Genotype-response correlation in DRIVE PK, a phase 2 study of AG-348 in patients with pyruvate kinase deficiency

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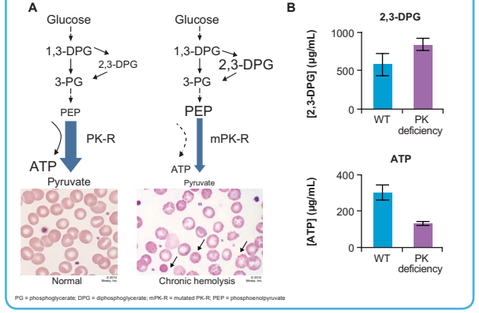
INTRODUCTION

- Pyruvate kinase (PK) deficiency is a congenital hemolytic anemia caused by mutations in the *PKLR* gene, leading to a deficiency of the glycolytic enzyme red cell PK (PK-R) (**Figure 1**).[1,2]
- AG-348 is an orally available small-molecule allosteric activator of PK-R that activates the wild type (WT) and a range of mutant PK-R enzymes associated with PK deficiency (**Figure 2**).[3,4]
- Increased PK-R activity and ATP levels in patient red blood cells treated with AG-348 *ex vivo* may be linked to *PKLR* genotype and/or PK-R protein level (**Figure 3**).[4]
- In a phase 2 clinical study of patients with PK deficiency (DRIVE PK; NCT02476916), 26 of 52 patients (50%) experienced a maximum Hb increase of >1.0 g/dL (mean maximum increase, 3.4 g/dL; range, 1.1–5.8 g/dL), including 25 of 42 patients (59.5%) with at least one missense mutation (**Figure 4**).[5]
  - In most cases, Hb increases were rapid and sustained, and seen across a wide dose range from 5 to 300 mg twice daily (BID) (**Figure 5**).
  - Hemolysis markers (reticulocytes, indirect bilirubin, haptoglobin) improved in patients who experienced a maximum Hb increase of >1.0 g/dL.
  - Hb increases were observed in patients with a variety of *PKLR* mutations, and increases were associated with the presence of at least one missense mutation.
- Because PK deficiency is a genetically heterogeneous disease, with over 200 described mutations, we sought to understand in greater detail the molecular parameters associated with Hb increases in patients treated with AG-348.

Figure 1. Metabolic defects in PK deficiency[6]

(A) The role of the PK-R enzyme in glycolysis. Defective glycolysis in PK-deficient red blood cells results in the accumulation of the upstream metabolites 2,3-DPG and PEP and the depletion of ATP and pyruvate, and decreased red blood cell lifespan. (B) Levels of 2,3-DPG and ATP in whole blood from healthy volunteers and patients with PK deficiency.



PG = phosphoglycerate; DPG = diphosphoglycerate; mPK-R = mutated PK-R; PEP = phosphoenolpyruvate

Figure 2. AG-348 is an allosteric activator of PK-R[3,4]

(A) Chemical structure of AG-348. (B) Recombinant WT PK-R enzyme activity was assessed after incubation with or without AG-348 (2 µM) in the presence of increasing concentrations of PEP. (C) Crystal structure of AG-348 bound to PK-R tetramer.

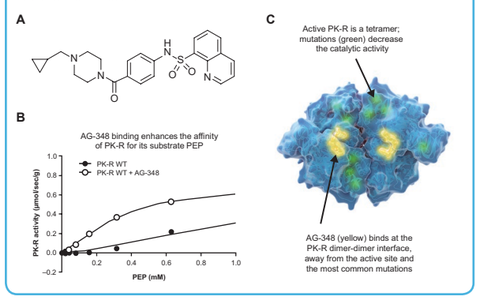


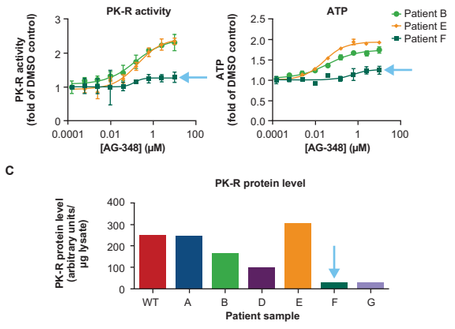
Figure 3. *Ex vivo* response may be linked to genotype and/or PK-R protein level[4]

(A) Genotype of patient samples. (B) PK-R activity and ATP levels in red blood cells from patients with PK deficiency; cells were incubated with AG-348 for 24 hr. (C) PK-R protein levels in red blood cells from healthy volunteers (indicated by WT) and patients with PK deficiency as measured by Meso Scale assay.

