Familial Support Group Meeting

Overview of ALN-TTR, a Novel RNAi Therapeutic for TTR Amyloidosis

October 29, 2011
Jared Gollob, M.D.
Agenda

- Background on RNAi and TTR Amyloidosis
- ALN-TTR Program: Preclinical Data
- Natural History Study of Serum TTR Levels
- ALN-TTR01 Phase 1 Study Design and Status
- Summary and Next Steps
RNAi Cellular Mechanism

Natural Process of RNAi

Synthetic siRNA

dsRNA

dicer

Cleavage

Strand separation

RISC

Complementary pairing

mRNA

(A)_n

Cleavage

(A)_n

Targeted Gene Silencing

mRNA degradation
Lipid Nanoparticles (LNPs) for Systemic RNAi

- Multi-component lipid formulation
  - Amino lipid
  - Structural lipid
  - PEG lipid
  - Cholesterol

- Highly efficient for liver delivery
  - Hepatocyte-specific gene silencing achieved

- Low surface charge
- Small uniform size particle <100 nm
• TTR synthesized predominantly in liver, circulates as ~55 KDa tetramer
• Binds and transports serum retinol binding protein (RBP)/vitamin A and minor fraction of serum thyroxine (T4)
• Knock-out mice have mild peripheral phenotype
  » Reduced levels of serum retinol, RBP, thyroid hormone without significant physiological effects
    – Normal vitamin A metabolism, total liver retinol unchanged (Wei et al., 1995)
    – Thyroid function normal (Palha et al., 1994)
  » Modest sensorimotor changes may represent developmental effect (Fleming et al., 2007)
Transthyretin (TTR) Amyloidosis

- Autosomal dominant disease
- Deposition of mutant and wild-type TTR in tissues outside of liver
  - Nerves and/or heart are main target organs depending on mutation
- Familial amyloidotic polyneuropathy (FAP)
  - Prevalence of ~10,000 cases worldwide
  - V30M most common mutation
- Familial amyloidotic cardiomyopathy (FAC)
  - Prevalence of ~40,000 cases worldwide
  - V122I most common mutation
- Liver transplant current standard of care for early stage V30M FAP
- Drugs in development
  - TTR tetramer stabilizers (tafamidis, diflunisal)
  - Agents targeting hepatic TTR synthesis (siRNA, ASO)
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• Summary and Next Steps
## ALN-TTR Summary

<table>
<thead>
<tr>
<th>Molecule</th>
<th>Formulation and Dosing</th>
<th>Molecular Hypothesis</th>
<th>Therapeutic Hypothesis</th>
<th>Potential Indications</th>
<th>Stage Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-TTR siRNA, modified duplex</td>
<td>Formulated in lipid nanoparticle</td>
<td>An RNAi therapeutic targeting TTR gene will inhibit mutant and wild type TTR production in the liver</td>
<td>Decreasing hepatic production of mutant and wild type TTR will decrease amyloid deposition in tissues and facilitate amyloid clearance from tissues, thereby halting progression or improving end-organ dysfunction</td>
<td>Familial amyloidotic polyneuropathy (FAP) Familial cardiac amyloidosis (FAC)</td>
<td>ALN-TTR01 Phase I trial initiated in July 2010; Advancing ALN-TTR02 with 2nd generation LNP</td>
</tr>
</tbody>
</table>
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
~140 siRNAs screened to identify lead candidate

- Assays performed in HepG2 cells
- TTR mRNA quantified by RT-PCR 24hr post-transfection

\[ IC_{50} = 3 \text{ pM} \]
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 48hr post-dose

**Liver mRNA**

<table>
<thead>
<tr>
<th>ALN-TTR (mg/kg)</th>
<th>Control siRNA (mg/kg)</th>
<th>TTR/GAPDH mRNA (relative to control siRNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0.03</td>
<td>1.6</td>
</tr>
<tr>
<td>0.3</td>
<td>0.3</td>
<td>1.4</td>
</tr>
<tr>
<td>3</td>
<td>0.3</td>
<td>1.0</td>
</tr>
</tbody>
</table>

**Serum Protein**

<table>
<thead>
<tr>
<th>ALN-TTR (mg/kg)</th>
<th>Control siRNA (mg/kg)</th>
<th>TTR serum levels (relative to control siRNA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>0.03</td>
<td>1.4</td>
</tr>
<tr>
<td>0.3</td>
<td>0.3</td>
<td>1.2</td>
</tr>
<tr>
<td>3</td>
<td>0.3</td>
<td>1.0</td>
</tr>
</tbody>
</table>

