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636.MYELODYSPLASTIC SYNDROMES-BASIC AND TRANSLATIONAL

Biochemical and Metabolomic Analysis of Glycolytic Activity in Red Blood Cells from Low-Risk Myelodysplastic Syndromes (LR-MDS) Patients and in-Vitro Effect of the Pyruvate Kinase Activator AG-946

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Background: Anemia is the most common cytopenia occurring in 80%-85% of patients (pts) with LR-MDS and therapeutic strategies remain limited. Beyond defective maturation, preliminary data has shown decreased glycolytic activity in MDS-RBCs. Glycolytic pathway is the prominent metabolic cascade in RBCs and is currently targeted with PK activators in clinical trials of congenital anaemias which are also marked by ineffective erythropoiesis like MDS. AG946 is an investigational, potent activator of PK with potential to enhance RBC functionality and survival by increasing glycolysis.
Aim: Evaluate enzymatic activity of the glycolytic pathway in pts with anemia due to LR-MDS and in-vitro effect of pyruvate kinase (PK) activator (AG946) in RBCs cultures of LR-MDS pts.
Methods
In part A, 66 anemic pts (i.e., Hb<11 g/dL) diagnosed with MDS according to WHO 2022 were enrolled in the study and sampled for peripheral blood >4 weeks after the last RBC unit to reduce blood donor contamination. The determination of activities of RBC enzymes hexokinase (HK) and PK (the initial and terminal steps of glycolytic pathway), and of ATP levels was performed by standard spectrophotometric method according to Beutler et al, 1984. 2,3DPG levels were tested by (2,3-BPG) ELISA kit (ElisaGenie, IE).
In part B, purified RBC or whole blood of 10 LRMDS pts and 5 healthy controls (HC) were incubated for 6 and 24 h at 37°C in presence or absence of AG946 in phosphate-buffered saline containing 1% glucose, 170 mg/L adenine, and 5.25 g/L mannitol (AGAM, pH 7.40). RBC were incubated with 1, 5 and 50 uM of AG946 for up to 24 h, at 37°C. After 6 and 24 h, enzymatic activities (HK, PK) and ATP levels were measured. Additionally, metabolomic analysis was performed by LC-MS on extracts from blood samples for each condition.
Results
Part A: Pts were mainly elderly males (median age 80yr, range 58-92), and mostly belonged to IPSS-R low (78%) and very low/low groups (85%). PK activity (median 13.7 IU/gHb, 7.5-35.9) was reduced in 35% of cases and HK activity (median 2.3 IU/gHb, 0.8-8.1) was increased in 91%, resulting in an abnormally reduced PK/HK ratio in majority of pts (95%). Notably, ATP levels (median 3.4 umol/gHb, 1.3-6.5) were also reduced in 33/56 tested patients (59%), whilst 2,3DPG levels were close to the upper limit of the normal range (median 389 nmol/gHb, 318-639).

Conclusions: Our data demonstrates decreased glycolytic activity in a large cohort of anemic pts with LR-MDS versus HC, with reduced PK activity in 1/3 of cases, decreased PK/HK ratio in nearly all subjects, and reduced ATP in 2/3 of pts. Furthermore, in vitro incubation with AG946 led to an increase in PK activity and PK/HK ratio and stable ATP levels in RBCs of LR-MDS pts across all WHO classifications. Metabolomic analysis confirmed an altered RBC-metabolic status of MDS versus HC at baseline with possible modulation of glycolysis by AG946, further supporting a potential therapeutic role of PK Activation with AG946 in LR-MDS.

Disclosures Fattizzo: *Agios:* Consultancy, Research Funding, Speakers Bureau; *Janssen:* Speakers Bureau; *Zenas Biopharma:* Research Funding; *Sobi:* Speakers Bureau. **Patel:** *Agios Pharmaceuticals, Inc.:* Current Employment, Current equity holder in publicly-traded company. **Wind-Rotolo:** *Agios Pharmaceuticals, Inc.:* Current Employment, Current equity holder in publicly-traded company. **Bianchi:** *Agios Pharmaceuticals, Inc.:* Other: Scientific advisor. **Barcellini:** *Novartis:* Consultancy, Honoraria, Speakers Bureau; *Alexion, AstraZeneca Rare Disease:* Consultancy, Membership on an entity's Board of Directors or advisory committees, Research Funding.

Figure 1A. Significantly enriched metabolic pathways between patients and controls at baseline.

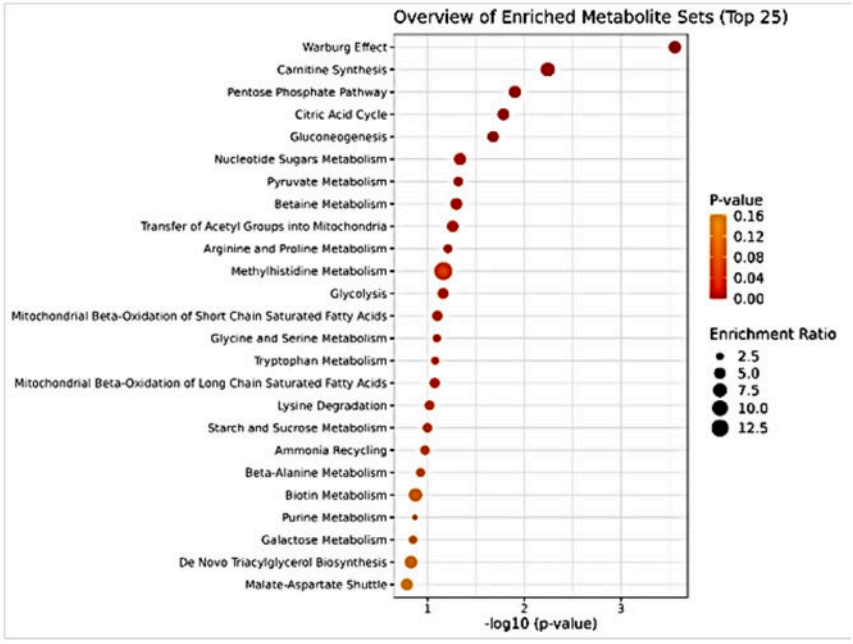
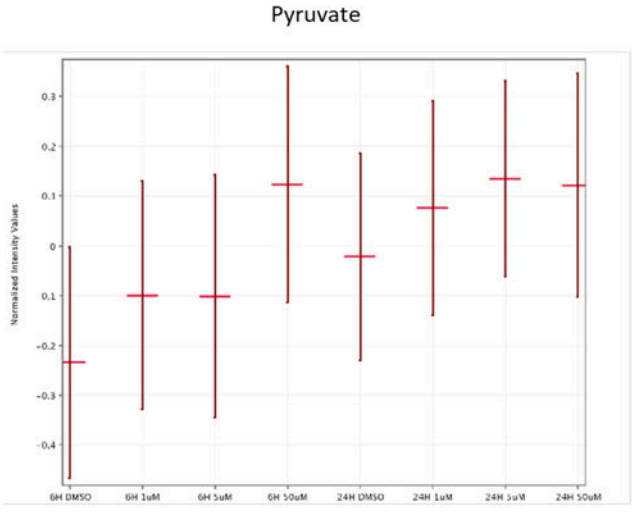


Figure 1B. Pyruvate levels in MDS patients at baseline and after incubation with AG946 1, 5, and 50 uM at 6 and 24 h.



Profile plots with average normalized intensity values ± Standard Error

Figure 1

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