AG-519 is a potent activator of mutant pyruvate kinase associated with hemolytic anemia

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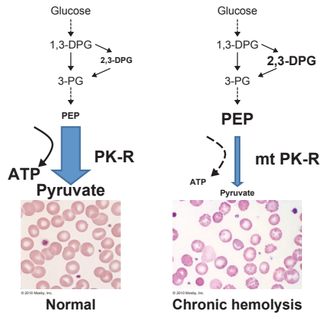
BACKGROUND

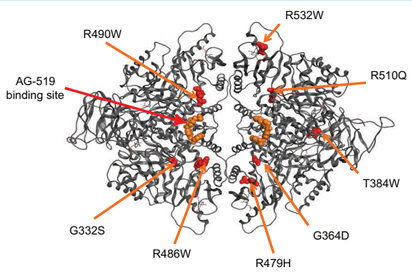
- Pyruvate kinase (PK) deficiency is an autosomal recessive enzymopathy, and is the most common cause of hereditary nonspherocytic hemolytic anemia.
- PK deficiency is an inborn error of metabolism resulting in life-long hemolytic anemia associated with severe comorbidities.[1]
- It is hypothesized that mutations in the red cell isoform of PK (PK-R) result in insufficient energy production, in the form of adenosine triphosphate (ATP), to maintain red cell membrane homeostasis (**Figure 1**).
- Treatment is generally supportive, focusing on the resultant anemia,[2] and there are no approved drugs that directly target mutated PK-R (mt PK-R).
- AG-519 is a potent, highly selective and orally bioavailable second PK-R activator shown preclinically to have no aromatase inhibitory effects (**Figure 2**).
- An ongoing randomized, double-blind, phase 1 study of AG-519 in healthy volunteers (NCT02630927) aims to identify a safe and pharmacodynamically active dose and schedule to be used in subsequent clinical studies enrolling subjects with PK deficiency.

Figure 1. Mutations in PK-R result in dysregulated red cell metabolism and chronic hemolysis



3-PG, 3-phosphoglycerate; DPG, diphosphoglycerate; mt PK-R, mutant PK-R; PEP, phosphoenolpyruvate.

Figure 2. AG-519 is an allosteric regulator of PK-R activity



Tetrameric crystal structure of PK-R enzyme. Brown arrows highlight a subset of mutation sites associated with PK deficiency. Recombinant enzymes with these mutations were expressed and tested with AG-519 (**Figure 3**). AG-519 binds at the dimer-dimer interface of the PK-R tetramer, as indicated by red arrow.

OBJECTIVES

- We describe the mechanism of action and cellular effects of AG-519 in *in vitro* and *ex vivo* settings on mutant PK-R proteins associated with PK deficiency, and *in vivo* in C57Bl6 mice.

METHODS

- Mutant PK-R proteins were expressed in E. coli and the kinetic parameters of the purified enzymes were evaluated in the presence or absence of AG-519.
- For thermostability studies, mutant enzymes were pre-incubated with control or AG-519 and then subjected to elevated temperature (53°C) followed by assessment of residual activity over time.
- C57Bl6 mice were administered AG-519 BID by oral gavage for 3 days, followed by evaluation of blood PK-R activity and ATP/2,3-DPG levels.
- Peripheral blood was obtained from patients with PK deficiency and the red cells were incubated with AG-519 for up to 24 hr, followed by assessment of PK-R activity and ATP levels.

References

1. Zanella A et al. *Blood Rev* 2007;21:217–31.
2. Grace R et al. *Am J Hematol* 2015;90:825–30.

Disclosures

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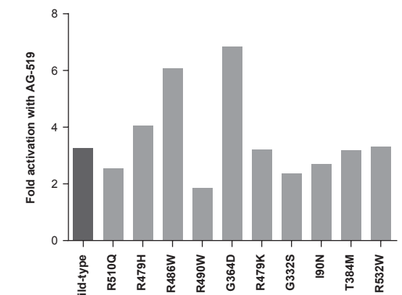
RESULTS

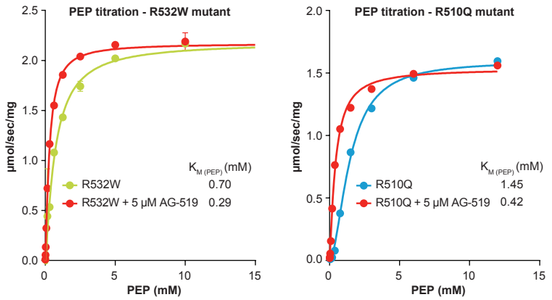
Figure 3. AG-519 activates tested recombinant mt PK-R enzymes in *in vitro* biochemical assays



AC_{50} (nM)	19	43	16	53	12	57	15	34	44	32	20

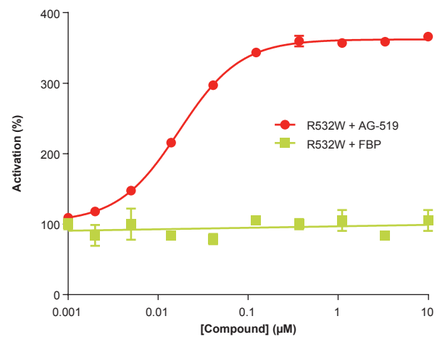
AG-519 activates tested recombinant mt PK-R enzymes by ~2-fold or greater, with AC_{50} values as indicated.

