Preclinical pharmacokinetics and pharmacodynamics of AG-519, an allosteric pyruvate kinase activator

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### Background: Pyruvate kinase (PK) deficiency

#### Description
- A rare genetic disease causing chronic hemolytic anemia
- Symptoms vary in severity
- Current treatments are supportive only

#### Etiology
- Caused by mutations in the red blood cell isoform of PK (PK-R), a key enzyme in red blood cell glycolysis

#### Biology
- Leads to increases in the upstream metabolite 2,3-DPG and decreases in the product ATP in blood

#### Therapeutic concepts
- Activation of mt PK-R could repair the metabolic defect
- Increase hemoglobin levels and decrease hemolysis, leading to patient benefit

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Glucose → **2,3-DPG** → ATP

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Glucose → **2,3-DPG** → ATP

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**Glycolysis in healthy red blood cell**

**Defective glycolysis in mt PK-R red blood cell**

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2,3-DPG = 2,3-diphosphoglycerate; ATP = adenosine triphosphate; mt PK-R = mutant PK-R
PK-R activators for the treatment of PK deficiency

- Activation of PK-R resulting in increases in ATP and decreases in 2,3-DPG in healthy volunteers has been observed with an earlier molecule (AG-348)

- Early AG-348 clinical data demonstrate proof-of-concept with rapid and sustained Hb increases in patients with PK deficiency\(^1\)

- AG-519 is a potent, highly selective, orally bioavailable second PK-R activator developed with the aim of eliminating off-target aromatase inhibitory effects of AG-348

\(^1\) Presentation S466. Hb = hemoglobin
Objectives

- To explore the pharmacokinetic/pharmacodynamic (PK/PD) relationships of AG-519 with PK-R activity, ATP and 2,3-DPG in wild type PK-R mice

- To use data from animal studies to project the pharmacokinetic profile and efficacious dose of AG-519 in humans
Human efficacious dose and dosing regimen projection

- Human pharmacokinetics projection
  - Pharmacokinetic studies in different species
  - *In vitro* metabolism
  - Plasma protein binding and *in vitro* $CL_{\text{int}}$

- Human efficacious exposure estimation
  - Cell biology and biochemistry studies
  - PK/PD studies

Modeling simulation for human projection

$CL_{\text{int}} = \text{intrinsic clearance}$
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Modeling simulation for human projection

\( CL_{\text{int}} = \text{intrinsic clearance} \)
Comparable AG-519 pharmacokinetics across species

- Moderate clearance (1.13–2.51 L/hr/kg), moderate to high volume of distribution (2.08–6.44 L/kg) and similar plasma protein binding (79.3% - 87.3%) in mouse, rat, dog and monkey
- Rapid absorption ($T_{\text{max}} \leq 1.2 \text{ h}$) and moderate oral bioavailability (6.9–19.5%)
- Good *in vitro* to *in vivo* correlation in the CL estimates across species

**CL** = clearance; $T_{\text{max}}$ = time to maximum plasma concentration
Human pharmacokinetic projections

- Pharmacokinetic parameters in mouse, rat, dog and monkey used for human pharmacokinetic projection
- *In vitro* metabolism data used as a correction factor
- Allometric scaling conducted for human pharmacokinetic projection

**Projected human pharmacokinetic parameters:**
- CL: 0.4 L/hr/kg
- $V_{SS}$: 3.0 L/kg
- Effective $t_{1/2}$: 4 – 7 hr
- Bioavailability: 22%

Eh = hepatic extraction ratio; CL = clearance; $V_{SS}$ = volume of distribution at steady state; $t_{1/2}$ = half-life
Human efficacious dose and dosing regimen projection

- **Human pharmacokinetics projection**
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**Modeling simulation for human projection**

$CL_{int} =$ intrinsic clearance
Mouse PK/PD study design

Female C57/BL6 mice
PK-R WT

Oral AG-519

Dosing
Vehicle*  1 mg/kg  10 mg/kg  50 mg/kg  150 mg/kg

Single dose  5 doses (BID)  13 doses (BID)

