ALN-TTR, an RNAi Therapeutic for the Treatment of Transthyretin-Mediated Amyloidosis

Rene Alvarez, Ph.D.
**RNAi to treat significant orphan disease**

- Transthyretin (TTR)-mediated amyloidosis (ATTR)
  - Caused by mutation in TTR gene
  - Amyloid deposits in nerves and heart
    - Familial Amyloid Polyneuropathy (FAP)
    - Familial Amyloid Cardiomyopathy (FAC)
  - ~50,000 patients with significant morbidity and mortality

- Clinical pathology
  - Typical onset ~40 to >60 yr
  - Fatal within 5-15 years
  - Loss of autonomic function, painful neuropathy
  - Congestive heart failure

- Liver transplant current standard-of-care for subset of FAP patients

- Initiate Phase I trial H1, 2010
RNA Interference

**Synthetic siRNA**

- dsRNA
- dicer
- Cleavage
- Strand separation
- Complementary pairing
- mRNA

**Reduced protein expression**

- Target-specific mRNA degradation
Unformulated siRNAs are rapidly cleared from circulation

Lipid nanoparticle formulations (SNALP) of siRNAs prolong half-life and enable hepatic delivery

Systemic administration of SNALP-formulated siRNAs results in

- Dose-dependent mRNA and protein suppression of hepatocyte-expressed disease targets
- Suppression maintained for 2 to 4 weeks
- Has been demonstrated in multiple species (rodents, non-human primate) for multiple targets (apoB, TTR, Factor VII, PCSK9)*

*Zimmerman et al., Nature, 2006; Akinc et al., Nature Biotech, 2008; Frank-Kamenetsky et al., PNAS, 2008
• Effective delivery of siRNA to hepatocytes with current LNP platform
  » Chemically modified TTR siRNA
  » Formulated in SNALP for systemic delivery
• Hepatocytes primary site of TTR expression
  » Mutant and wild-type TTR proteins pathogenic
    – Liver transplant can stabilize or improve V30M FAP patients
    – However, cardiac disease accelerates in other ATTR patients due to increased production of wild-type TTR
  » Production of both wild-type and mutant TTR ideally targeted
• Target well validated with human genetics
  » ~90% FAP caused by V30M mutation
  » FAC caused primarily by V122I mutation
ALN-TTR siRNA Selection

- >100 Mutations identified in TTR gene
- ALN-TTR targets region of TTR mRNA common to wild-type and all known mutant forms of TTR
Durable Suppression of TTR *In Vivo*

**Durable reduction of TTR mRNA with rodent TTR siRNA analog**
- Single i.v. infusion of rodent TTR analog or control siRNA; 1 mg/kg dose
- Liver mRNA levels measured on Days 3, 8, 11, 15, 19, 22, 25 and 29 post-dose

![Graph showing relative TTR mRNA levels](image)

- **p < 0.01, ***p < 0.001 (one-way ANOVA, Bonferroni post-hoc test)
ALN-TTR Reduces TTR mRNA
Non-Human Primates

ALN-TTR shows dose dependent silencing of TTR mRNA
- Single i.v. infusion of ALN-TTR or control siRNA
- Liver mRNA levels measured 48 hr post-dose

ED$_{50}$ ~ 0.3 mg/kg

*** p < 0.001
(one-way ANOVA, Dunn’s post-hoc test)
Durability of Reduction of Wild-Type TTR \textit{In Vivo} in NHP

<table>
<thead>
<tr>
<th>Control siRNA</th>
<th>3 mg/kg</th>
<th>0.3 mg/kg</th>
<th>1 mg/kg</th>
<th>3 mg/kg</th>
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<tbody>
<tr>
<td>ALN-TTR</td>
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Day | 0 | 1 | 2 | 3 | 4 | 5 | 7 | 10 | 14 
---|---|---|---|---|---|---|---|-----|-----

3 mg/kg
ALN-TTR Silences Mutant Human TTR
V30M TTR Transgenic Mouse Model

ALN-TTR silences human V30M TTR mRNA and suppresses mutant protein levels
- Single i.v. dose of ALN-TTR or control siRNA
- Liver mRNA and serum TTR levels measured 48 hr post-dose

