A Drug-Drug Interaction Study to Investigate the Effect of Givosiran on the activity of 5 major drug metabolizing CYP450 enzymes in Subjects with Acute Intermittent Porphyria (AIP) who are Chronic High Excreters (CHE)

Daphne Vassiliou¹, Eliane Sardh¹, Pauline Harper¹, Nader Najafian², Amy Simon², Amy Burke², Jae Kim², Pushkal Garg², Gabriel Robbie², Sagar Agarwal²

¹Porphyria Centre Sweden, Centre for Inherited Metabolic Disorders, Karolinska University Hospital, Karolinska Institutet, Sweden; Alnylam Pharmaceuticals²
Givosiran: Investigational RNAi Therapeutic for AHP

Therapeutic Hypothesis

- Reduction of Liver ALAS1 Protein to Lower ALA and PBG

ALA induces porphyria symptoms

Givosiran results in reduction of ALAS1 and lowers ALA/PBG production to prevent attacks and disease symptoms
Givosiran Has Potential to Impact Activity of Drug Metabolizing Enzymes

- Givosiran inhibition of ALAS1 mRNA, the first and rate limiting enzyme in heme biosynthesis pathway in liver, could potentially lower hepatic heme content

- Givosiran does not impact heme biosynthesis in bone marrow which is controlled by ALAS2; givosiran does not inhibit ALAS2

- Lowered hepatic heme levels could reduce activity of heme-dependent proteins in liver such as drug metabolizing cytochrome P450 (CYP450) enzymes
  - Majority of hepatic heme is incorporated in CYP450 enzymes

- Results from in vivo monkey studies were inconclusive but suggested potential impact on CYP3A4 activity
Pharmacokinetic Drug-Drug Interactions

- CYP450s are the major family of drug metabolizing enzymes
  - 5 enzymes (CYP 3A4, 2C19, 1A2, 2C9, 2D6) metabolize ~80% of clinically used drugs\(^1\)
  - Age, sex, ethnicity, genetic polymorphisms, and disease influences activity
- Pharmacokinetic (PK) drug-drug interaction (DDI) important for drugs metabolized by CYP450s
  - Increased CYP450 activity can decrease drug exposure → potential altered effectiveness
  - Reduced CYP450 activity can increase drug exposure → potential altered safety profile
- Polypharmacy common in AHP patients due to multiple comorbidities, such as chronic pain, depression and hypertension

\(^1\) Zanger UM. Pharmacol Ther. 2013 Apr;138(1):103-41.
Design of Givosiran DDI Study

- Inje cocktail used to simultaneously evaluate effect of givosiran on 5 major CYP450 enzymes\(^1\)
- CHE subjects have relevant enzyme defect and elevated ALAS1/ALA/PBG enabling evaluation of givosiran pharmacodynamics and are on less medications than AHP patients with attacks
- Statistical analysis determined 10 subjects sufficient to detect a significant change in exposure of probe substrates
- Sequential (each patient their own control) study in 10 CHE subjects
- Dose of Inje cocktail on Day 1 (Baseline) and Day 36 (post-givosiran)
  - 2.5 mg/kg single dose of givosiran on Day 8
  - 28-day window post givosiran administration to enable maximal ALAS1/ALA reduction

**Inje Cocktail Components:**
- 5 mg Midazolam (CYP3A4)
- 40 mg Omeprazole (CYP2C19)
- 200 mg Caffeine (CYP1A2)
- 50 mg Losartan (CYP2C9)
- 30 mg Dextromethorphan (CYP2D6)

