Pre-clinical Development of ALN-AS1 RNAi Therapeutic for the Treatment of Acute Intermittent Porphyria
Acute Intermittent Porphyria (AIP) Program
Unmet Need and Product Opportunity

AIP is autosomal dominant disorder
- Loss-of-function mutations in porphobilinogen deaminase, key step in heme synthesis
- Ultra-rare orphan disease
  - ~5,000 Patients with annual attacks US/Europe
  - ~500 Patients with recurrent attacks US/Europe

High unmet need and cost
- Patients present with acute or recurrent attacks
  - Hospitalization with severe abdominal pain, peripheral and autonomic neuropathy, and neuropsychiatric symptoms
- Limited treatment options
  - Blood-derived hemin given centrally
    - Slow onset, severe thrombophlebitis, iron overload, resistance, and risk of liver cancer
  - No prophylactic treatment to prevent attacks
    - Often monthly in women associated with menses
Therapeutic Hypothesis
Targeting Key Trigger of Toxic Heme Intermediate Production

Neurotoxicity
- Altered GABA Signaling
- Iron mediated oxidation and ROS production

Brennan et al., Nature; 280:514-515 (1979)
Pharmacologic validation of ALAS-1 as target

- ALAS-1 mRNA strongly upregulated during attack
- Panhematin down modulates ALAS-1
- Addition of heme to liver cells in culture leads to reduced ALAS-1 mRNA

Liver targeting of ALAS-1 should be sufficient

- Liver transplant is curative
- Domino transplants induce symptoms
- Liver derived metabolites drive attacks

Dar et al., Hepatobiliary Pancreat Dis Int.; 9:93-6 (2010)
Wu et al., Genes Dev; 23:2201-2209 (2009)
RNAi Therapeutic Targeting ALAS-1
Potential Use for Prophylaxis and Acute Treatment

**Recurrent Attack Setting (Prophylaxis)**

- Chronic suppression will blunt recurrent ALAS-1 upregulation that drives attacks
- Will result in chronic suppression of ALA/PBG
- Yields reduction in number and severity of attacks

**Acute Attack Setting (Treatment)**

- Halt flux through pathway
- Quick reduction in ALA/PBG
- Rapid improvement in clinical symptoms
Rodent Models of AIP

Mouse AIP Model
- PBGD compound heterozygous KO
- ~30% Residual PBGD activity
- ~2x increase in basal ALA/PBG
- ~30-100X increase in ALA/PBG following qd x 3-4 phenobarb (PB)
- Older animals have axonal degeneration and impaired motor function

Rat AIP Model (in house)
- Transient PBGD siRNA KD in liver
- ~15% Residual PBGD mRNA
- 10-50x increase in ALA/PBG following qd x3 phenobarb induction

siRNA Inhibits Induction of ALAS-1 and ALA/PBG in Mouse AIP Model

Prophylaxis

Control or ALAS-1 siRNA 1.0 mg/kg

PB (IP x 3)

Liver-ALAS mRNA and Protein

Day 1  Day 2  Day 3  Day 4  Day 5

Plasma-ALA/PBG (LC-MS)

ALAS-1 mRNA

ALAS Protein

Plasma ALA/PBG

Urinary ALA/PBG

Relative ALAS1 mRNA Levels

β-actin

siRNA: Ctrl  ALAS1

PB: wt/wt  AIP

ALA

PBG

Urinary ALA or PBG (umol/l)

ALA

PBG

Urinary ALA or PBG (umol/l/mg creatinine)
Metabolite Inhibition is Durable Prophylaxis

ALAS-1 siRNA 1mg/kg
PB IP x 3 Plasma-ALA/PBG

Week 0

Week 2

Week 4

Plasma ALA or PBG (μmol/L)

Control

Week 0

Week 2

Week 4
Faster Onset of Action with siRNA vs. Heme Treatment

PB + DDC IP x 3

Heme or siRNA

D1

D2

D3

D4

Plasma-ALA/PBG (LC-MS)

Int'l Congress of Porphyrins and Porphyrias, May 2013; Mount Sinai Collaboration
Monitoring Liver RNAi Activity with cERD

“Circulating Extracellular RNA Detection”

- RNAi activity in liver typically monitored through:
  - mRNA or 5’RACE product in tissue
  - Circulating secreted protein
- Serial biopsies required to monitor ALAS-1 kinetics in NHP
- Exosomes are shed into bodily fluids from many different cell types and contain mRNA and miRNA derived from tissue of origin
- Exosomes can be used to monitor RNAi mechanism in circulation
  - Important when no secreted biomarker exists

Serum from treated animals

↓ Filtration with low speed spin

↓ Spin at 160,000g, for 2 hours to pellet

↓ Extract RNA and analyze ALAS-1 levels by qPCR
cERD Correlates with Liver mRNA Data

Results with ALAS-1 GalNAc-siRNA in NHP

Study Day:

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ALAS-1 Transcript
- by cERD
- by liver biopsy

ALAS-1 GalNAc-siRNA Dosing

ALAS-1 GalNAc-siRNA (mg/kg)

- PBS
- 1.25 mg/kg
- 2.5 mg/kg
- 5.0 mg/kg

Normalized ALAS-1

Normalized ALAS-1 (Fraction Pre-dose)

Days

PBS
1.25 mg/kg
2.5 mg/kg
5.0 mg/kg
ALN-AS1 Development Candidate Shows Robust Efficacy
Rat Model of AIP

ALN-AS1 (BiW) 2.5 or 5 mg/kg

Phenobarb (PB)

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<th>D14</th>
<th>D18-19 (urine)</th>
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<td>D7</td>
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<td>D18-19 (urine)</td>
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Rat Urine PBG (mmol/mol Creatinine)
Relative To PBS=1

Rat Urine ALA (mmol/mol Creatinine)
Relative To PBS=1

PBS PBS 2.5 PBS 5.0

PBS PBS 2.5 PBS 5.0

+ PB

+ PB
ALN-AS1 Program
Initial Clinical Development Plan (CDP)*

Phase I study in “high excreter” AIP patients
- Asymptomatic AIP patients with elevated plasma ALA and PBG
- Randomized, double-blind, placebo-controlled, multi-dose, dose-escalation study
  » ~20 Patients
- Demonstrate decreased levels of plasma ALA and PBG

Phase II/III study in AIP patients with recurrent attacks
- Cross-over study design
  » <50 Patients
- Demonstrate reduced frequency and severity of AIP attacks

*Subject to change
Alnylam is developing ALN-AS1 for treatment of AIP and other hepatic porphyrias

- Generated pre-clinical proof of concept for targeting ALAS-1 with an RNAi therapeutic
  - Blunted ALAS-1 mRNA up-regulation in liver and impaired ALA/PBG production in rodent models
  - Effective with prophylaxis or acute treatment
- Identified ALN-AS1, a GalNAc-siRNA conjugate, as Development Candidate
  - Potent suppression of ALAS-1 with subcutaneous dosing
  - Complete blunting of ALA/PBG up-regulation at doses ≤ 2.5 mg/kg
- Plan to file IND/CTA in 2014
- Rapid CDP focused on prophylaxis in recurrent attack patients with highest unmet need