The Pyruvate Kinase Activator Mitapivat Ameliorates Anemia and Prevents Iron Overload in a Mouse Model of Hereditary Spherocytosis

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Hereditary Spherocytosis (HS)

- **Hereditary Spherocytosis (HS)** is the most common hemolytic anemia due to inherited RBC membrane defects with a prevalence estimated from 1: 2,000 to 1: 5,000 births.

- HS is due to mutation on genes encoding for red cell membrane or cytoskeleton proteins such as ankyrin, band 3, band 4.2 or α-, β-spectrin.

- HS clinical presentation is characterized by hemolytic anemia, reticulocytosis, jaundice, cholelithiasis and splenomegaly.

HS red cells are characterized by membrane mechanical and metabolic instability

• In HS, the absence/reduction in one of the membrane/cytoskeleton key proteins promotes membrane mechanical instability, resulting in:
  – red cell membrane exposure of phosphatidylserine (PS),
  – release of erythroid microvesicles,
  – generation of spherocytes.

• HS red cells display decrease ATP content when exposed to oxidation or after 24hr incubation.

The oral PK activator Mitapivat improves anemia in PK deficiency and thalassemia

• Mitapivat (AG-348) is an oral small-molecule activator of pyruvate kinase.

• Two phase 2 clinical trials of mitapivat, one in adult patients with pyruvate kinase deficiency and the other in non transfusion-dependent thalassemia, demonstrated rapid and sustained increase in Hb levels

• Mitapivat ameliorates murine β-thalassemic anemia with a beneficial effect on iron homeostasis.

Aim of the study

To Investigate the effects of the PK activator, Mitapivat, on red cell metabolism and hematologic phenotype of band 4.2-/- mice, a model of HS.
Study design

- Two to eight months-old female Band 4.2−/− and C57BL6/J mice were used (n=6-11 animals in each group);

- Mitapivat (100 mg/Kg/d) was administrated for 6 months;

- CBC and reticulocytes were determined with a Sysmex Hematology Analyzer;

- Erythroid Annexin-V positivity was evaluated by FACS;

- Perls’ staining was carried out on fixed spleen and liver;

- Spleen and liver iron concentration were measured;

- Immunoblot analysis was carried out on mouse red cells.
Mitapivat ameliorates the anemia of band 4.2−/− mice

*p<0.05 compared to WT mice

°p<0.05 compared to vehicle treated mice
Mitapivat reduces hemolysis in band 4.2−/− mice

*°p<0.05 compared to WT mice
°p<0.05 compared to vehicle treated mice
Mitapivat improves 4.2⁻/⁻ mouse red cell membrane mechanical stability

In 4.2\(^{-/-}\) mice, Mitapivat reduces the release of erythroid microvesicles.
In band 4.2⁻/⁻ mice, Mitapivat reduces splenomegaly and spleen iron-overload.

*SIC

*p<0.05 compared to WT mice

°p<0.05 compared to vehicle treated mice
In band 4.2\(^{-/-}\) mice, Mitapivat decreases liver iron-overload

*\(p<0.05\) compared to WT mice
°\(p<0.05\) compared to vehicle treated mice
Conclusions

• Band 4.2\(^{-/-}\) mice treated with Mitapivat show:
  – Reduced hemolysis and amelioration of anemia
  – Improved red cell membrane mechanical stability
  – Reduction in spleen and liver iron overload

• Mitapivat might represent an interesting and novel therapeutic option for HS patients