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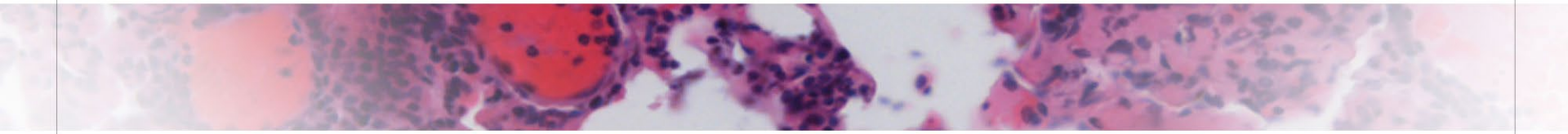

The Pyruvate Kinase Activator Mitapivat Ameliorates Anemia and Prevents Iron Overload in a Mouse Model of Hereditary Spherocytosis

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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.

Iolascon A et al. BJH, 2019; Mohandas N et al. Blood, 2008

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.

Iolascon A et al. BJH, 2019; Bozzi A et al Biochem Mol Biol Int 32: 95-103, 1994; De Jong K et al Blood 94: 319, 1999; Mohandas N et al. Blood 112: 3939, 2008

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.

Grace RF et al. NEJM 381: 933, 2019; Yang H et al. Clin Pharmacol Dev 8: 246, 2019; Kung C et al Blood 130: 1347-1356, 2017; Matte A et al. Haematologica 101:18, 2016; Kuo KHM, et al. HemaSphere. 2020;4(S1):Abstract S297.