Liver mRNA

| Control siRNA (mg/kg) | ALN-TTR (mg/kg) | TTR/GAPDH mRNA
|----------------------|----------------|------------------|
| 3                    | 0.03           | **p < 0.001** (one-way ANOVA, Dunn's post-hoc test)
| 0.3                  | 3              |

Serum Protein

| Control siRNA (mg/kg) | ALN-TTR (mg/kg) | TTR serum levels
|----------------------|----------------|------------------|
| 3                    | 0.03           | **p < 0.001** (one-way ANOVA, Dunn's post-hoc test)
| 0.3                  | 3              |

ED$_{50}$ ~ 0.15 mg/kg
ALN-TTR Silencing is Durable
V30M TTR Transgenic Mouse Model

ALN-TTR efficacy is both rapid and durable
- Single i.v. bolus of ALN-TTR or control siRNA; 1 mg/kg
- Liver mRNA and serum protein levels measured on Days 3, 8, 15 and 22 post-dose

<table>
<thead>
<tr>
<th></th>
<th>Control siRNA</th>
<th>Day 3</th>
<th>Day 8</th>
<th>Day 15</th>
<th>Day 22</th>
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<tr>
<td><strong>Liver mRNA</strong></td>
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<tr>
<td>TTR/GAPDH mRNA</td>
<td>1.6</td>
<td>1.2</td>
<td>0.8</td>
<td>0.4</td>
<td>0.2</td>
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<tr>
<td>(relative to Control siRNA)</td>
<td>1.0</td>
<td>1.4</td>
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<td><strong>Serum Protein</strong></td>
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![Liver mRNA and Serum Protein Graphs](Image)
Therapeutic Efficacy for ALN-TTR
V30M TTR Transgenic Mouse Model

ALN-TTR blocks pathogenic accumulation of mutant human TTR in peripheral tissues
- Multi-dose i.v. bolus of ALN-TTR or control siRNA, 3 mg/kg (d0, 14, 28)
- Quantitation of TTR deposition by immunohistochemistry on day 56

Collaboration with M. Saraiva
Therapeutic Efficacy for ALN-TTR
V30M TTR Transgenic Mouse Model

Marked reduction of mutant TTR in tissues associated with human disease
- Mutant V30M TTR quantified with immunohistochemistry
ALN-TTR Therapeutic Efficacy
V30M TTR Transgenic Mouse Model

ALN-TTR promotes regression of pathogenic mutant human TTR deposits in peripheral tissues

- >90% Regression of existing V30M hTTR tissue deposits
- Multi-dose IV bolus of ALN-TTR01 or control siRNA, 3 mg/kg (week 0, 2, 4, 6, 8, 10)
- Quantitation of TTR deposition by immunohistochemistry (week 11)

**Relative TTR Tissue Levels**

- Esophagus: 100% Control siRNA, 97% ALN-TTR
- Colon: 98.8% Control siRNA, 97% ALN-TTR
- Stomach: 98.5% Control siRNA, 98.8% ALN-TTR
- Sciatic nerve: 97% Control siRNA, 98.8% ALN-TTR
- Dorsal root ganglion: 98% Control siRNA, 98.8% ALN-TTR

Dorsal Root Ganglion

Control siRNA

ALN-TTR01
We have identified a lead siRNA targeting wild-type and all mutant forms of TTR with an IC50 of 3 pM in hepatocyte cell lines. ALN-TTR reduced TTR mRNA levels (ED50 ~ 0.3 mg/kg) in non-human primate, with significant reduction of TTR serum protein levels and durability of suppression >14 days post-single administration. ALN-TTR reduced mutant TTR mRNA and plasma protein levels > 90% in the hTTR V30M transgenic mouse model, with suppression lasting > 21 days post-single administration. ALN-TTR, when administered to hTTR V30M transgenic mice, prevents TTR deposition (young mice) and leads to regression (old mice) of pre-existing TTR deposits in key target tissues, including: dorsal root ganglia, sciatic nerve, stomach, and intestines. These findings demonstrate the potential therapeutic benefit of an RNAi therapeutic targeting TTR for the treatment of TTR-mediated amyloidosis (ATTR).