FUNCTIONAL AND MULTI-OMICS SIGNATURES OF MITAPIVAT EFFICACY UPON ACTIVATION OF PYRUVATE KINASE IN RED BLOOD CELLS FROM PATIENTS WITH SICKLE CELL DISEASE

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INTRODUCTION

2,3-diphosphoglycerate (DPG) in red blood cells (RBCs) stabilizes the deoxy conformation of hemoglobin (Hb) to promote oxygen off-loading and counteract hypoxia. In sickle cell disease (SCD), mutation of glutamate 6 to valine in the beta chain of Hb results in HbS that polymerizes, causing RBCs to sickle upon deoxygenation. Elevated DPG promotes sickling by stabilizing HbS fibers. DPG is an intermediate substrate in the glycolytic pathway in which pyruvate kinase (PK) is a rate-limiting enzyme.

In a previous Phase 1 study (NCT04000165, Xu et al, 2022), we established proof-of-concept for activating PK as a viable anti-sickling therapeutic approach. Mitapivat, an investigational oral allosteric activator of PK, decreased DPG, increased adenosine triphosphate (ATP) levels, and improved hematologic and sickling parameters in patients (pts) with SCD (HbSS).

AIM

To investigate the impact of activating PK on RBCs from SCD patients on long term mitapivat in extended study (NCT04610866) using multi-omics.

METHOD

Fifteen pts with HbSS (aged 25 – 57 years, 10 males) were evaluated. All pts started mitapivat at 50 mg twice daily (BID), escalating after 4 wks to 100 mg BID; dose adjustments were performed for safety and tolerability, per PI discretion. At the time of data cutoff (March 23, 2023), all 15 pts completed the core period of 24 wks, 14 pts completed 48 wks ,10 pts completed 72 wks and 6 pts completed 92 wks. Leuko-depleted RBCs (LDRBCs) obtained from fresh whole blood in EDTA at baseline (V1, prior to drug initiation) and various longitudinal time points over the course of the study were flash-frozen in a mixture of ethanol and dry ice, and kept frozen at -80°C until analysis. Clinical measurements of DPG, ATP, red cell PK (PKR) protein and activity levels, oxygen affinity (p50) and sickling kinetics (t50) were accompanied by multi-omics (metabolomics, lipidomics, proteomics) characterization of the LDRBCs. In total, 150 (6x12, 4x10, 4x8 and 1x6) timepoint samples were analyzed (Fig 1A).

Multi-omics analyses were performed through a stepwise extraction of metabolites, lipids and proteins on 50 µl of packed LDRBC pellets. Extracts were separated via ultra-high-pressure liquid chromatography coupled to mass spectrometry, prior to peak picking and identification of ddMS2 data as previously described (Thomas et al 2020). Statistical analyses were conducted using MetaboAnalyst v 5.0 and Rstudio upon normalization of relative quantitative data to visit 0 levels for each variable. Network analysis and pathway analysis were performed in OmicsNet v 2.0.

RESULTS

Mitapivat therapy had significant effects on the metabolome, lipidome and proteome of SCD RBCs. Overall, the temporal trends of omics responses to mitapivat treatment across visits was evident, especially when focusing on the top 50 metabolites/lipids or proteins by time series ANOVA. Specifically, results suggested a positive correlation between mass spectrometry-based quantitation of RBC mitapivat levels and DPG consumption, ATP accumulation, PKR activity (measured via enzymatic assays), and PKR protein levels, with internal consistency between omics data and antibody-based or enzymatic assays. The treatment also promoted activation of the Lands cycle, transient elevation of lysophosphatidylcholines and oxylipins, and depletion in free fatty acids within the first 6 months, suggestive of an effect on membrane lipid remodeling. Mass spectrometry detected mitapivat levels in the erythrocytes, suggesting successful drug delivery. ATP and carnitine levels were identified as the top two metabolites with the strongest positive and negative responses to the treatment across all patients through the whole duration of the study (Fig 1B). In keeping with the activation of PKR and elevation in glycolytic fluxes, we observed decreases in DPG and increases in ATP and lactate (Fig1C). SCD RBCs abnormally retain mitochondria; here, we showed a consistent decrease in the levels of residual mitochondrial proteins in RBCs within 4 wks of mitapivat treatment (Fig. 1D). Spearman correlation analyses of omics data confirmed the specificity of mitapivat on targeting late glycolysis (Fig 2 A,B), with glycolytic metabolites ranking as the top correlates to parameters of oxygen affinity (p50) and sickling kinetics (t50) during mitapivat treatment (Fig. 2C, D).

