retention in SCA.[8] In PK deficiency, patients lack the PKR isoform, but this is during early-stage erythropoiesis and is likely compensated by expression of the PKM2 isoform. Nonetheless, shortages in pyruvate or ATP in late-stage erythropoiesis might impair reticulocyte maturation and mitophagy[9] and could underlie the extreme reticulo-

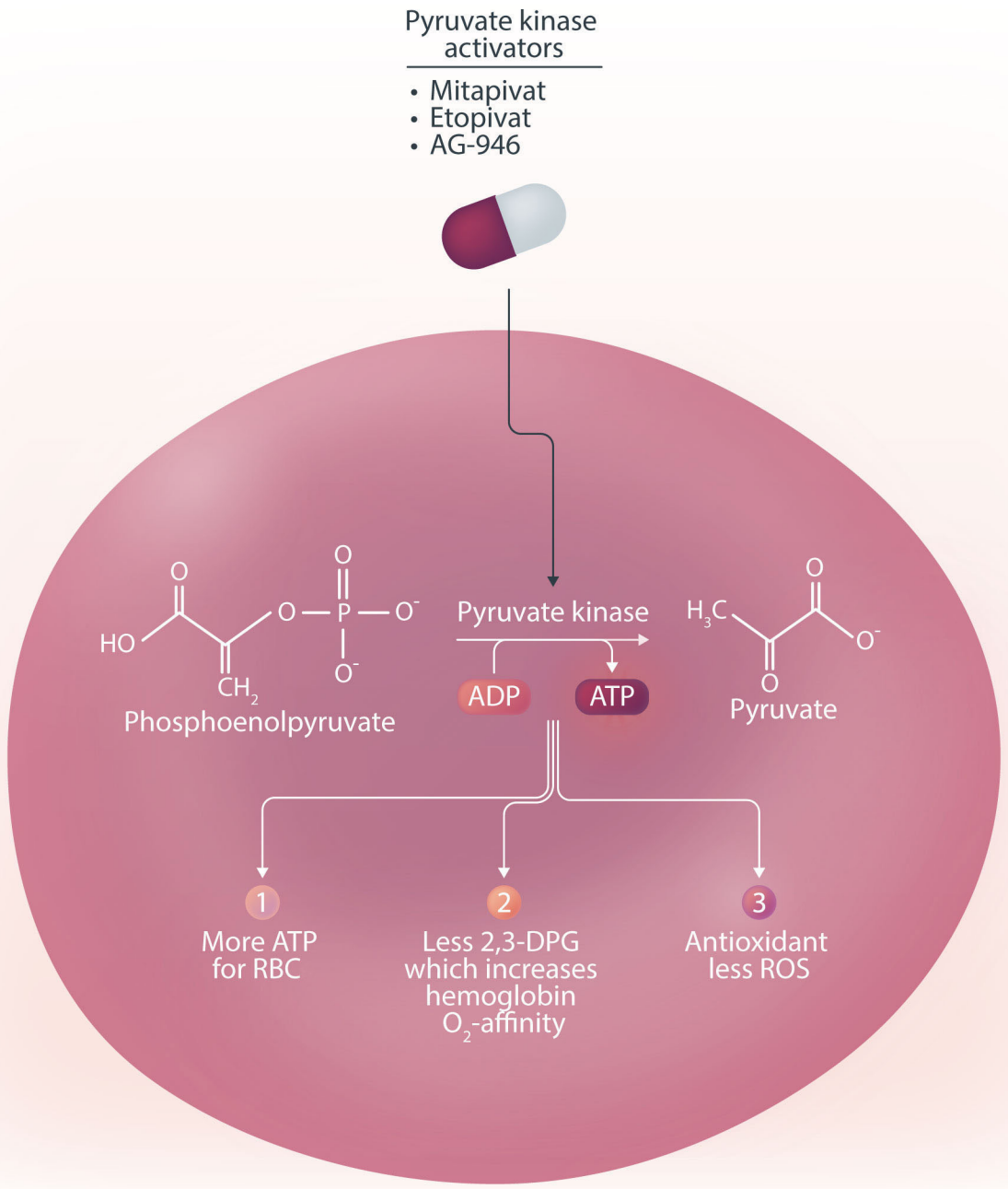

Figure 1. Pyruvate kinase activation in sickle cell anemia. Pyruvate kinase catalyzes the glycolytic pathway conversion of phosphoenolpyruvate to pyruvate. This facilitates three key outcomes: (i) increased adenosine triphosphate availability for red blood cells, improving energy supply and cellular functions; (ii) decreased levels of 2,3-diphosphoglycerate, leading to increased oxygen affinity of hemoglobin, a well-known anti-sickling mechanism; and (iii) augmented antioxidant capacity and reduced reactive oxygen species, contributing to a decrease in oxidative stress within the red blood cells. 2,3-DPG: 2,3-diphosphoglycerate; ADP: adenosine diphosphate; ATP: adenosine triphosphate; RBC: red blood cells; ROS: reactive oxygen species.

cytosis measured in patients with PK deficiency after splenectomy.[7] If so, replenishing pyruvate and ATP with PK activators should promote mitophagy. This seemed to be the case in a preclinical study of Townes mice – a well-known model of SCA – in which mitapivat ameliorated both mitochondrial retention and oxidative stress.[10] As the authors speculate, mitochondrial proteins in RBC might play a role in SCA by promoting inflammation, adding another potential benefit of PK activation in SCA to be studied further.

One might argue that the extended lifespan of RBC in this study might lead to a decrease in mitochondrial proteins merely by reducing the fraction of reticulocytes. The authors claim that the leukocyte depletion used minimized this problem by removing most reticulocytes. This was, however, not formally demonstrated in the study.

Interestingly, mivapivat levels measured in RBC correlated with a range of measures of positive outcomes such as glycolytic activation (including higher ATP and lower 2,3-DPG) and acyl-carnitines. The depleted carnitine pools found could be interpreted as a rationale for testing supplementation on top of mitapivat, but this remains speculative.

A significant and noted limitation of the study was lack of control for RBC age, which complicates the precise evaluation of mitapivat's effects, one example being the effect on the PK enzyme itself, which decreases during the RBC lifespan. Ongoing and future studies will explore the potential of PK activation across various hemolytic anemias, hopefully adding more details to the multi-omics

effects associated with PK activation while adjusting for RBC lifespan variations during treatment.
Collectively, the insights provided by D'Alessandro *et al.* are a significant step forward, enhancing our understanding of the complex remodeling provided by *in vivo* PK activation.

Disclosures
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