RoxyScan Assesses Pyruvate Kinase Activator's Effect on Oxidative Stress Sensitivity in β-Thalassemia Patients: An Ancillary Study of the ENERGIZE/ ENERGIZE-T clinical trials.

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Background

Thalassemia is a hereditary anemia characterized by ineffective erythropoiesis, chronic hemolysis, and oxidative stress. Current treatments primarily consist of blood transfusions and iron chelation.

Mitapivat, an oral pyruvate kinase (PK) activator, enhances glycolysis and ATP production, and has been shown to improve red blood cell (RBC) function in several RBC disorders, such as sickle cell disease. Whether it also improves the RBC's capability to handle oxidative stress is unknown. The ENERGIZE (NCT04770753) and ENERGIZE-T (NCT04770779) trials evaluate mitapivat in non-transfusion-dependent (NTDT) and transfusion-dependent (TDT) thalassemia patients, respectively. Here we present results of an ancillary study with the Roxyscan in patients participating in these trials enrolled in the UMC Utrecht, the Netherlands.

Methods

The RoxyScan is a novel application of the Lorrca MaxSis (RR Mechatronics, Zwaag). It measures changes in membrane deformability and rheological properties of RBCs exposed to oxidant (cumene hydroperoxide, CHP, 80 µM) during continuous shear stress (30 Pa), for 1800 seconds[1]. Deformability of RBCs is expressed as the elongation index(EI) (Figure 1).

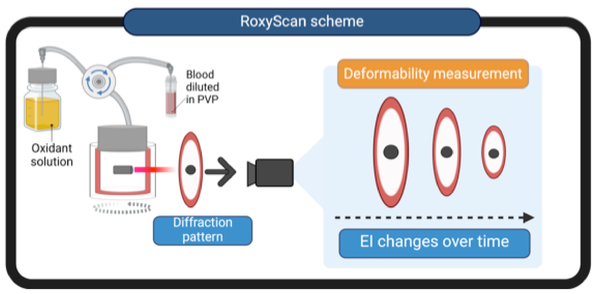

Figure 1. Schematic overview of the RoxyScan technique. *Created with BioRender.com*

Exploratory outcome parameters of the RoxyScan are:

T-POD, Time to Point of Oxidant-induced change in Deformability, *i.e.,* the timepoint at which a 10% decrease in deformability (EI_{Max}) is reached.

EI_{Min}, Minimum deformability.

Pyruvate kinase activity was measured spectrophotometrically, and RBC age was determined with density separation gradient.

Note: the higher the T-POD and/or EI_{Min} the higher the resistance to oxidant-induced rheologic changes as quantified by ektacytometry.

Results: RoxyScan parameter TDT and NTDT groups

RBCs from TDT and NTDT patients both exhibited significantly lower T-POD and EI_{Min} values at baseline than healthy controls (T-POD: NTDT 31% SD± 13%, TDT 51% SD± 19% of healthy controls (100%), $p < 0.01$; EI_{Min}: NTDT 16% SD± 43%, TDT 67% SD± 23% of healthy controls (100%), $p < 0.01$(Figure 2). Both T-POD and EI_{Min} (not shown) are clearly associated with RBC age with older fractions having lower outcomes compared to younger fractions ($p<0.05$)

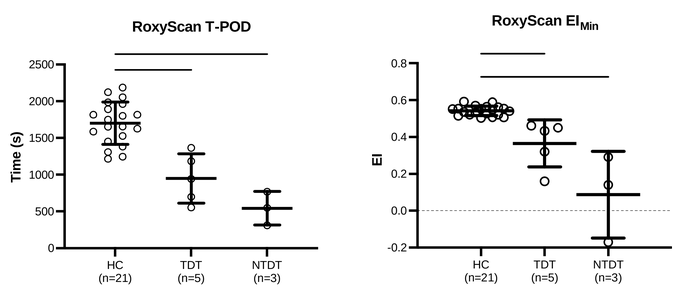

Figure 2. RoxyScan parameters T-POD and EI_{Min} in healthy controls versus TDT and NTDT patient groups. The Kruskal Wallis test was used for the comparison among 3 groups (level of significance=0.05)

T-POD significantly increased in both TDT (15.6%) and NTDT (15.9%) groups ($p < 0.001$) after Mitapivat treatment. EI_{Min} did not significantly increase in TDT (1.1%) or NTDT (3.7%) groups ($p > 0.05$) with Mitapivat treatment (Figure 3).

