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.
- AG-519 is a potent activator of wild-type and mutant PK-R.

METHODS
- Peripheral blood was obtained from patients with PK deficiency and the red cells were incubated with AG-519.
- For thermostability studies, mutant enzymes were pre-incubated with control or AG-519 and then subjected to elevated temperature (53°C) and 3000 rpm for 1 hr. Remaining activity was pre-incubated with a dose titration of AG-519 for 1 hr. The enzyme was then incubated at 37°C for 60 min and PK-R activity was assessed.

OBJECTIVES
- To identify a safe and pharmacodynamically active dose and schedule to be used in subsequent clinical studies.

RESULTS
- AG-519 activates tested recombinant PK-R enzymes in vitro (Figure 3).
- AG-519 activates tested recombinant PK-R enzymes by 10 fold or more, with half-maximal stimulation (AC50) of 0.22 µM (Figure 4).
- AG-519 increases PK-R activity and modulates ATP/2,3-diphosphoglycerate (2,3-DPG) levels in wild-type mice (Figure 5).
- AG-519 increases PK-R activity and modulates ATP/2,3-DPG levels in PK-deficient patients (Figure 6).
- AG-519 increases PK-R activity and modulates ATP/2,3-DPG levels in PK-deficient patients (Figure 7).

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 S380 (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 S456 on 11 June).