A

Genotype

Patient	Mutations (nucleotide)	Mutations (protein)
A	1529 G>A/1532 G>A	R510Q/G511R
B	1456 C>T/1168 G>A	R486W/D390N
C	1529 G>A/721 G>T	R510Q/E241stop
D	1483 G>A/721 G>T	A495V/E241stop
E	1456 C>T/1022 G>A	R486W/G341D
F	1529 G>A/1241 C>G	R510Q/P414R
G	401T>A/1487 T>G	V134D/V496G



DMSO = dimethyl sulfoxide

Figure 4. Maximum Hb change by genotype in DRIVE PK patients[5]

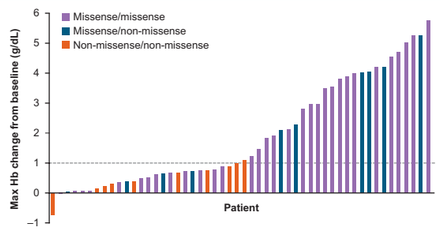
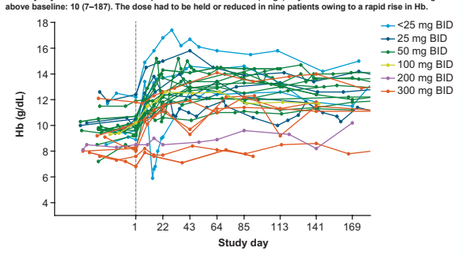


Figure 5. Hb change over time in DRIVE PK patients who had a maximum Hb increase of >1.0 g/dL[5]

The majority of Hb increases were rapid and sustained. Median (range) days to the first Hb increase of >1.0 g/dL above baseline: 10 (7–187). The dose had to be held or reduced in nine patients owing to a rapid rise in Hb.



These are the treatment doses that each patient received for the longest duration during the core period

OBJECTIVE

- To analyze the relationship between Hb increase and patient genotype, biochemical response to AG-348 treatment, and baseline PK-R protein level.

METHODS

- Whole blood samples were collected from patients with PK deficiency enrolled in the phase 2 DRIVE PK study.
- Patient genotypes were determined by Centogene AG (http://www.centogene.com).
- Levels of PK-R protein were quantitated using a Meso Scale assay as described previously (antibodies from Abcam, Cambridge, UK [ab89071] and Aviva Systems Biology, London, UK [OAGA00912]).[4]
  - The signal was normalized to a reference control sample from a subject without PK deficiency.
  - For PK-R protein-level testing, the sample was obtained on Day 0 prior to the initiation of AG-348 treatment, except in a single patient for whom the sample from Day 15 was used.
- Patient consent was received for all testing procedures.

RESULTS

Figure 6. An association between baseline PK-R protein level and maximum Hb change was observed in DRIVE PK patients

(A) Correlation plot between maximum Hb change observed in DRIVE PK patients and normalized PK-R protein level (r^2 = 0.39, p<0.0001). Dots represent individual patients. (B) PK-R protein levels in DRIVE PK patients categorized by maximum Hb change.