Clinical Trials: NCT03505853
### Subject Disposition and Demographics

<table>
<thead>
<tr>
<th>Patients Enrolled</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients Completed</td>
<td>9 (1 discontinued treatment due to non-safety reasons)</td>
</tr>
<tr>
<td>Age, median (range)</td>
<td>49 years (39-59)</td>
</tr>
<tr>
<td>Sex</td>
<td>7 Females, 3 Males</td>
</tr>
<tr>
<td>Race</td>
<td>Caucasian</td>
</tr>
<tr>
<td>Baseline ALAS1 Levels</td>
<td>2.13$^1$</td>
</tr>
<tr>
<td>Baseline ALA Levels$^2$</td>
<td>7.14 mmol/mol Cr</td>
</tr>
<tr>
<td>Baseline PBG Levels$^2$</td>
<td>14.6 mmol/mol Cr</td>
</tr>
<tr>
<td>CYP2C9 Phenotype</td>
<td>Normal (n = 6), Intermediate Metabolizers (n = 4)</td>
</tr>
<tr>
<td>CYP2C19 Phenotype</td>
<td>Normal (n = 8), Intermediate Metabolizers (n = 2)</td>
</tr>
<tr>
<td>CYP2D6 Phenotype</td>
<td>Normal (n = 6), Intermediate Metabolizers (n = 4)</td>
</tr>
</tbody>
</table>

---

$^1$ Ratio relative to healthy subjects

$^2$ Upper Limit of Normal: ALA=1.5 mmol/mol Cr; PBG=0.14 mmol/mol Cr determined based on samples collected from 150 normal healthy subjects analyzed by LC-MS/MS. LC-MS/MS assay performed at a central laboratory (Covance, Utah).
• Maximum PD effect of givosiran was achieved by Day 36, when DDI was evaluated
  - Urine ALAS1 mRNA levels lowered by 64% compared to baseline, urine ALA levels lowered by 89% and PBG levels lowered by 93% compared to baseline
  - Residual ALAS1 mRNA, ALA and PBG post-givosiran dosing similar to levels achieved in AHP patients with attacks

Ratio of 1 for ALAS1 represent healthy levels since ALAS1 levels in urine are normalized to healthy subject levels
Upper Limit of Normal (ULN) for ALA = 1.47 and PBG = 0.137 based on determination of levels in healthy subjects
Chan A. Mol Ther Nucleic Acids. 2015 Nov 3;4:e263.
Impact of Givosiran Treatment on CYP450 Enzyme Activity

Solid lines are baseline and dotted lines are post-givosiran treatment.

Givosiran Treatment had Variable Impact on CYP450 Enzyme Activity

- Moderate inhibitory effect on CYP1A2 and 2D6
  - Caffeine AUC increased 3.1-fold and dextromethorphan AUC increased 2.4-fold after givosiran treatment

- Weak inhibitory effect on CYP3A4 and 2C19
  - Midazolam and omeprazole AUC increased 1.6-fold and 1.5-fold, respectively, after givosiran treatment

- No effect on CYP2C9
  - Losartan AUC was unchanged after givosiran treatment

FDA/EMEA guidance: moderate inhibitors increase the AUC ≥2 to <5-fold; weak inhibitors increase AUC ≥1.25 to <2-fold
Medications Commonly Used in Symptomatic AHP Patients

- Highest impact of DDI may be on drugs primarily metabolized by CYP2D6
  - CYP2D6 metabolized drugs typically titrated slowly given genetic polymorphisms common in population, leading to extensive variation in CYP2D6 activity (e.g. 50-fold variability in tricyclic antidepressant levels)
- Very few drugs metabolized by CYP1A2
- Minor impact on CYP3A4 and CYP2C19 is unlikely to be clinically significant/relevant
Summary

• Givosiran treatment had a variable impact on CYP450 enzyme activity
  o Moderate reduction in activity of CYP1A2 and CYP2D6
  o Weak reduction in activity of CYP2C19 and CYP3A4
  o No effect on activity of CYP2C9

• Patients on drugs with a narrow therapeutic index primarily metabolized by CYP2D6 or CYP1A2 may need to be monitored more frequently to determine if dose adjustment of a concomitant medication is required
We would like to acknowledge Dr Marika Kvarnström, RN Caroline Bäck, and the staff at Karolinska Trial Alliance for expert assistance in accomplishing this study.

This study would not have been possible without the collaboration of the Porphyria community. We are very grateful to the study patients for the time and effort they invested in this project.

**Funding:** This study was sponsored by Alnylam Pharmaceuticals.
Impact of Givosiran Treatment on CYP450 Enzyme Activity

- Baseline concentration-time profiles comparable between CHE subjects and healthy subjects, suggesting that CYP450 enzyme activity is consistent between CHE subjects and healthy controls (grey line)