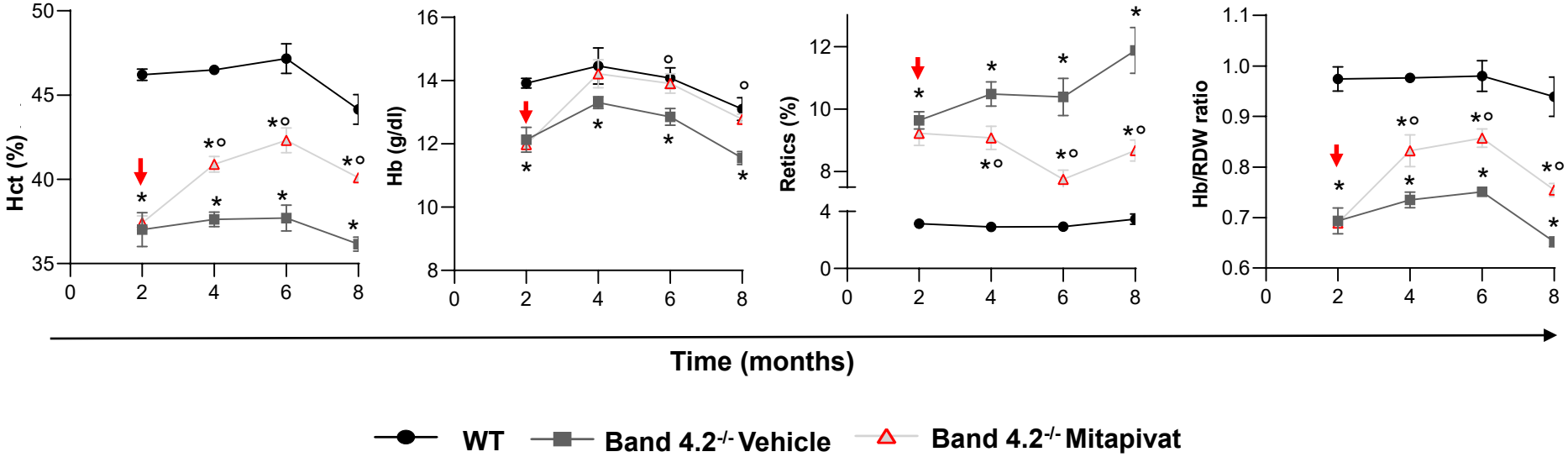
Aim of the study

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

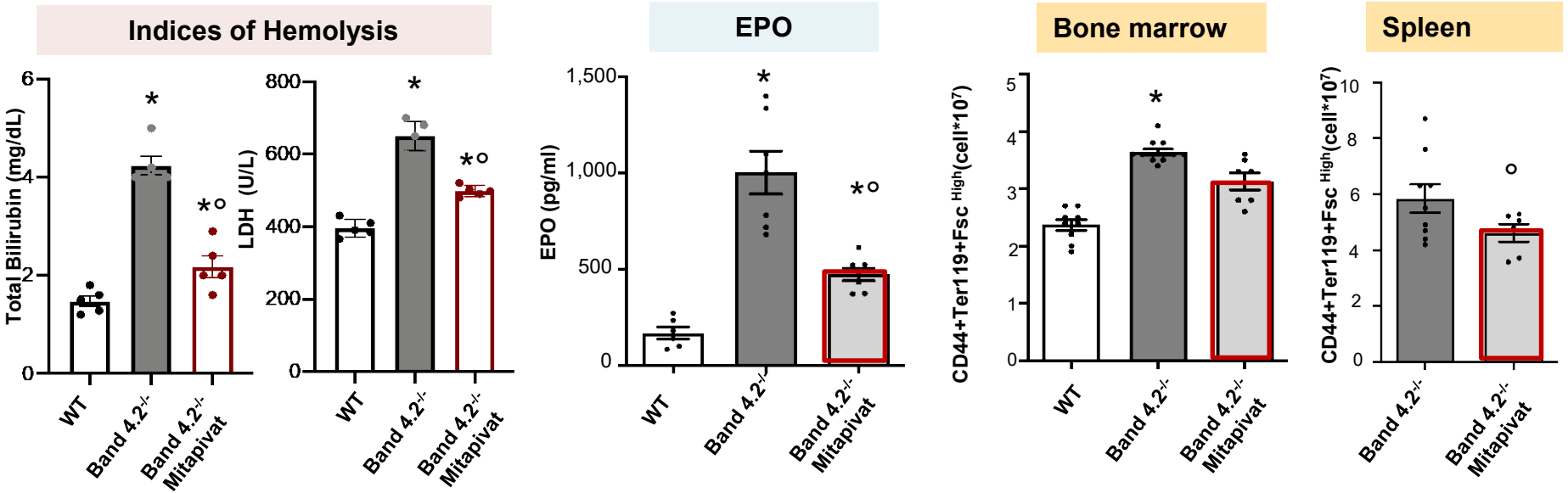
- Two to eight months-old female Band $4.2^{-/-}$ and C57BL6/J mice were used (*n*=6-11 animals in each group);
- Mitapivat (100 mg/Kg/d) was administrated for 6 months;
- CBC and reticulocytes were determined with a Sysmex Hematology Analyzer;
- Erythroid Annexin-V positivity was evaluated by FACS;
- 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



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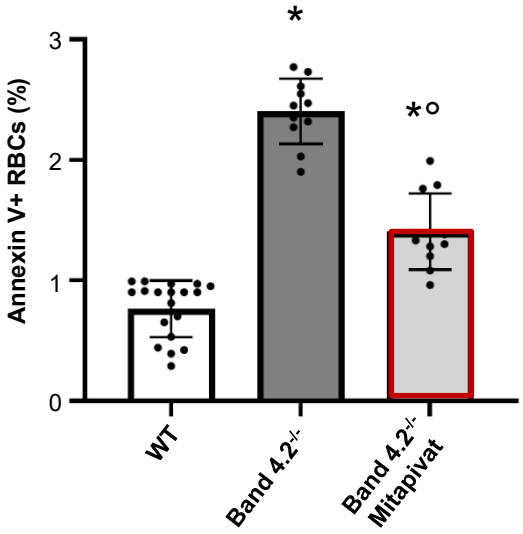
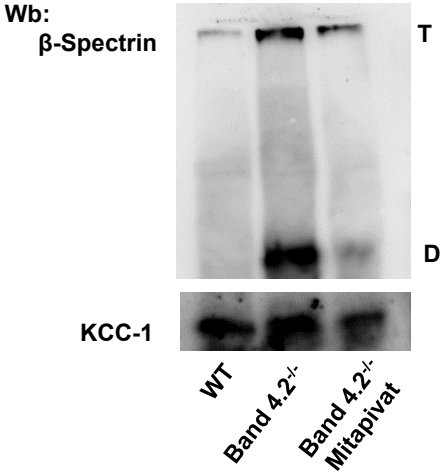
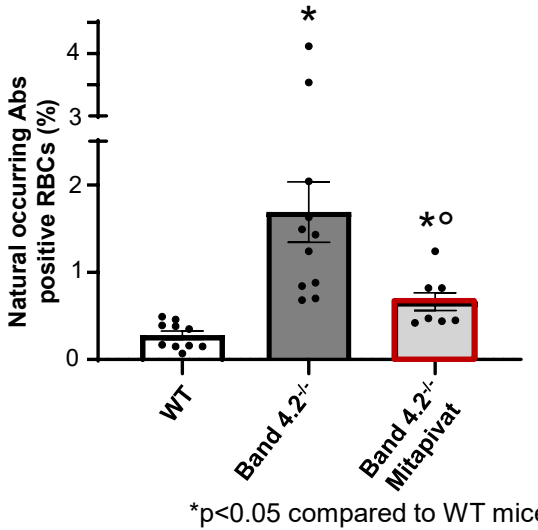
Mitapivat improves 4.2$^{-/-}$ mouse red cell membrane mechanical stability