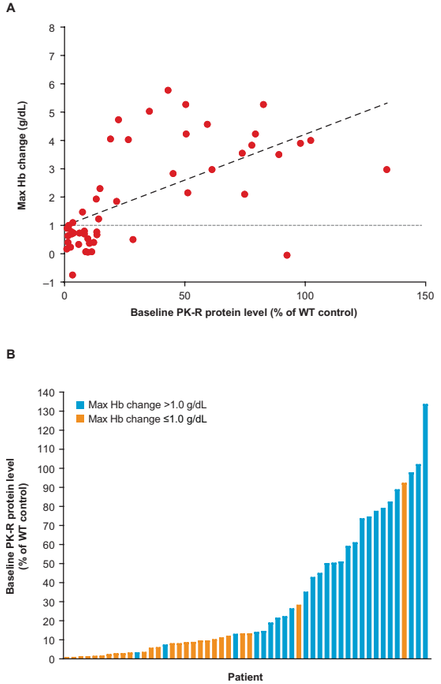


Figure 7. Distribution of mutations among 52 DRIVE PK patients

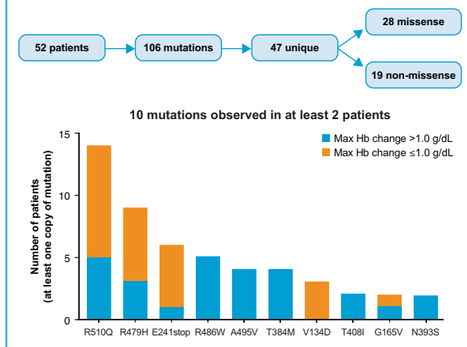


Figure 8. Patients with an Hb increase of >1.0 g/dL have greater average PK-R protein levels

PK-R protein levels in DRIVE PK patients (expressed as % of WT control sample) categorized by Hb change. Horizontal lines and percentage values indicate the mean, error bars show the standard deviation, and each symbol represents an individual patient.

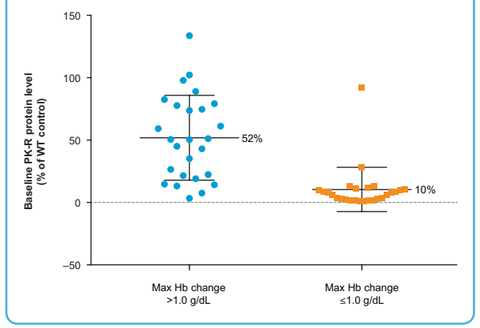


Figure 9. Patients with two non-missense mutations have significantly lower PK-R protein levels

PK-R protein levels in patients with at least one missense mutation vs those with two non-missense change. Horizontal lines and percentage values indicate the mean, error bars show the standard deviation, and each symbol represents an individual patient.

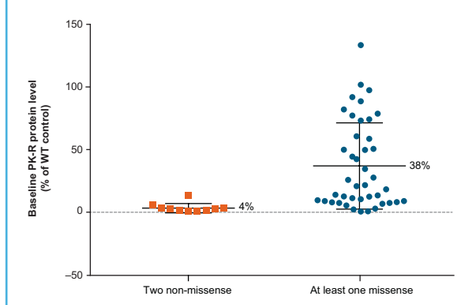
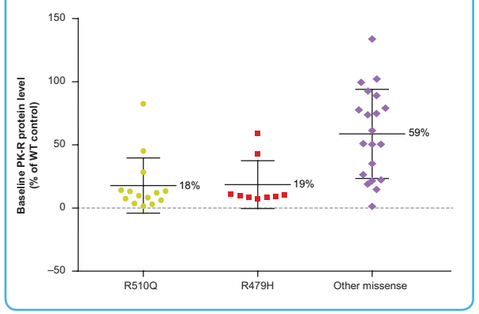


Figure 10. Patients with at least one R510Q or R479H missense mutation have lower PK-R protein levels than patients with other missense mutations

PK-R protein levels in all patients with at least one missense mutation, stratified into those with at least one R510Q mutation, at least one R479H mutation, or neither. Horizontal lines and percentage values indicate the mean, error bars show the standard deviation, and each symbol represents an individual patient.



SUMMARY AND CONCLUSIONS

- A statistically significant correlation was observed between baseline PK-R protein level and Hb increases in patients with PK deficiency treated with AG-348.
- This correlation is evidence that AG-348 is working via its proposed mechanism of action of stimulating the residual activity of the mutant enzyme.
- Although neither genotype nor PK-R protein level could predict Hb increases with absolute precision, some trends were observed:
  - Patients with two non-missense mutations had lower protein levels than those with at least one missense mutation.
  - Patients with R479H or R510Q mutations had lower protein levels than patients with other missense mutations.
- These preliminary findings will be examined further in the ongoing phase 3 studies of AG-348 (NCT03548220 and NCT03559699).

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Disclosures

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