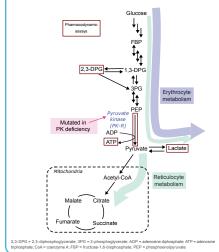
Characterization of metabolic response to AG-348, an allosteric activator of red cell pyruvate kinase, in healthy volunteers and pyruvate kinase deficiency patients

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BACKGROUND

- **PK deficiency**
- Pyruvate kinase (PK) deficiency is a glycolytic enzymopati that causes lifelong chronic hemolytic anemia. PK deficiency is caused by abnormalities of the PK red blood
- cell isoform R (PK-R) due to mutations in the PKLR gene. Mutations in PK-R typically affect protein stability, catalytic
- activity, or both, which adversely affects glycolysis and leads to severe energy starvation in red blood cells.

Figure 1. Glycolytic pathway



igure 2. AG-348 is a first-in-class allosteric activator of PK-R and is in clinical ent to treat PK deficiency

Phase 1 studies of AG-348 in healthy volunteers (NCT02108106, NCT02149966) have been completed, and a phase 2 study in patients with PK deficiency is ongoing (DRIVE PK: NCT02476916).



AG-348 13 9 55 16 59 22 14 41 26

PK-R crystal structure showing location of AG-348 binding site (orange) and some mutations observed in PK deficiency (red)

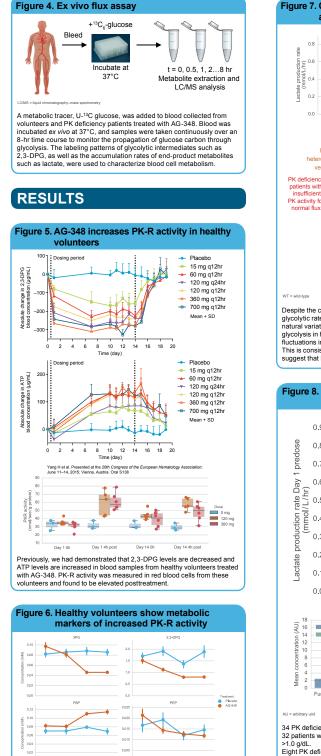
Recombinantly expressed wildtype or mutant PK-R proteins were incubated with AG-348. Shown are fold-activation and the concentration of drug that gave 50% of maximal activation (AC_{50}).

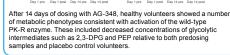
METHODS

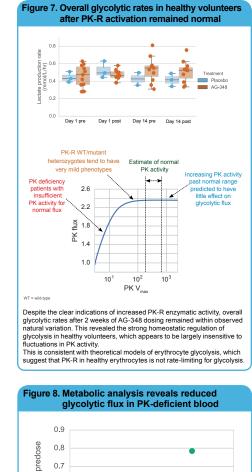


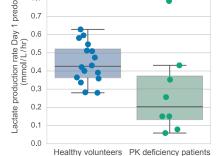
Day 14 Day 43 Day 113 Day 16 AG-348 healthy volunteer MAD (NCT02149966) AG-348 phase 2 in PK deficiency patients (DRIVE PK: NCTO

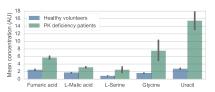
Blood samples were collected at the indicated times (up to 14 days for health volunteers and up to 6 months for PK deficiency patients) to assess PK-R activity, levels of ATP/2,3-DPG, and PK-R flux, and for metabolomic profiling. Dose levels evaluated in this study include 120 mg BID and 360 mg BID (healthy volunteers) and 50 mg BID and 300 mg BID (PK deficiency patients)











34 PK deficiency patients have enrolled in the DRIVE PK study, and 15 of 32 patients with ≥3 weeks of data had a maximal increase in hemoglobin

Eight PK deficiency patients have undergone extensive metabolic characterization to determine unique qualities of metabolism in PK deficiency. Compared with healthy volunteers. PK deficiency patient blood cells showed significantly decreased lactate production rates, consistent with a metabolic bottleneck in glycolysis caused by the PK-R mutation. Additionally, PK deficiency patients exhibited a number of other distinct metabolic qualities, such as high concentrations of nucleotides, aming acids, and tricarboxylic acid cycle metabolites