Sampling

Serial sample collections for PK/PD evaluation up to 72 hr after last dose

Read out
Plasma AG-519  Whole blood ATP  Whole blood 2,3-DPG  Whole blood PK-R activity

PK/PD steady state achieved after 5 BID doses

*0.5% methyl cellulose in water

n=4 mice per time point in each dose group
Using mouse PK/PD to estimate human efficacious exposure

- Drug exposure-dependent response observed for all three markers
- The exposure-response relationship is described by an $E_{\text{max}}$ model
- $EAUC_{90}$ (421 hr•ng/mL) for ATP increase used for human efficacious dose prediction

<table>
<thead>
<tr>
<th>Parameter (13 BID doses)</th>
<th>PK-R activity</th>
<th>2,3-DPG</th>
<th>ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td>AG-519 free $AC_{50}$ (nM)</td>
<td>0.55</td>
<td>0.32</td>
<td>0.72</td>
</tr>
<tr>
<td>AG-519 $EAUC_{90}$ (hr•ng/mL)</td>
<td>320</td>
<td>187</td>
<td>421</td>
</tr>
</tbody>
</table>

$AC_{50}$ = half-maximal activity concentration; $EAUC_{90}$ = area under the curve at 90% maximum effect
AG-519 human dose projection

- Favorable pharmacokinetics in multiple species
- Clear PK/PD relationship established in the mouse model enabled the prediction of the AG-519 efficacious dose in humans
- Projected human efficacious dose and dosing schedule:
  - 62–134 mg, orally twice daily
- These data supported the decision to bring AG-519 into a phase 1 healthy volunteer study
AG-519 has favorable clinical pharmacokinetics: Poster 752

- Rapid absorption, moderate variability
- Exposure is dose-proportional or slightly greater than dose-proportional
- Effective t½ of approximately 6 hr

Effective t₁/₂: elimination t₁/₂ in the therapeutic relevant concentration range, in this case t₁/₂a
Comparison: projected vs actual human pharmacokinetics

- Human pharmacokinetic profile was simulated using animal data, the projected human pharmacokinetic parameters, and a 2-compartment model.
- Animal AG-519 pharmacokinetic data translate well to healthy volunteers.
- The actual human pharmacokinetic profile is similar to the simulated:
  - Slightly higher than projected $C_{\text{max}}$; good $C_{\text{trough}}$ projection.
  - As projected, pharmacokinetic profile supported oral BID dosing regimen.

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<tr>
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<th>Projected</th>
<th>Actual</th>
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<tbody>
<tr>
<td>CL/F, L/hr/kg</td>
<td>1.8</td>
<td>0.66–1.0</td>
</tr>
<tr>
<td>Effective $t_{1/2}$, hr</td>
<td>4–7</td>
<td>6</td>
</tr>
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BID = twice-daily; CL/F = oral clearance; $C_{\text{max}}$ = maximum plasma concentration; $C_{\text{trough}}$ = lowest plasma concentration.
Dose-dependent changes in ATP and 2,3-DPG blood levels are consistent with PK-R activation: Poster 752

Mean (+ SD) change in blood concentration-time profiles of ATP following multiple oral doses of AG-519 (cohorts 1 and 2 only)

- Placebo
- 125 mg AG-519 q12h
- 375 mg AG-519 q12h

- 62% increase from baseline

Dosing period
Conclusions

- AG-519 shows favorable pharmacokinetic profiles in multiple species
- Preclinical PK/PD relationship and favorable pharmacokinetics enabled prediction of human efficacious dose and dosing regimen
  - AG-519 has favorable pharmacokinetic profile in humans
  - Dose-dependent PD response consistent with PK-R activation
  - AG-519 has a favorable safety profile to date, and it does not demonstrate the inhibition of aromatase previously observed with AG-348
- The PK/PD data from healthy subjects will inform dose selection for future studies of AG-519 in patients with PK deficiency

Please see Posters 752 and 742. PD = pharmacodynamic
Acknowledgements

- Agios PK-R discovery team
- Agios PK-R development team
- The volunteers taking part in the AG-519 phase 1 study