ED$_{50}$ ~ 0.15 mg/kg

RNAi, MicroRNAs, 2009
Collaboration with M. Saraiva
ALN-TTR Silencing is Durable
V30M TTR Transgenic Mouse Model

ALN-TTR efficacy is both rapid and durable
- Single i.v. dose 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

Serum Protein

AALSD: Liver Meeting, Nov 2009
Collaboration with M. Saraiva
ALN-TTR Prophylactic Efficacy
V30M TTR Transgenic Mouse Model

ALN-TTR blocks pathogenic accumulation of mutant human TTR in peripheral tissues
- >95% Reduction of V30M hTTR deposition
- Multi-dose i.v. injections of ALN-TTR or control siRNA, 3 mg/kg (days 0, 14, 28)
- Quantitation of V30M hTTR deposition by immunohistochemistry (day 56)

Keystone: RNA Silencing, Jan. 2010
Collaboration with M. Saraiva
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-TTR or control siRNA, 3 mg/kg (d0, 14, 28, 42, 56, and 70)
- Quantitation of TTR deposition by immunohistochemistry (day 77)

**Graph:**
- X-axis: Relative TTR Tissue Levels
- Y-axis: 0 to 70
- Y-axis labels: Esophagus, Colon, Stomach, Sciatic nerve, Dorsal root ganglion
- **Control siRNA**
  - Esophagus: 100%
  - Colon: 99%
  - Stomach: 99%
  - Sciatic nerve: 97%
  - Dorsal root ganglion: 99%
- **ALN-TTR**
  - Esophagus: 100%
  - Colon: 99%
  - Stomach: 99%
  - Sciatic nerve: 97%
  - Dorsal root ganglion: 99%

**Legend:**
- Control siRNA
- ALN-TTR

**Immunohistochemistry Images:**
- Dorsal Root Ganglion
  - Control siRNA
  - ALN-TTR

*XII International Symposium on Amyloidosis, Apr. 2010*
Collaboration with M. Saraiva
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 48hr post-dose

![Graph showing the effect of ALN-TTR on TTR mRNA levels.

- **ED50 ~ 0.3 mg/kg**
- **p< 0.001** (one-way ANOVA, Dunn’s post-hoc test)

TTR/GAPDH mRNA (Relative to Control)

- **Control siRNA (mg/kg):** 3
- **0.3 ALN-TTR (mg/kg):** 47%
- **1.0 ALN-TTR (mg/kg):** 62%
- **3.0 ALN-TTR (mg/kg):** 82%
2nd Generation ALN-TTR Program
ALN-TTR02

ALN-TTR02 shows >10-fold improved in vivo efficacy

- Single i.v. infusion
- Liver mRNA levels measured 48 hr post-dose
- Potent, dose-dependent TTR silencing

<table>
<thead>
<tr>
<th>TTR mRNA Levels (Relative to Control)</th>
<th>1st Generation</th>
<th>2nd Generation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>ALN-TTR01 (mg/kg)</td>
<td>0.3</td>
<td>0.03</td>
</tr>
<tr>
<td>ALN-TTR02 (mg/kg)</td>
<td>1.0</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>0.3</td>
</tr>
<tr>
<td>* p&lt;0.05</td>
<td></td>
<td>* p&lt;0.05</td>
</tr>
</tbody>
</table>

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

ED$_{50}$ ~ 0.03 mg/kg

BRI Translational Genetics Series, Feb. 2011
Suppression of TTR Protein by ALN-TTR02 is Durable Non-Human Primates

ALN-TTR02 demonstrates durable, dose-dependent suppression of TTR serum protein levels greater than 28 days in NHP

- Single 15 min IV infusion (0.03, 0.1, 0.3 mg/kg)
- Serum TTR protein levels measured by ELISA on days 0, 1, 2, 4, 7, 14, 21, and 28

- ALN-TTR02 ED$_{50}$ ~ 0.03 mg/kg, ED$_{80}$ ~ 0.2 mg/kg
- Maximal TTR protein suppression at ~ 7-14 days

BRI Translational Genetics Series, Feb. 2011
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• Summary and Next Steps
Study Design / Objective

» Non-intervention / natural history study

» Assess variability of serial serum TTR levels in patients and carriers using:
  - ELISA-based assay
  - Isoelectric focusing gel (IEF)
  - LCMS/MS

» Weekly blood draws
  - 4 consecutive weeks at clinic or at subject’s home

» PI: Dr. John Berk (Boston University)

