A Randomized, Placebo Controlled, Phase 1 Study of ALN-AS1, an Investigational RNAi Therapeutic for the Treatment of Acute Hepatic Porphyrias

Interim presentation of Safety and Pharmacodynamic Results

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Acute Hepatic Porphyrias Pathophysiology

**Acute Hepatic Porphyrias (AHP)**
Inborn errors of heme synthesis resulting from enzyme defects in the liver

- **Acute Intermittent Porphyria (AIP)**
  - Most common AHP: prevalence 2-5 per 100,000, ~5-10% with manifest disease
  - Autosomal dominant mutation in the HMBS (PBGD) gene: 50% of activity

- **Disease Pathophysiology**
  - ALA synthase (ALAS1) is induced, leading to accumulation of toxic heme intermediates ALA and PBG that cause nerve damage and acute attacks

- **Affecting**
  - Central Nervous System (seizures, PRES)
  - Autonomous Nervous System (abdominal pain, hypertension, tachycardia)
  - Peripheral Nervous System (muscle weakness, paralysis)

- **Treatment**
  - Human hemin
ALN-AS1: RNAi Therapeutic Hypothesis
Knockdown of Liver ALAS1 Protein to Reduce ALA/PBG

ALAS1 protein 

ALA/PBG induce porphyria symptoms

ALAS1 protein

ALN-AS1 knockdown of ALAS1 reduces ALA/PBG production and prevents attacks

ALN-AS1

ALAS1 siRNA

Liver targeting ligand

Sardh: A Randomized, Placebo Controlled, Phase 1 Study of ALN-AS1, Sep 07 2016
ALN-AS1 Phase 1 Study: AIP patient populations

Asymptomatic High Excreters (ASHE)
• Persistently elevated ALA/PBG
• More clinically relevant than healthy volunteers
• Ability to measure key biomarkers
• Medically stable patient population for initial safety evaluation (studied in prior enzyme replacement trial)

Recurrent attack patients
• Highest unmet medical need
• Evaluate safety and dose regimen in small subset of patients

Studies A and B (SAD/MAD) in ASHE patients

**Study Design**
- Randomized, single-blind, placebo-controlled *single and multiple ascending dose* study in ASHE patients

**Primary Objective**
- Safety and tolerability of ALN-AS1

**Secondary Objectives**
- Characterize ALN-AS1 pharmacokinetics (PK) and pharmacodynamics (PD), i.e. ALA and PBG lowering

**Exploratory Objectives**
- Characterize circulating ALAS1 mRNA from the liver in urine and serum using a circulating RNA detection (cERD) assay

Part C (MD) in recurrent attack patients

**Study Design**
- Placebo-controlled *multiple dose study* in recurrent attacks patients

**Primary Objective**
- Safety and tolerability of ALN-AS1

**Secondary Objectives**
- Characterize ALN-AS1 PK and PD

**Exploratory Objectives**
- Clinical activity of ALN-AS1 on attack characteristics and treatment, and patient quality of life
- Characterize circulating ALAS1 mRNA from the liver in urine and serum
ALN-AS1 Phase 1 Study: Key Eligibility Criteria

**Part A and B Inclusion**
- Male or female, ages 18-65 years
- Acute intermittent porphyria (AIP), with genetic diagnosis of HMBS mutation
- Urine PBG > 4 mmol/mol creatinine at screening

**Part A and B Exclusion**
- Attack* within 6 months of screening
- Heme use in past 6 months
- Subjects with new prescription medication regimen within 3 months of screen

**Part C Only Inclusion**
- Experienced at least 2 porphyria attacks in past 6 months or on heme prophylaxis to prevent attacks
- If on heme prophylaxis, willing to stop during study

*Attack definition: intense abdominal or back pain requiring hospitalization, heme use or treatment consisting of increased carbohydrate intake or pain medication
**ALN-AS1 Phase 1 Study Progress**

**Part A: Single-Ascending Dose (SAD)** | Randomized 3:1, Single-blind, Placebo-controlled in ASHE

- 0.035 mg/kg x 1 SC, N=4
- 0.10 mg/kg x 1 SC, N=4
- 0.35 mg/kg x 1 SC, N=4
- 1.0 mg/kg x 1 SC, N=4
- 2.5 mg/kg x 1 SC, N=4

*The 0.035 mg/kg SAD cohort was dosed after the 0.10 and 0.35 mg/kg cohorts.

