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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Purpose

To evaluate red blood cell (RBC) pyruvate kinase (PK) and cellular properties of patients with myelodysplastic syndrome (MDS), and to determine the effect of *ex vivo* treatment with the PK activator AG-946.

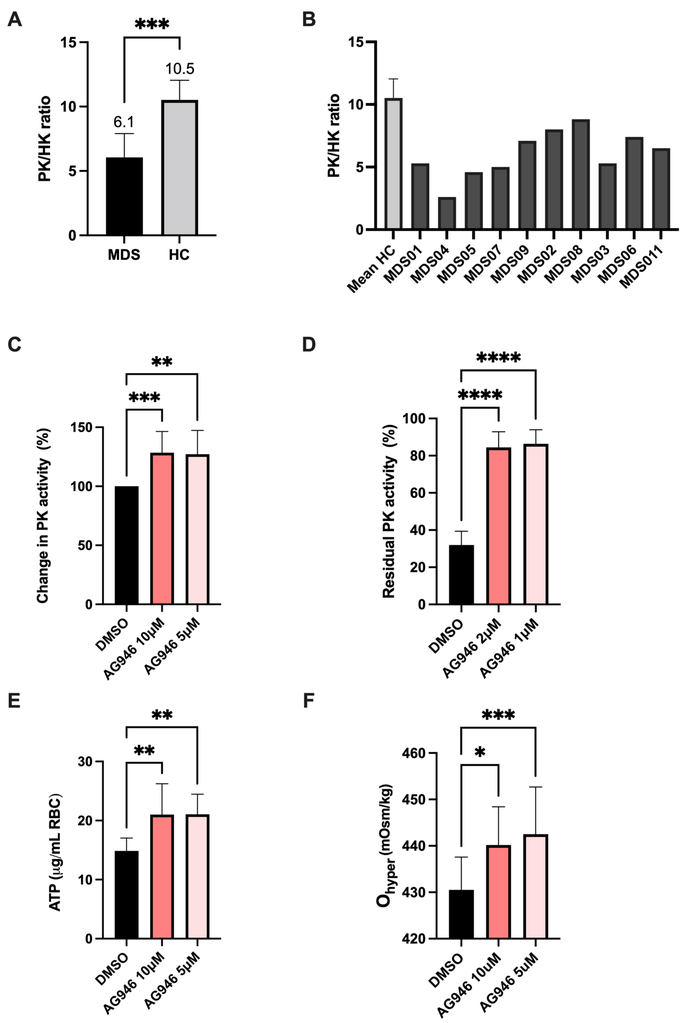
Introduction

- Patients with MDS frequently suffer from anemia, which directly affects their quality of life. Therapeutic options for these patients are limited.
- Decreased activity of RBC PK, a key regulatory enzyme of glycolysis, has been described previously in MDS patients.
- In light of current advances in PK activation therapies (including a clinical trial (NCT05490446)), we studied properties of RBC PK in MDS, as well as the effect of *ex vivo* treatment of MDS RBCs with the PK activator AG-946.

Methods

- Eleven non-transfusion dependent MDS patients and six healthy controls (HCs) were studied.
- PK activity and thermostability were measured, as well as hexokinase (HK) activity, to evaluate PK activity in relation to mean RBC age (PK/HK ratio).
- *Ex vivo* treatment with MDS RBCs with PK activator AG-946 (10uM or 5uM), compared to blanc (DMSO). After incubation of 16 hours at 37 °C, the following assays were performed:
  - PK activity
  - ATP levels (LC-MS/MS) (*N*=10)
  - Osmotic gradient ektacytometry (Lorrca Maxsis)
- Effect of PK activation on PK thermostability was assessed by incubating RBC lysates (DMSO, AG-946 2uM, AG-946 1uM), after which lysates were incubated at 53 °C for 60 minutes (PEP 0.5mM).
- Effect of PK activation on erythroid development was assessed by culturing peripheral blood mononuclear cells MethoCult™ H4434 medium for 14 days in absence or presence of AG-946 (10uM or 625nM, DMSO as blank).

Figure 1. PK is affected in MDS RBCs, yet can be restored upon *ex vivo* treatment with PK activator AG-946, subsequently improving RBC metabolism.



Results

- Mean PK/HK ratio was significantly decreased in MDS compared to HCs (6.1 versus 10.5), as well as baseline PK thermostability (expressed as residual activity, 68% versus 79%). (**Figure 1A,B**).
- *Ex vivo* treatment with the PK activator AG-946 led to an increase in both PK activity and residual PK activity incubation at 53 °C at 60 minutes (**Figure 1C,D**).
  - PK activity: 10µM AG-946, mean increase 29%; 5µM AG-946, 27%.
  - PK thermostability: DMSO, 32% residual activity; 2µM AG-946, 84%; 1µM AG-946, 86%.
- Upon PK activation, ATP levels also significantly increased (**Figure 1E**).
- RBC functionality improved upon treatment with AG-946, as reflected by the increase in Ohyper, indicating improved hydration status (**Figure 1F**).
- To date, colony forming culture assays have been performed in 4/11 patients. Interestingly, in one of four there was 36% increase in number of burst forming units-erythroid (non-dose-related) upon treatment with AG-946.

Conclusion

Our findings suggest that RBCs from MDS patients show a decrease in PK activity and thermostability. Furthermore, we show that ex vivo treatment with AG-946 increases PK activity, ATP levels and stabilizes PK. RBC hydration was shown to be improved upon treatment with AG-946. The preliminary results of the culture assay could indicate that in certain patients, dyserythropoiesis may be ameliorated upon PK activation. 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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