Effects of AG-348, a pyruvate kinase activator, on anemia and hemolysis in patients with pyruvate kinase deficiency: early data from the DRIVE-PK study

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- Rachael F Grace: Agios – study co-principal investigator and scientific advisor
- Christian Rose: Agios – study investigator
- D Mark Layton: Agios – study investigator
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- Bruce Silver, Marvin Cohen: Agios – consultants
- Bertil Glader: Agios – study co-principal investigator and scientific advisor
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Pyruvate kinase (PK) deficiency: a severe congenital anemia

**Description**
- Presents in childhood with severe hemolytic anemia

**Etiology**
- Caused by mutations in the *PK-LR* gene coding for erythrocyte pyruvate kinase (PK-R)

**Disease Burden**
- Lifelong hemolytic anemia
- Iron overload and jaundice
- Infection risk post-splenectomy

**Diagnosis/Treatment**
- PK-R enzyme activity and/or genetic testing
- Supportive treatment: transfusions, splenectomy, iron chelation

**Type of PK-LR mutations found in 74 unrelated cases enrolled in the PK deficiency natural history study**
- Missense/missense 53%
- Missense/non-missense 25%
- Non-missense/non-missense 22%

AG-348: allosteric activator of wild-type and mutant PK-R

Active PK-R is a tetramer; mutations (green) decrease the catalytic activity

AG-348 (yellow) binds at the PK-R dimer-dimer interface, away from the active site and the most common mutations

AG-348 activates mutant PK-R in blood from PK deficient patients

Kung C et al. 55th ASH Annual Meeting 2013, Abstract 2180; Kung C et al. 56th ASH Annual Meeting 2014, Abstract 4010

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DMSO AG-348 2 μM

Study design

Open-label, global phase 2 study: 14 centers in the US, Canada, and EU

Transfusion-independent PK-deficient adults
(ClinicalTrials.gov NCT02476916) n=25 in each arm

Randomization
Stratified by PK-R genotype (none excluded)

Arm 1
300 mg BID

Arm 2
50 mg BID

6 month core dosing period

Assessment points (weeks)
1 2 3 6 9 12 16 20 24

Primary endpoints:
- Safety and tolerability

Secondary endpoints:
- Pharmacokinetics of AG-348
- PD response: ATP, 2,3-DPG
- Indicators of clinical activity: hemoglobin, reticulocyte count, and other hematologic parameters

Extension arm

Transfusion independence = no more than 3 units of red blood cells transfused in 12 months prior to the first day of study dosing and no transfusions within 4 months of first day of study dosing

All patients provided written informed consent.
BID = twice daily; PD = pharmacodynamic
The study was initiated in June 2015; data cut-off 27 March 2016
18 patients have been treated, with no early discontinuations
Three patients have completed the 24-week core period
  - Two remain on treatment in the extension arm

Demographics and disposition

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>50 mg BID, n=9</th>
<th>300 mg BID, n=9</th>
<th>Total, N=18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Men/Women, n</td>
<td>7/2</td>
<td>5/4</td>
<td>12/6</td>
</tr>
<tr>
<td>Age in years, mean (range)</td>
<td>25.9 (19–41)</td>
<td>35.3 (22–61)</td>
<td>30.6 (19–61)</td>
</tr>
<tr>
<td>Racea white, n</td>
<td>8</td>
<td>9</td>
<td>17</td>
</tr>
<tr>
<td>Hemoglobin baseline, g/dL, mean (SD)</td>
<td>10.0 (1.5)</td>
<td>8.5 (1.6)</td>
<td>9.3 (1.7)</td>
</tr>
<tr>
<td>Duration of treatment, weeks, median (range)</td>
<td>10.7 (3.0–24.4)</td>
<td>10.9 (3.0–24.0)</td>
<td>10.8 (3.0–24.4)</td>
</tr>
<tr>
<td>Splenectomized, n</td>
<td>6</td>
<td>7</td>
<td>13</td>
</tr>
</tbody>
</table>

aNot reported in 1 subject
Safety and adverse event (AE) summary

- AG-348 was generally well tolerated; the majority of AEs were grade 1–2
  - One subject received a dose reduction due to rapidly increasing hemoglobin
  - One grade 2 ‘allergic reaction’, successfully re-challenged with lower dose
  - One grade 2 AE of osteoporosis has been reported since the cut-off date
- No significant changes in ECGs and clinical safety laboratory parameters