**Part B: Multiple-Ascending Dose (MAD)** | Randomized 3:1, Single blind, Placebo-controlled in ASHE

- 0.35 mg/kg, qMx2 SC, N=4
- 1.0mg/kg, qMx2 SC, N=4

**Part C: Multiple-Dose (MD)** | Randomized 3:1, Double-blind, Placebo-controlled in AIP patients with recurrent attacks

- Run-in Observation (1 to 6 months)
- Cohort 1 N=4
- Cohort 2 N=4
- Cohort 3 N=4

ongoing
### ALN-AS1 Phase 1 Study Part A and Part B
Demographics and Baseline Disease Characteristics

<table>
<thead>
<tr>
<th>Part A and B</th>
<th>Characteristic</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of Patients</td>
<td>N=23* (ALN-AS1:Placebo=21:7)</td>
</tr>
<tr>
<td></td>
<td>Median Age (range)</td>
<td>47 years (30-64)</td>
</tr>
<tr>
<td></td>
<td>Gender</td>
<td>18 Females, 5 Males</td>
</tr>
<tr>
<td></td>
<td>Race n</td>
<td>22 White/Caucasian, 1 Asian</td>
</tr>
<tr>
<td></td>
<td>Genotype (n)</td>
<td>8 different mutations identified**:&lt;br&gt;• HMBS_593G&gt;A (13)&lt;br&gt;• HMBS_87+1G&gt;A (4)&lt;br&gt;• HMBS_499-1G&gt;A (1)&lt;br&gt;• HMBS_517C&gt;T (1)&lt;br&gt;• HMBS_647G&gt;A (1)&lt;br&gt;• HMBS_847_848delTG (1)&lt;br&gt;• 673C&gt;T variant exon 11 (1)&lt;br&gt;• Exon 3 shift IVS3+1G&gt;T (1)</td>
</tr>
<tr>
<td></td>
<td>Mean baseline ALA (range)</td>
<td>11.0 mmol/mol Cr (2.9-24.6) ^</td>
</tr>
<tr>
<td></td>
<td>Mean baseline PBG (range)</td>
<td>22.0 mmol/mol Cr (4.5-50.5) ^</td>
</tr>
</tbody>
</table>

*5 subjects had >1 treatment assignment: 2 subjects repeated Part A; 3 subjects enrolled in Part A and Part B<br>** 3 mutations added to database post data cut and W198X collapsed into HMBS_593G>A

Data in database as of 28 Jun 2016

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Part A (SAD) and Part B (MAD) Safety and Tolerability

ALN-AS1 was generally well-tolerated in Parts A and B of Study ALN-AS1-001

No drug-related SAEs or discontinuations due to AEs

- Two SAD subjects (0.035 and 0.10 mg/kg dose groups) were hospitalized for SAE of “abdominal pain”. Both events were assessed as unlikely related to ALN-AS1 by the investigators
- One MAD subject (1 mg/kg dose group) experienced a miscarriage 7 weeks post-conception (90 days post-ALN-AS1) during the extended follow-up period which was assessed as unlikely related by the investigator

SAD: Total of 49 AEs reported (10 AEs in 5 PBO subjects; 39 AEs in 11 ALN-AS1 subjects)

- All AEs were mild or moderate in severity with the exception of one severe AE of abdominal pain (same subject noted above with SAE at 0.10 mg/kg dose).
- AEs reported in ≥2 subjects were abdominal pain, diarrhea, and hypoesthesia
- 8 related or possibly related AEs were reported in 5 subjects
  - Diarrhea, dyspepsia, hematochezia, hypoesthesia, injection site erythema, injection site pain, decreased GFR and elevated Cr
- Injection site reactions (erythema and pain) were seen in 2 subjects—both mild and transient

MAD: Total of 29 AEs reported (4 AEs in 1 PBO subject; 25 AEs in 6 ALN-AS1 subjects)

- All reported AEs were mild or moderate in severity
- AEs reported in ≥2 subjects were nasopharyngitis and rash
- 8 related or possibly related AEs were reported in 3 subjects
  - Pruritus only (1 subject), rash only (1 subject) and pruritus and rash (1 subject)
- No injection site reactions were reported

No clinically significant changes in vital signs, EKG, clinical laboratory or physical examination

*All Safety Data in database as of 28 Jun 2016 with the exception of the MAD SAE which was as of 03 Sept 2016*
ALN-AS1 Phase 1 Study Interim Results
SAD Pharmacodynamic Data: Serum ALAS1 mRNA by cERD

ALAS1 mRNA induced approximately 3-fold in ASHE compared to normal healthy volunteers

Rapid, dose-dependent, and durable ALAS1 mRNA lowering after single dose
• 64 ± 1% mean (SEM) maximal reduction in 2.5 mg/kg dose group
• Remaining ALAS1 mRNA levels after highest dose similar to levels in normal healthy individuals

Data in database as of 28 Jun 2016
ALN-AS1 Phase 1 Study Interim Results
SAD Pharmacodynamic Data: Urinary ALA and PBG

Rapid, dose-dependent, and durable ALA and PBG lowering after single dose
- Mean (SEM) maximal reduction in 2.5 mg/kg group: 86± 2% (ALA) and 95± 0.4% (PBG)
- Prolonged ALA and PBG lowering with single dose supporting monthly or quarterly dosing
- Normalization of ALA/PBG achieved at higher dose levels

