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**

**Natural Process of RNAi**

1. **dsRNA**
   - dicer
   - Cleavage
   - Strand separation
   - Complementary pairing
   - mRNA \((A)_n\)
   - Cleavage

2. **Synthetic siRNA**
   - Reduced protein expression
   - Target-specific mRNA degradation

**Synthetic siRNA**

**Natural Process of RNAi**
Systemic RNAi Delivery
Lipid Nanoparticles

- 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_url)

- **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

![Bar chart showing dose-dependent silencing of TTR mRNA](chart.png)

- **ED$_{50}$ ~ 0.3 mg/kg**
- *** p< 0.001 (one-way ANOVA, Dunn's post-hoc test)

TTR/GAPDH mRNA (Relative to Control)

<table>
<thead>
<tr>
<th>Control siRNA (mg/kg)</th>
<th>ALN-TTR (mg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0.3</td>
</tr>
<tr>
<td>47%</td>
<td>62%</td>
</tr>
</tbody>
</table>
Durability of Reduction of Wild-Type TTR *In Vivo* in NHP

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

<table>
<thead>
<tr>
<th>Control siRNA (mg/kg)</th>
<th>ALN-TTR (mg/kg)</th>
<th>TTR/GAPDH mRNA (relative to control siRNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0.03</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>0.0</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Serum Protein

<table>
<thead>
<tr>
<th>Control siRNA (mg/kg)</th>
<th>ALN-TTR (mg/kg)</th>
<th>TTR serum levels (relative to control siRNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0.03</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>0.0</td>
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<tr>
<td></td>
<td>3</td>
<td>0.2</td>
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</tbody>
</table>

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

Liver mRNA and Serum Protein levels over time:
- **Liver mRNA**:
  - Control siRNA: Day 3 (1.6), Day 8 (0.4), Day 15 (0.6), Day 22 (0.8)
  - ALN-TTR: Day 3 (0.2), Day 8 (0.1), Day 15 (0.1), Day 22 (0.1)

- **Serum Protein**:
  - Control siRNA: Day 3 (1.4), Day 8 (0.6), Day 15 (0.4), Day 22 (0.2)
  - ALN-TTR: Day 3 (0.2), Day 8 (0.4), Day 15 (0.6), Day 22 (0.8)
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

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control siRNA</th>
<th>ALN-TTR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dorsal Root Ganglion</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>Sciatic Nerve</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>Stomach</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
<tr>
<td>Intestine</td>
<td>![Image]</td>
<td>![Image]</td>
</tr>
</tbody>
</table>
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)
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).
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