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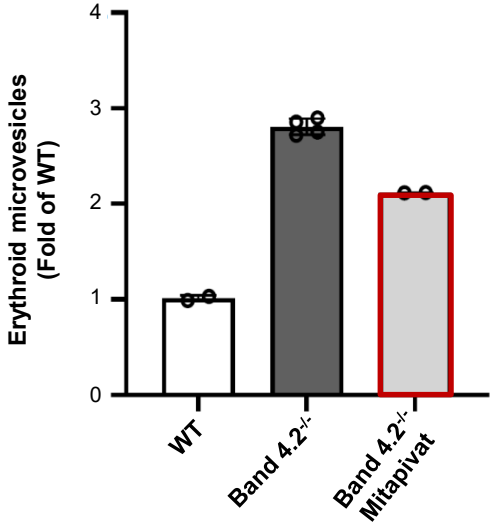
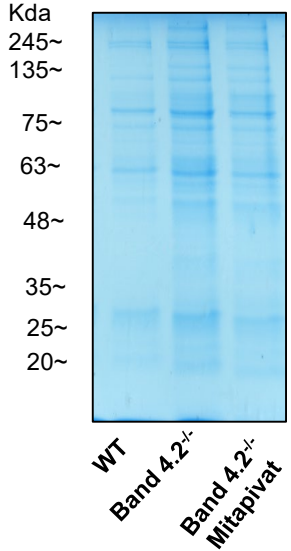
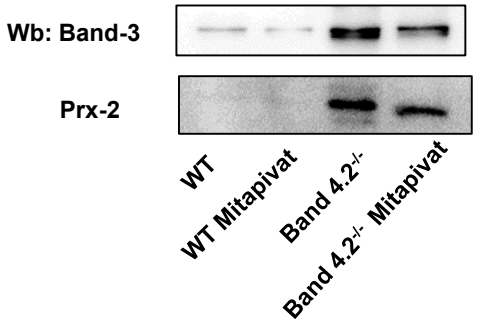
Blanc L et al Biochemistry 49: 4516, 2010; An X et al. JBC 277: 31796, 2002; Lutz HU et al Frontiers Physiol doi:org/10.3389/fphs.2013.00387, 2013; Zaninoni A et al. Frontiers Immunol doi org 10.3389/fimmu.2020.01389, 2020; Reliene R et al Blood 100: 2208, 2002

In 4.2$^{-/-}$ mice, Mitapivat reduces the release of erythroid microvesicles

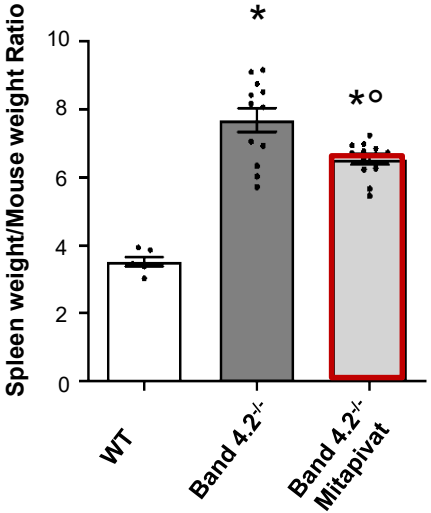


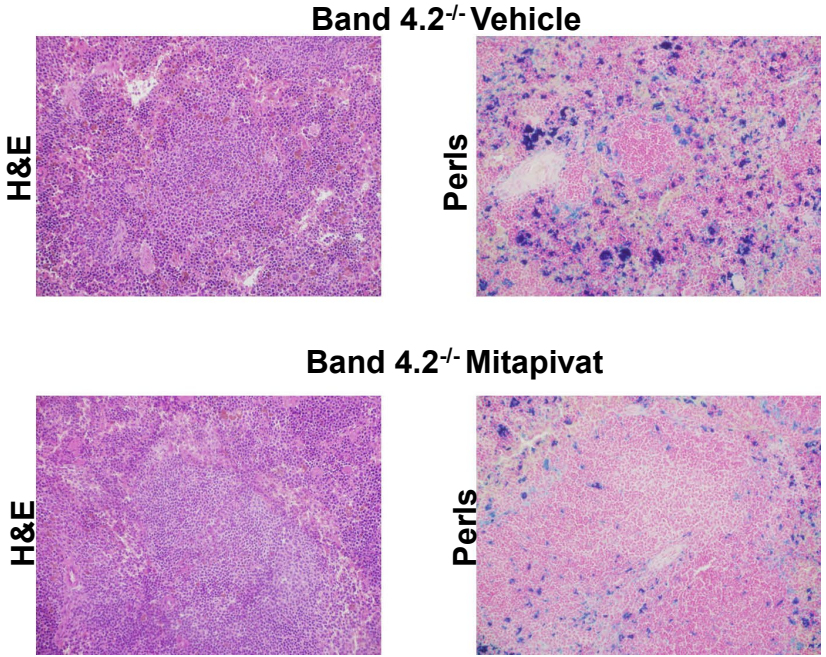
Erythroid microvesicles and protein composition