Figure 4. AG-519 activates mutant alleles R532W and R510Q by increasing their affinity for PK-R substrate PEP



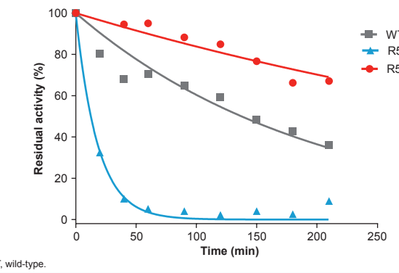
PEP, phosphoenolpyruvate.

Figure 5. AG-519 activates the R532W mutant enzyme that is insensitive to FBP, an endogenous activator of PK-R



FBP, fructose 1,6-bisphosphate.

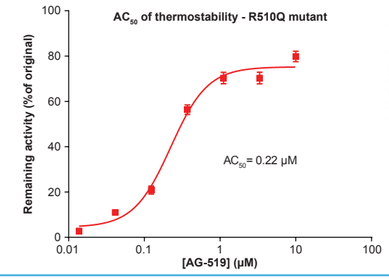
Figure 6. Binding of AG-519 can enhance the stability of the PK-R tetramer



AG-519 improves thermostability of the R510Q mutant enzyme upon incubation at 53°C. Half-life ($t_{1/2}$) values: PK-R WT, 137.5 min; R510Q, 12.5 min; R510Q + AG-519 (10 μM), >200 min.

WT, wild-type.

Figure 7. AG-519 is a potent stabilizer of the R510Q mutant enzyme



Recombinantly-expressed R510Q protein was pre-incubated with a dose titration of AG-519 for 1 hr. The enzyme was then incubated at 53°C for 60 min and residual PK-R activity was assessed.

Figure 8. AG-519 increases PK-R activity and modulates ATP/2,3-diphosphoglycerate levels in wild-type mice

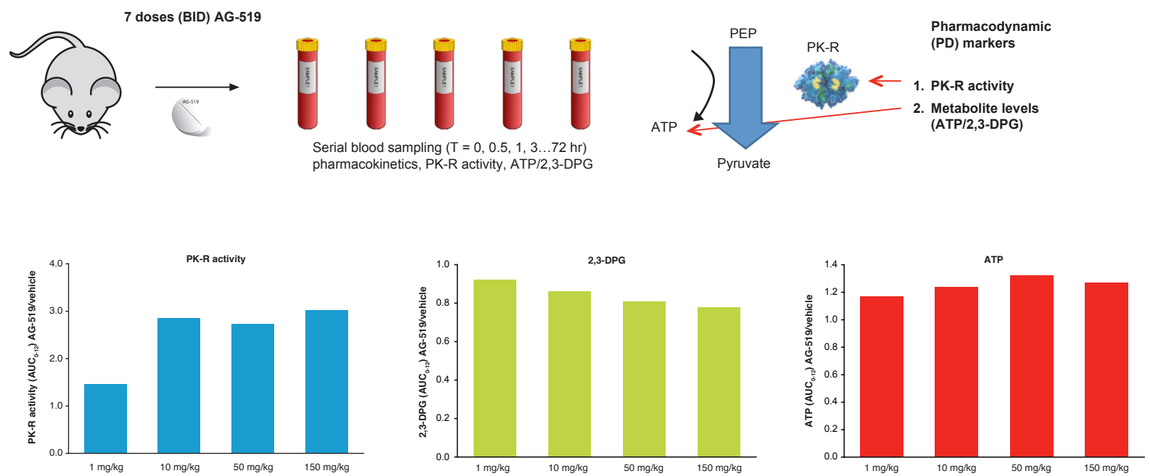
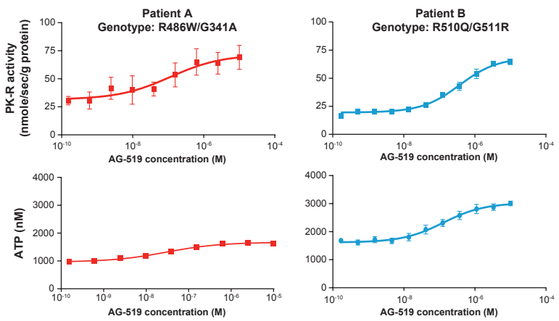


Figure 9. *Ex vivo* treatment with AG-519 increases PK-R activity and ATP levels in PK deficient patient cells



Red cells from PK-deficient patients were treated with a dose titration of AG-519 and incubated overnight at 37°C, followed by assessment of PK-R activity and ATP levels.

CONCLUSIONS

- AG-519 is a potent activator of wild-type and mutant PK-R (mt PK-R) enzymes associated with PK deficiency.
- AG-519 improves catalytic efficiency and protein stability of mt PK-R enzymes.
- AG-519 can activate mt PK-R in red cells from patients with PK deficiency.
- AG-519 shows excellent *in vivo* activity and potency in mice.
- Please see Poster 752 (11 June) for data from the AG-519 phase 1 healthy volunteer study, and Oral Presentation S830 (12 June) for discussion of preclinical cross-species PK/PD.
- The potency and activity of AG-519 as an activator of both wild-type and mutant forms of PK-R is similar to that of AG-348, a PK-R activator currently in phase 2 testing in patients with PK deficiency (NCT02476916; Oral Presentation S466 on 11 June).

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