Excluding subject 201-0002, Day 0: 0-6 hour ALA measurement

Data in database as of 28 Jun 2016

Data Assay URL: < 3.9 or 3.8 mmol/mol Cr at sites 101 or 201
PBG Assay URL: < 1.6 or 1.5 mmol/mol Cr at sites 101 or 201
ALN-AS1 Phase 1 Study Interim Results
SAD: Changes in Serum ALAS1 mRNA and Urinary ALA/PBG Highly Correlated

**ALAS1 mRNA vs ALA**

- Percent Change Normalized ALAS1 mRNA (SERUM)
- Regression Line
- SAD Placebo
- 0.035 mg/kg ALN-AS1
- 0.10 mg/kg ALN-AS1
- 0.35 mg/kg ALN-AS1
- 1.0 mg/kg ALN-AS1
- 2.5 mg/kg ALN-AS1

R² = 0.79, p<0.001

**ALAS1 mRNA vs PBG**

- Percent Change Normalized ALAS1 mRNA (SERUM)
- Regression Line
- SAD Placebo
- 0.035 mg/kg ALN-AS1
- 0.10 mg/kg ALN-AS1
- 0.35 mg/kg ALN-AS1
- 1.0 mg/kg ALN-AS1
- 2.5 mg/kg ALN-AS1

R² = 0.87, p<0.001

Data in database as of 28 Jun 2016

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ALN-AS1 Phase 1 Study Interim Results
MAD Pharmacodynamic Data: Serum ALAS1 mRNA by cERD

Rapid, dose-dependent, and durable ALAS1 mRNA lowering
• 54 ± 2% mean (SEM) maximal reduction relative to baseline in 1.0 mg/kg dose group
• ALAS1 mRNA reduction seen with 2 doses similar to single dose

Data in database as of 28 Jun 2016
Rapid, dose-dependent, and durable ALA and PBG lowering after multiple doses

- Mean (SEM) maximal reduction in 1 mg/kg group: 84 ± 2% (ALA) and 89 ± 5% (PBG)
- Multiple doses without additive effect in either dose group
- Normalization of ALA/PBG achieved at higher dose levels

**ALN-AS1 Phase 1 Study Interim Results**

**MAD Pharmacodynamic Data: Urinary ALA and PBG**

**ALA**

- MAD Placebo (N=2)
- 0.35 mg/kg ALN-AS1 (N=3)
- 1.0 mg/kg ALN-AS1 (N=3)

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>ALA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>1/2</td>
</tr>
<tr>
<td>0.35</td>
<td>1/3</td>
</tr>
<tr>
<td>1.0</td>
<td>3/3</td>
</tr>
</tbody>
</table>

**PBG**

- MAD Placebo (N=2)
- 0.35 mg/kg ALN-AS1 (N=3)
- 1.0 mg/kg ALN-AS1 (N=3)

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>PBG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>0/2</td>
</tr>
<tr>
<td>0.35</td>
<td>0/2</td>
</tr>
<tr>
<td>1.0</td>
<td>2/3</td>
</tr>
</tbody>
</table>

**Data in database as of 28 Jun 2016**

**ALA Assay URL:** < 3.9 or 3.8 mmol/mol Cr at sites 101 or 201

**PBG Assay URL:** < 1.6 or 1.5 mmol/mol Cr at sites 101 or 201
ALN-AS1 Phase 1 Study Initial Results

Summary

ALN-AS1 generally well tolerated with single or multiple (2) doses
- No drug-related SAEs or discontinuations due to AEs
- No dose-dependent AEs or clinically significant changes in vital signs, EKG, clinical laboratory or physical examination

Non-invasive cERD assay to quantify liver ALAS1 mRNA expression demonstrated
- ASHE have 3-fold ALAS1 mRNA induction compared to normal healthy individuals
- Rapid, dose-dependent, and durable ALAS1 mRNA lowering with single and multiple doses of ALN-AS1; highly correlated with changes in ALA and PBG
  - 64% with a single 2.5 mg/kg dose and 54% with multiple 1.0 mg/kg doses

Rapid, dose-dependent, and durable lowering of urinary ALA and PBG with single and multiple doses of ALN-AS1
- 86% and 95%, respectively, with a single 2.5 mg/kg dose
- 84% and 89%, respectively with multiple 1.0 mg/kg doses

Next Steps
- Part A/B, (SAD/MAD) continue to monitor ALA, PBG and ALAS1 recovery
- Part C, MD portion in recurrent attack patients ongoing in Sweden, UK and US

Data in database as of 28 Jun 2016
Acknowledgements

Phase 1 Investigators

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- Rena Denoncourt

The AIP patients who participated In this Phase 1 Study

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