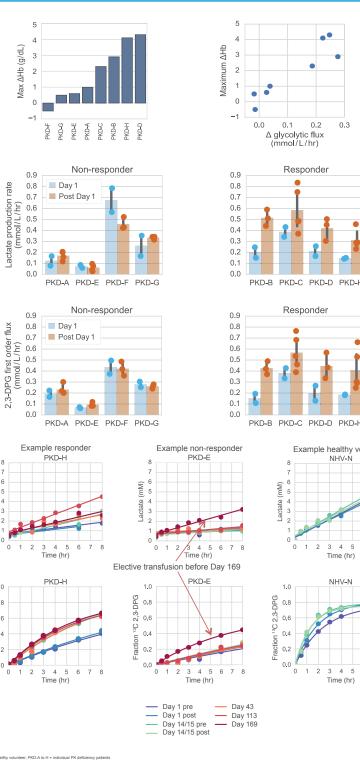


Figure 9. AG-348 rescues metabolic phenotypes in responsive patients

Of the eight PK deficiency patients undergoing extensive metabolic characterization, four had a maximal increase in hemoglobin of >1.0 g/dL In those patients, we observed a >0.1 mmol/L/hr (>50%) increase in glycolytic rates. Additionally, we observed increased incorporation of 13C Into glycolytic intermediates such as 2,3-DPG, suggesting an increase in metabolically active erythrocytes. None of the four PK deficiency patien that did not have a hemoglobin increase of >1.0 g/dL showed significant metabolic changes.

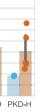
Agios Pharmaceuticals, Inc., Cambridge, MA, USA

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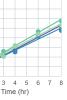
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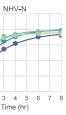


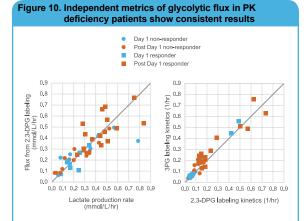




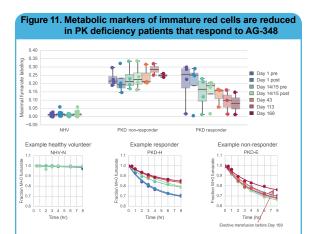
Example healthy voluntee NHV-N







Two independent metrics were used to assess glycolytic flux in PK deficiency patients: the total lactate production rate and the rate of labeling in 2,3-DPG. Use of 2,3-DPG first-order kinetics was justified by the fact that it accounts for the vast majority of the pool of glycolytic metabolites, and that metabolites in lower glycolysis showed virtually identical labeling kinetics. The two metrics gave highly consistent results.



An additional observation from the metabolic flux analysis of PK deficiency patient blood was the significant propagation of ¹³C from glucose into tricarboxylic acid cycle metabolites such as citrate, fumarate, and malate. This labeling, which was absent in healthy volunteers. strongly suggested the presence of active mitochondrial metabolism. Since erythrocytes lose their mitochondria during the development process, this metabolism is likely performed by reticulocytes, consistent with the fact that PK deficiency patients tend to have hyperactive bone marrow and increased reticulocyte counts in peripheral blood. Additionally, incomplete labeling of glycolytic intermediates demonstrated the existence of a metabolically inactive cell subpopulation, further suggesting that reticulocytes play an important role in maintaining blood metabolism in PK deficiency patients.

CONCLUSIONS

- A >50% increase in glycolytic flux was observed in PK deficiency patients treated with AG-348 who had a hemoglobin increase >1.0 g/dL, but was not observed in patients without such an increase
- Metabolic markers of immature red cells are reduced in PK deficiency patients that respond to AG-348.
- · Strong homeostatic regulation of overall rates of glycolysis was observed in healthy volunteers, even in the presence of activated PK-R.
- These data demonstrate that hemoglobin increases in PK deficiency patients treated with AG-348 are associated with increased red cell glycolysis

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