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POSTER ABSTRACTS

636.MYELODYSPLASTIC SYNDROMES-BASIC AND TRANSLATIONAL

The Pyruvate Kinase (PK) Activator AG-946 Improves PK Properties and Red Blood Cell (RBC) Characteristics upon *Ex Vivo* treatment of RBCs from Patients with Myelodysplastic Syndromes

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Background: Myelodysplastic syndromes (MDS) consist of a heterogenous group of clonal myeloid malignancies, characterized by dysplasia, ineffective hematopoiesis and cytopenias. MDS patients frequently suffer from anemia, directly affecting quality of life. The limited therapeutic options often do not result in the desired clinical improvement. Interestingly, in MDS the activity of the red blood cell (RBC) enzyme pyruvate kinase (PK), a key regulatory enzyme of glycolysis, may be decreased. The activation of PK via small molecules is hypothesized to be beneficial in a wide range of anemias. Currently, a phase 2a, open-label, proof of concept trial is running on the use of the PK activator AG-946 in low-risk MDS patients (NCT05490446). In light of these developments, we investigated the effect of *ex vivo* treatment of MDS RBCs with AG-946.
Objectives: To evaluate RBC PK and cellular properties of patients with MDS, and to determine the effect of *ex vivo* treatment with the PK activator AG-946.
Methods: Eleven low-risk non-transfusion dependent MDS patients and six healthy controls (HCs) were studied. Baseline PK activity and PK thermostability were measured in RBC lysates of purified RBCs, under V_{max} conditions (phosphoenolpyruvate (PEP) 5mM final concentration). Hexokinase (HK) activity was included to relate PK activity to mean RBC age. *Ex vivo* treatment was performed by incubating MDS RBCs for 16 hours at 37 °C in presence or absence of the PK activator AG-946 (10μM or 5μM) using dimethylsulfoxide (DMSO) as a blank. Effect was assessed by measuring PK activity (using a final concentration of PEP 0.5mM) and levels of adenosine triphosphate (ATP) (LC-MS/MS). Functional RBC analysis was performed by osmotic gradient ektacytometry (Lorrca MaxSis). To investigate the effect on PK thermostability, RBC lysates were incubated with AG-946 (2μM or 1μM) or DMSO, after which residual PK activity was measured (PEP 0.5mM). The effect of AG-946 on erythroid development was studied using peripheral blood mononuclear cells, cultured in MethoCult ™ H4434 medium for 14 days in the presence or absence of AG-946 (10μM or 625nM, DMSO as blank). Differences between conditions were determined using Unpaired T-test, ANOVA with Dunnett's test or Kruskal-Wallis with Dunn's test via GraphPad Prism.
Results: Mean PK/HK ratio was significantly lower in RBCs from MDS patients compared to HCs (6.1 (SD 1.8) versus 10.5 (SD 1.5), $p<0.001$). Upon *ex vivo* treatment, PK activity significantly increased (independent of dosage) when compared to DMSO (10μM AG-946, mean increase 30% ($p<0.001$); 5μM, 28% ($p<0.0001$)) (Figure 1A). MDS RBCs displayed decreased PK thermostability at baseline under V_{max} conditions (HCs 79% residual activity versus 68% in MDS, $p<0.01$). This was restored by *ex vivo* treatment with AG-946 (DMSO, 33% residual activity, 2μM AG-946, 85% ($p<0.0001$); 1μM, 87% ($p<0.0001$)). The increase in PK activity was accompanied by significantly increased ATP levels (N=10, DMSO, mean ATP 14.9 μg/mL RBC; 10μM AG-946, 21.0 μg/mL RBC ($p<0.01$); 5μM, 21.1 μg/mL RBC ($p<0.01$)) (Figure 1B).
Functionally, RBC properties improved upon *ex vivo* treatment with AG-946 as reflected by the modest yet significant increase in O_{hyper}, indicating improved hydration status (10μM AG-946, mean increase 2.3% ($p<0.001$); 5μM, 2.9% ($p<0.0001$)). To date, culture assays were performed in 8 patients; 3/8 showed an increase in the number of burst forming units-erythroid (BFU-Es) upon treatment with AG-946 when compared to DMSO. The percentual increases differed per patient: increase of 25% with 10μM AG946 and 150% with 625nM, increase of 40% (10μM) and 39% (625nM), and for the third an increase of 22% (10μM) and 39% (625nM).
Conclusion: Our findings show that RBCs from MDS patients have decreased PK activity and thermostability. We demonstrate that *ex vivo* treatment with AG-946 increases PK activity and ATP levels and restores PK thermostability. Moreover, *ex*

vivo treatment with AG-946 improves RBC hydration, suggesting that improved energy status directly improves RBC functional properties. The preliminary results of the culture assay could indicate that in certain patients, dyserythropoiesis may be ameliorated upon PK activation. To better understand the effects on erythropoiesis additional experiments are required. In conclusion, our data might support a rationale for the use of PK activators as a novel therapeutic option for MDS.

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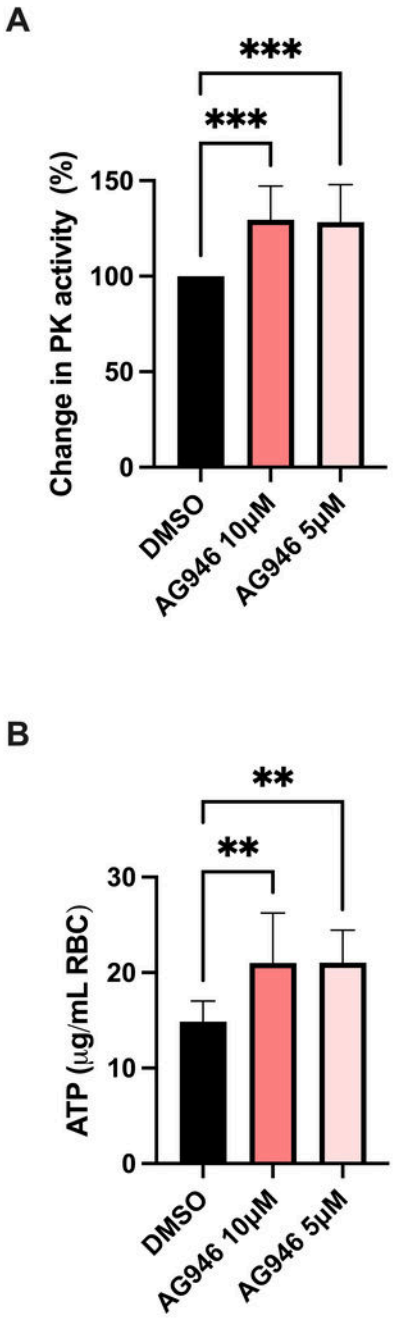

Figure 1 - Effects of *ex vivo* treatment with the pyruvate kinase (PK) activator AG946 on red blood cells (RBCs) from patients with myelodysplastic syndrome (MDS). After treatment of RBCs with the PK activators for 16 hours at 37 °C, PK activity significantly increased (N=11) (Panel A). An increase in adenosine triphosphate (ATP) levels was found upon the increase in PK activity after *ex vivo* treatment of RBCs (N=10) (Panel B). Error bars represent standard deviation. $^{**}p$ <0.01, $^{***}p$ <0.001. ATP adenosine triphosphate; DMSO dimethylsulfoxide; PK pyruvate kinase; RBC red blood cell.

Figure 1

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