<table>
<thead>
<tr>
<th></th>
<th>50 mg BID n=9</th>
<th>300 mg BID n=9</th>
<th>Total N=18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subjects experiencing at least 1 AE, n</td>
<td>7</td>
<td>8</td>
<td>15</td>
</tr>
<tr>
<td>Most frequent AEs (≥3 subjects), n</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>4</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Headache</td>
<td>2</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Hot flush</td>
<td>1</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Insomnia</td>
<td>0</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Subjects experiencing at least 1 drug-related AE, n</td>
<td>6</td>
<td>7</td>
<td>13</td>
</tr>
<tr>
<td>Subjects experiencing at least 1 serious AE</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Subjects experiencing at least 1 grade ≥3 AE&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Subjects experiencing at least 1 grade ≥3 drug-related AE</td>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

AEs were graded using National Cancer Institute Common Terminology Criteria, version 4.03
<sup>a</sup>Two grade 3 events (unrelated hypertension, related hypertriglyceridemia)
Effect of AG-348 on sex steroids

- Sex steroids were assessed at baseline, week 12 and week 24
- An upward trend in free testosterone and a downward trend in estradiol were observed in male patients

Hormone levels in male patients with available data

Normal reference low and high limits for men shown as horizontal dotted lines
Clinical Activity Results
Maximum change in hemoglobin (Hb) in the 50 and 300 mg BID dose groups

- 9 of 18 patients had an increase in Hb >1.0 g/dL
Hb increases are rapid and sustained

- Median time to a Hb increase >1.0 g/dL was 1.9 weeks (range, 1.1–9.1 weeks)
- In patients who had Hb increases >1.0 g/dL:
  - The mean maximum increase was 3.4 g/dL (range, 2.3–4.9 g/dL)

N=18, each line represents an individual patient
Genotype profile and Hb change

- Across all genotypes, 9 of 18 patients had an increase in Hb >1.0 g/dL
- Of 13 patients with at least one missense mutation, 9 had an increase in Hb >1.0 g/dL

![Graph showing genotype profile and Hb change](image-url)
Pharmacodynamic Results
Effect of AG-348 on pharmacodynamic marker 2,3-DPG

- Levels of 2,3-DPG and ATP in whole blood assessed at baseline and weeks 2, 3, 6, 9, 12, 16, 20 and 24
- No discernible effect on ATP observed
- More data are needed to clarify if any correlation exists between 2,3-DPG decreases and Hb increases >1.0 g/dL

![Graph showing change in 2,3-DPG concentration over time for 50 mg BID and 300 mg BID dosages.](image-url)
DRIVE-PK conclusions

- AG-348 is a novel, first-in-class, PK-R activator in clinical testing as a disease-altering therapy to restore metabolic function in patients with PK deficiency
- Daily dosing with AG-348 for up to 6 months is well tolerated
- AG-348 demonstrates proof-of-concept with rapid and sustained Hb increases in patients with PK deficiency
- Preliminary genotype correlations were observed:
  - Patients with at least one missense mutation are more likely to have a Hb increase of >1.0 g/dL
  - Non-missense/non-missense genotypes have not shown increases in Hb >1.0 g/dL
Acknowledgments

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AG-519 does not demonstrate off-target aromatase inhibition or effect steroid hormones

Male subjects receiving AG-519 375 mg BID. LLQ, lower limit of quantification; dotted red lines indicate reference range upper and lower limits.