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POSTER ABSTRACTS

112.THALASSEMIA AND GLOBIN GENE REGULATION

Mitapivat Treatment Increases β-Thalassemic Erythroblasts Energy Production and Responsiveness to Oxidative Stress

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β thalassemia (β thal) is a worldwide red blood cell (RBC) genetic disorder with limited therapeutic tools. Standard of care includes chronic RBC transfusions and iron chelation. In β thal, pathophysiologic studies have shown that oxidation plays a key role in both ineffective erythropoiesis and reduced survival of mature red cells in the peripheral circulation (Matte et al ARS 28: 1-14, 2018; Matte A et al. ARS 23: 1284, 2015). Recently, in a mouse model of β thal, we showed that the investigational pyruvate kinase (PK) activator mitapivat significantly improved anemia by targeting both ineffective erythropoiesis and hemolysis (Matte et al. JCI 131: e144206, 2021). We also found that mitapivat ameliorated the erythroid cell maturation index for *in vitro* CD34+ derived erythroblasts from β thal (cod ^{b039}) patients (Matte et al. JCI 131: e144206, 2021). The results of phase 2 proof-of-concept study (NCT03692052) in non-transfusion-dependent thalassemic patients support the beneficial effects of mitapivat on β thal (Kuo KHM et al Lancet 400: 493-501, 2022).

Here, we study CD34+ derived erythroblasts from either healthy controls or β thal (cod ^{b039}) patients (n=10). Mitapivat (2uM) was added to the cell cultures as previously described (Matte et al. JCI 131: e144206, 2021). Compared to healthy controls, we found up-regulation of *PKM* gene expression in the late phase of β thal erythropoiesis (14 days of culture). Whereas the expression of *PKLR* gene declined during erythroid maturation in both healthy and β-thal erythroblasts. We found higher total PK activity in β thal erythroblasts than in healthy cells. Using recombinant PKR and PKM2 isoforms (R&D systems), we found that mitapivat activates both PKR and PKM2 (EC50 13.6±3.3 and 30.1±1.5, respectively) (Kung C et al Blood 2017).

Studying erythropoiesis *in vitro* we confirmed that mitapivat significantly increased β thal erythroid maturation as supported by increased late-erythroblasts at 11 days of cell culture (Matte et al. JCI 131: e144206, 2021). The metabolomic analysis of β-thal erythroblasts at 14 days of culture confirmed that mitapivat activated PK, as gleaned by PGLY/DPG levels and increased fluxes through glycolysis, as inferred from steady-state measurements of glycolytic metabolites, DPG, ATP, and GTP. In addition, we observed higher levels of methionine oxidation in both mitapivat-treated healthy and β-thal erythroblasts, suggestive of a regulatory PTM effect secondary to higher ATP availability and, possible increased longevity of the cells.

The improvement in the intracellular milieu by mitapivat is also supported by the downregulation of peroxiredoxin-2 (Prx2) and heme-oxygenase-1 (Hmox1) gene expression, which are up-regulated in response to oxidative stress and free heme accumulation in vehicle treated β-thal erythroblasts (Matte et al. ARS 23: 1284, 2015). Noteworthy, we also observed a reduction in heat shock protein-70 (Arlet JB et al Nature 514: 242, 2014) in mitapivat treated β-thal erythroblasts, further supporting the protective effect of mitapivat during β-thal erythropoiesis.

In conclusion, our data show that both *pklr* and *pkm* isoforms are expressed in the late phase of β-thal erythropoiesis. The ability of mitapivat to activate both PKLR and PKM2 might represent an added value to limit oxidation during b-thal erythropoiesis, ensuring an improved β-thal erythroblasts maturation and survival.

Disclosures D'Alessandro: *Macopharma:* Consultancy; *Hemanext Inc:* Consultancy; *Omix Technologies Inc:* Current equity holder in private company. **Brugnara:** *Garuda Therapeutics:* Current equity holder in private company, Current holder of *stock options* in a privately-held company, Honoraria; *Pfizer:* Honoraria; *Sysmex:* Honoraria. **De Franceschi:** *Bristol Myers Squibb:* Research Funding; *Agios:* Research Funding; *F. Hoffmann-La Roche Ltd, Basel:* Membership on an entity's Board of Directors or advisory committees.

OffLabel Disclosure: Mitapivat is an oral activator of Pyruvate kinase, approved for the treatment of hemolytic anemia in adults with pyruvate kinase deficiency.

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