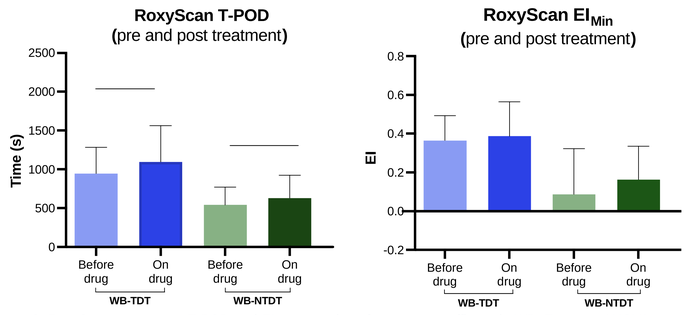

Figure 3. RoxyScan parameters T-POD and EI_{Min} as markers for treatment efficacy in WB. Two-way ANOVA was used to evaluate change between pre and post treatment in each group (level of significance=0.05). WB: Whole blood.

Results: PK activity and RoxyScan parameters correlation

Pyruvate kinase (PK) activity negatively correlated with T-POD ($r = -0.76$, $p = 0.03$) and EI_{Min} ($r = -0.76$, $p = 0.03$) in both TDT and NTDT (Figure 5).

Discussion: correlations of PK and PK activation with RBC age

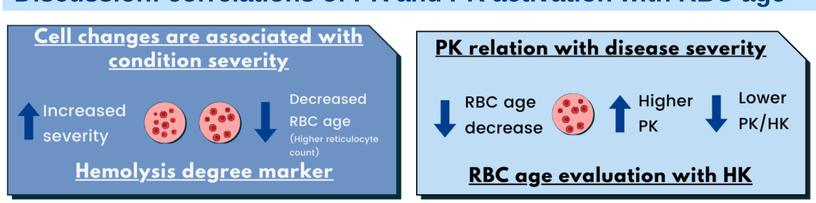

Figure 4. PK levels and RBC age relation associated to diseases severity and hemolysis scheme. *Created with Canva.com*

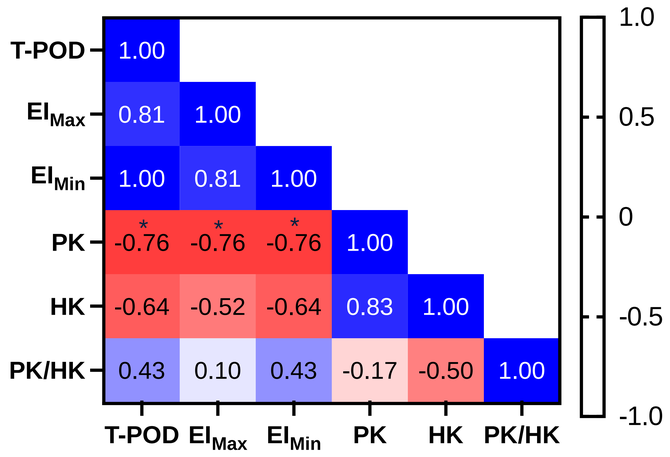

Figure 5. Correlation heatmap RoxyScan parameters with Pyruvate kinase and Hexokinase enzyme activity. The Spearman's correlation (r) was used to evaluate the monotonic relationship (level of significance=0.05)

Conclusion

RBC resilience to oxidative stress (high T-POD) was negatively associated with RBC age (decreased in denser fractions) and thus, absolute PK activity. However, red cell age corrected PK activity (PK/HK) was positively associated with high RBC oxidative stress resilience and improved upon mitapivat treatment both in TDT as well as NTDT patients. Further studies are warranted to clarify whether mitapivat effects in thalassemia are driven by RBC lifespan extension, erythropoiesis, or both.

[1]Larkin, S.K., Hernández, C., van Beers, E.J. et al. The RoxyScan is a novel measurement of red blood cell deformability under oxidative and shear stress. Sci Rep 14, 6344 (2024). https://doi.org/10.1038/s41598-024-56814-8