MITAPIVAT-INDUCED IMPROVEMENTS IN RBC DEFORMABILITY AND MEMBRANE INTEGRITY IN PATIENTS WITH SICKLE CELL DISEASE ARE SUSTAINED DURING EXTENDED THERAPY

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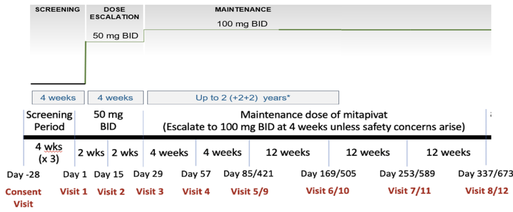
INTRODUCTION

- The ability of red blood cells (RBCs) to deform is a key factor in the pathophysiology of sickle cell disease (SCD) and is increasingly used as biomarkers of therapeutic efficacy in anti-sickling therapies.
- Mitapivat therapy in SCD has been shown to improve RBC deformability in response to shear stress and osmotic pressure, and decreasing oxygen tension, changes most likely related to improved membrane integrity.
- RBC tyrosine phosphorylation of band 3 (Tyr-p-bd3) impairs interaction with ankyrin-spectrin cytoskeleton
- In a Phase 1 study, we showed that mitapivat significantly reduced Tyr-p-bd3 in a dose-dependent manner in parallel with increased membrane-associated ankyrin-1 and intact (active) protein tyrosine phosphatase 1B (PTP1B).

AIM

- To evaluate the long-term effect of mitapivat on red cell flexibility by ektacytometry, and on RBC Tyr-p-bd3, PTP1B, and Ankyrin-1 levels using whole blood samples from patients enrolled in the extended, fixed-dose mitapivat study (Protocol 000049 / NCT04610866).
- To assess the sustainability of these mitapivat-induced changes, investigate their correlation and how they impact the hematologic parameters.



METHOD

- We studied 15 HbSS patients (aged 25–57 years; 10 males) currently enrolled under protocol NCT04610866 that evaluates long-term safety and tolerability of mitapivat in SCD patients.
- Laser-Optical Rotational Red Cell Analyzer (LORRCA, RR Mechatronics, Netherlands) assays were performed on fresh blood following standard procedures. The Elongation Index (EI) measures the cells' ability to deform under different shear stress (0.3 to 30 Pa), continuous osmolality 50 to 600 mOsm/kg) and oxygen pressures (pO_2, mmHg).
- Red cell membranes were isolated from stored frozen whole blood samples and subjected to Western blot for Tyr-p-bd3, ankyrin-1 and intact (active) PTP1B, quantified through densitometry.
- We analyzed the % change at each timepoint from baseline (V1) for each subject, and then derived the mean % change for each timepoint for all subjects.
- Significance testing was derived by Wilcox signed rank test. Correlations assessments were based on nonparametric Spearman correlation, with all analyses conducted using R (v4.2.3).

RESULTS

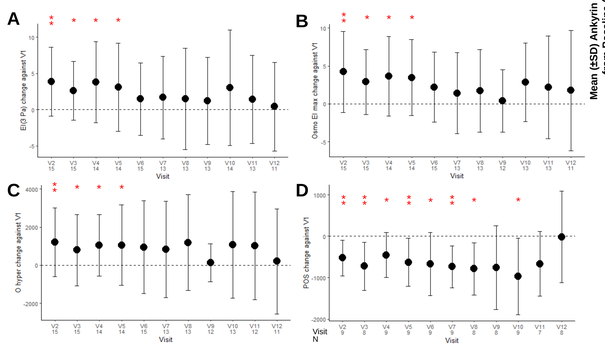


Figure 1. Mitapivat significantly improved red cel flexibility. The assays for deformability, OsmoScan, and OxygenScan showed the percentage changes compared to baseline before the treatment. (A) EI changes at shear stress at 3 Pa. (B) Osmo EImax changes. (C) O hyper changes of OsmoScan. (D) POS changes of OxygenScan. *: p<0.05; **: p<0.01.

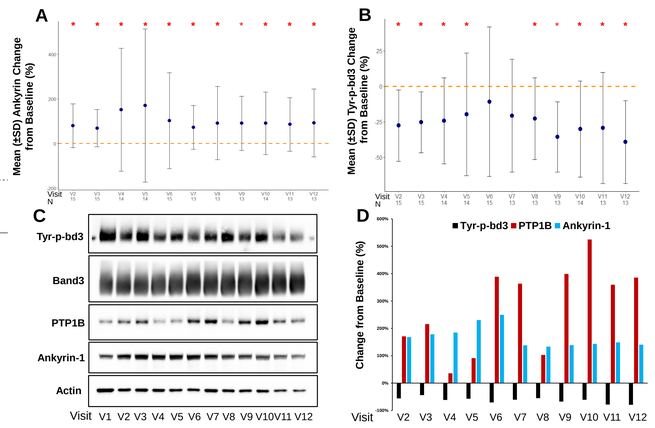


Figure 3. The membrane-bound ankyrin from sickle red cells increased and Tyr-p-band 3 decreased after treatment with mitapivat (A, B).
(C, D) a representative immunoblot profile of an individual treated with mitapivat. *: p< or = 0.05. Tyr-bd3: phosphorylated band 3.