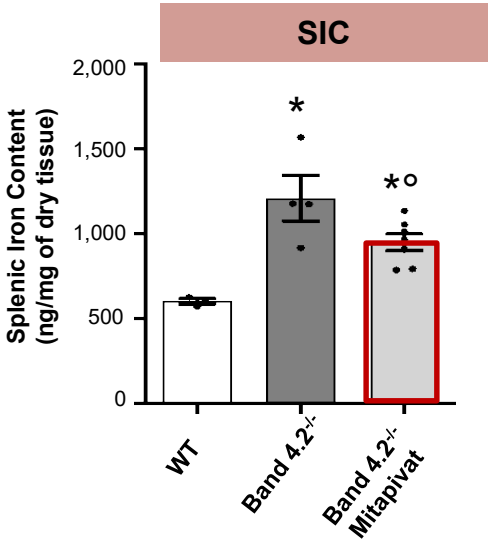




In band 4.2$^{-/-}$ mice, Mitapivat reduces splenomegaly and spleen iron-overload







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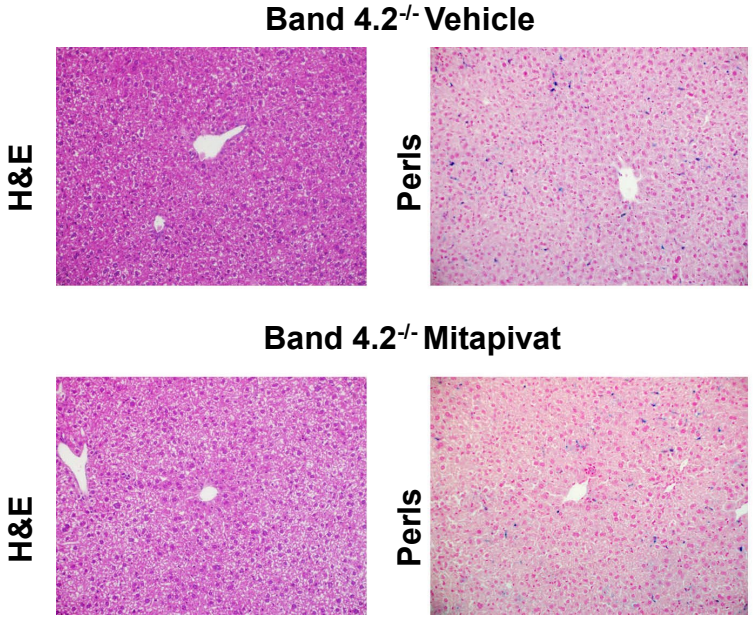
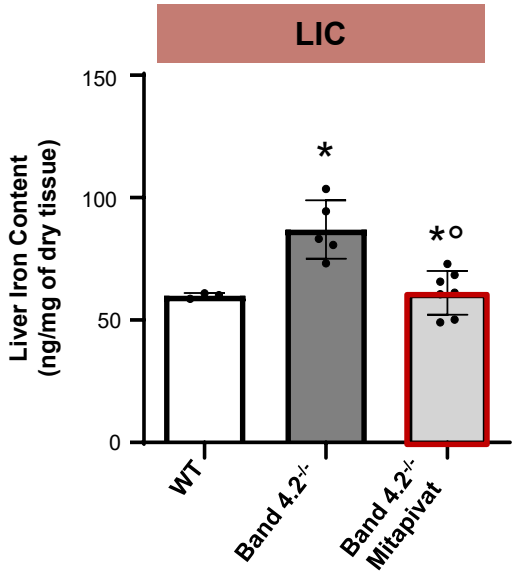
In band 4.2$^{-/-}$ mice, Mitapivat decreases liver iron-overload





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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