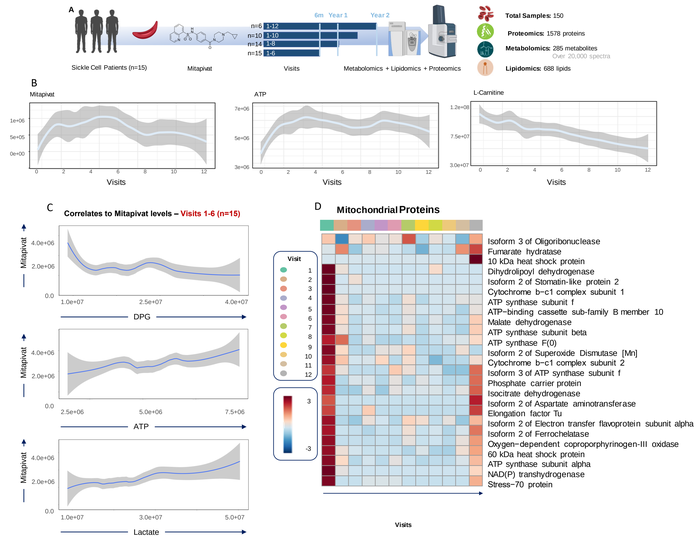

Figure 1. Impact of mitapivat on the metabolome and proteome of SCD RBCs. ***(A):*** *An overview of the clinical study. Fifteen sickle cell patients (SS genotype) were enrolled in this clinical trial, with all 15 patients being treated for up to 6 months, 14 for a whole year and 6 up to 2 years (visit 12). RBC samples underwent multi-omics and functional characterization for relevant parameters including DPG, ATP, PKR levels and activity, p50, T50.* ***(B):*** *Mass spectrometry analysis of RBCs showed successful drug delivery; ATP and carnitine were identified as the top two metabolites with the strongest positive and negative responses to the treatment throughout the whole duration of the study.* ***(C):*** *Metabolite correlates to mass spec-based measurements of mitapivat levels in RBCs during the first 6 months (visits 1-6, n =15) confirmed a strong positive association between mitapivat levels and glycolysis, with lactate and ATP ranking amongst the most significant positive correlates, and DPG as one of the top negative correlates.* ***(D):*** *Impact of mitapivat therapy on residual mitochondrial proteins in RBCs. Heat map of median values of peak areas for mitochondrial proteins (rows) during the course of the study consisting of up to 12 visits (columns). Several components of mitochondrial electron transport chain (e.g., ATP synthase subunit beta, ATPB) or other key cytosolic enzymes (e.g., maleate dehydrogenase – MDHM) with roles in apoptosis (e.g., cytochrome C – CYTC) were rapidly depleted upon the first treatment with mitapivat and remained low in most patients for the whole duration of the study.*

CONCLUSIONS

Multi-omics analysis of RBCs from HbSS patients treated with mitapivat identified benefits for glycolysis, DPG consumption and ATP generation, as well as activation of the Lands cycle.

CONFLICT OF INTEREST

AJW: Agios – employment and shareholder; SP: Agios – employment and shareholder; CH: Agios – employment and shareholder; AD is a founder of Omix Technologies Inc and Altis Biosciences, and an advisory board member for Hemanext Inc and Macopharma.

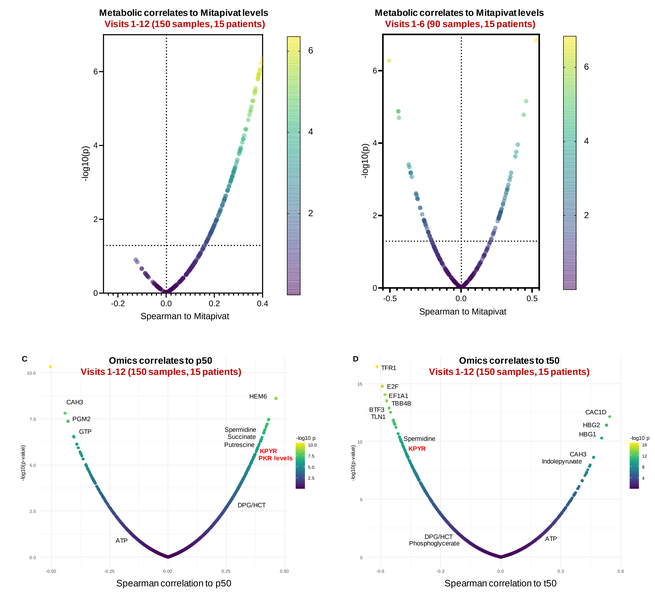

Figure 2. Spearman correlation analysis. (A and B): *Mass spec-detected mitapivat with metabolites during a 2-year period treatment (visits 1-12; n=6) or within the first 6 months for all subjects (n=15). Volcano plots indicate Spearman correlations (x axis) and -log10 of related p-values.* ***(C and D):*** *All omics with oxygen dissociation (p50) and sickling kinetics (t50) for all visits and all subjects (150 samples).*

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