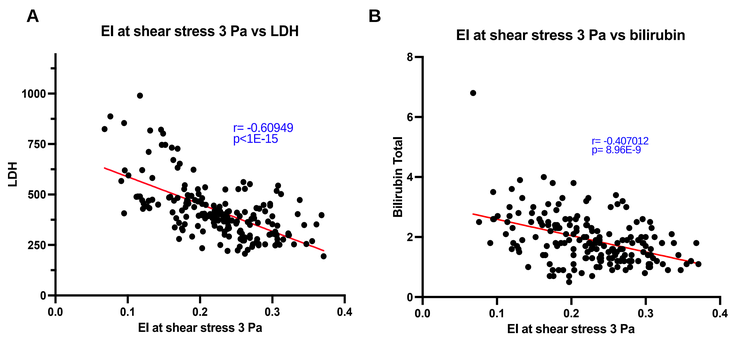


Figure 2. Mitapivat-induced RBC deformability are significantly associated with hemolysis markers, total bilirubin and LDH (A, B).
(C) Association between EI at 3 Pa of deformability assay and other hemological parameters.

C

Deformability, 3 Pa verse	Spearman r	P (two-tailed)	P value summary	P</= 0.05	Number of XY Pairs
Mean PKR Activity, nmole/sec/gram protein	-0.1822	0.02565	*	Yes	150
Mean PKR protein level/Hb (g/dL)	-0.2667	0.00033	***	Yes	177
2,3-DPG/HCT	-0.1883	0.01260	*	Yes	175
ATP/HCT	-0.0369	0.62753	ns	No	175
ATP/2,3-DPG RATIO	0.0971	0.20128	ns	No	175
P50 (torr)	-0.4221	5.37E-09	****	Yes	176
t50 (ML)	0.6346	<1E-15	****	Yes	178
Bilirubin Total	-0.4070	8.96E-09	****	Yes	185
Lactate Dehydrogenase (LDH)	-0.6095	<1E-15	****	Yes	182
Hemoglobin	0.3394	2.28E-06	****	Yes	185
Hematocrit	0.3404	2.13E-06	****	Yes	185
MCV	0.4472	1.75E-10	****	Yes	185
MCH	0.4185	3.06E-09	****	Yes	185
MCHC	-0.0755	0.30707	ns	No	185
RDW	-0.6852	<1E-15	****	Yes	185
Reticulocyte %	-0.3035	2.67E-05	****	Yes	185
Reticulocyte Abs Count	-0.2940	4.87E-05	****	Yes	185
Hemoglobin Fetal	0.5394	2.00E-15	****	Yes	185

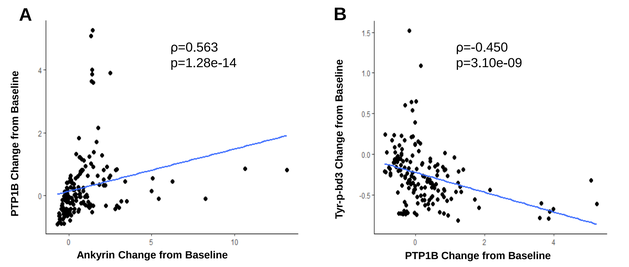


Figure 4. The membrane-bound ankyrin from sickle red cells is correlated with PTP1B and Tyr-p-band 3 and Tyr-p-band 3 in an extended treatment with mitapivat (A, B) .
Spearman rho and p values as shown.

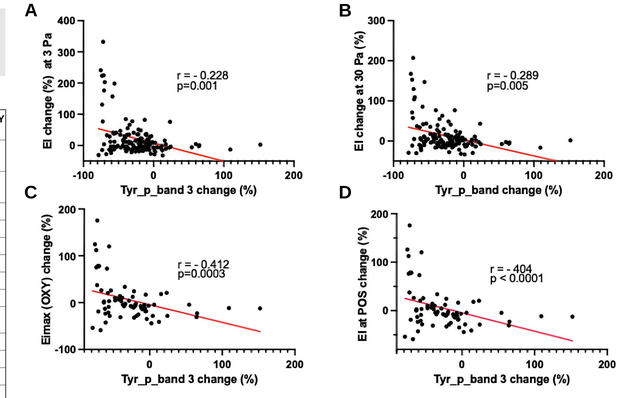


Figure 5. Red cell deformability is negatively correlated to band 3 tyrosine phosphorylation levels.
(A, B) EI changes at shear stress of 3 and 30 pascals are reversely correlated to changes of band 3 phosphorylation levels.
(C, D) Changes of Elmax of OxygenScan assay and POS are reversely associated with phosphorylation level changes. POS: point of sickling.

CONCLUSIONS

- Mitapivat-induced improvements in RBC deformability in SCD patients are both immediate and sustained throughout extended therapy, accompanied by a reduction in hemolysis.
- Mitapivat treatment decreased Tyr-p-bd3 levels in parallel with increased membrane-associated ankyrin-1 and intact PTP1B.
- Reduced Tyr-p-bd3, increased membrane-associated ankyrin-1 and intact PTP1B are observed within 2 weeks of initiating mitapivat and maintained throughout the course of treatment.
- Membrane associated ankyrin-1 is significantly positively correlated with intact PTP1B, and both are significantly negatively correlated with Tyr-p-bd3.
- Improvements in deformability and POS are associated with a notable reduction in Tyr-p-bd3, a critical factor in RBC integrity.
- Our findings confirm that activating PK in SCD improves RBC deformability, with a key mechanism being the reduction in band 3 phosphorylation, leading to an increased interaction with the anchoring protein, ankyrin.

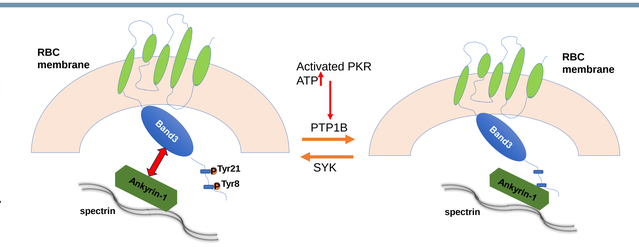


Figure 6. Proposed model for mitapivat-induced RBC changes in sickle cell disease.

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