Status/Demographics

» 27 subjects enrolled

» 26 completers
## Coefficient of Variation for Serum TTR
### All versus V30M Only

<table>
<thead>
<tr>
<th>Group included in analysis</th>
<th>Visits included in analysis</th>
<th>Number of Subjects</th>
<th>Number of Observations</th>
<th>Mean</th>
<th>Variance Component Estimate of Within Subject SD</th>
<th>Variance Component Estimate of Between Subject SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Carrier (All)</strong></td>
<td>All visits</td>
<td>11</td>
<td>44</td>
<td>194.9</td>
<td>33.2</td>
<td>42.6</td>
</tr>
<tr>
<td><strong>Patient (All)</strong></td>
<td>All visits</td>
<td>15</td>
<td>57</td>
<td>207.9</td>
<td>47.8</td>
<td>52.3</td>
</tr>
<tr>
<td><strong>Overall (All)</strong></td>
<td>All visits</td>
<td>26</td>
<td>101</td>
<td>202.2</td>
<td>42.0</td>
<td>47.7</td>
</tr>
<tr>
<td><strong>Carrier (V30M)</strong></td>
<td>All visits</td>
<td>4</td>
<td>16</td>
<td>230.7</td>
<td>26.5</td>
<td>55.1</td>
</tr>
<tr>
<td><strong>Patient (V30M)</strong></td>
<td>All visits</td>
<td>6</td>
<td>24</td>
<td>230.2</td>
<td>19.0</td>
<td>65.5</td>
</tr>
<tr>
<td><strong>Overall (V30M)</strong></td>
<td>All visits</td>
<td>10</td>
<td>40</td>
<td>230.4</td>
<td>22.3</td>
<td>58.2</td>
</tr>
</tbody>
</table>
• Serum TTR shows relatively modest intrapatient variability (~10-20% fluctuation from week to week)

• LCMS/MS analysis on V30M and V122I samples in progress

• Additional key factors to be analyzed in assessment of response to ALN-TTR include:
  » Serum RBP
  » Vitamin A levels
  » Kinetics of TTR/RBP/Vitamin A suppression
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ALN-TTR01 Phase I Study

Study Design

- Randomized, placebo-controlled, single-blind, single-dose escalation study
  - 3:1 randomization
  - 4 patients/cohort
- Up to 36 patients with ATTR
  - Conducted in Portugal (T. Coelho), Sweden (O. Suhr), France (D. Adams) and UK (P. Hawkins)
- Primary objective
  - Safety and tolerability
- Secondary objectives
  - Characterize plasma pharmacokinetics
  - Assess preliminary pharmacodynamic activity
    - Serum TTR, RBP, Vitamin A levels
      - TTR measured by ELISA and LCMS/MS
ALN-TTR01 Phase I Study (Cont’d)

Study Design

• Treatment regimen
  » Single 15-minute ALN-TTR01 i.v. infusion
  » Premedication with corticosteroids, H1/H2 blockers, acetaminophen
  » Dose levels: 0.01-1.0 mg/kg

• Status
  » Initiated Q3 ’10; actively enrolling
  » To present safety and pharmacodynamic data at VIIIth International Symposium on Familial Amyloidotic Polyneuropathy in Kumamoto, Japan on November 21st
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TTR Program
Summary and Next Steps

**ALN-TTR01**

- In Phase I clinical trial, actively enrolling
  - Targets wild-type TTR and all mutant forms of TTR
  - LNP formulation
  - Potent reduction of TTR *in vitro*
  - Potent and durable silencing of TTR *in vivo*
    - Wild-type TTR in rodents and non-human primates
    - Mutant human V30M TTR in transgenic mouse
  - Therapeutic efficacy measured by reduced TTR pathogenic tissue deposition in V30M transgenic mouse

**ALN-TTR02**

- Drug candidate using same siRNA as ALN-TTR01 formulated in second-generation LNP with enhanced potency, in development; IND/CTA filing in 2011
Acknowledgments
TTR Amyloidosis Program

Scientific Collaborators

• Maria Saraiva, Institute of Cellular and Molecular Biology, Porto, Portugal
• Yukio Ando and Hiro Jono, Kumamoto University, Japan

ALN-TTR01 Clinical Investigators

• Teresa Coelho
  » Hospital Geral de Santo Antonio, Porto, Portugal
• Ole Suhr
  » Umea University Hospital, Umea, Sweden
• David Adams
  » CHU Hospital Bicetre, Le Kremlin-Bicetre, France
• Tim Mant/Philip Hawkins
  » Quintiles Drug Research Unit at Guy’s Hospital, London