Phase I Safety, Pharmacokinetic and Pharmacodynamic Results for ALN-PCS

Preliminary Study Results

Amy Simon, M.D.
January 4, 2012
Agenda

- RNAi Background and Systemic Delivery with LNPs
- Rationale for Targeting PCSK9
- ALN-PCS Program: Pre-clinical Data
- ALN-PCS Phase I Trial
  - Study Design
  - Safety and Pharmacokinetic Data
  - Pharmacodynamic Data: Proof of Concept
- Summary and Next Steps
Mechanism of RNA Interference

A. dsRNA processed by Dicer
B. siRNAs loaded into RISC
C. Pairing w/ complementary mRNA
D. mRNA degradation/silencing
E. Protein knockdown
• siRNAs can silence mRNA and knockdown proteins involved in disease pathways
  » Includes targets previously “undruggable”
• Rapid drug discovery paradigm and platform
  » Ability to select development candidates within 3-6 months
• Many key technology “hurdles” solved
  » Major delivery advances have enabled rapid progress
  » 100s of published studies on pre-clinical efficacy
• RNAi therapeutics have entered clinical stages
  » ~15 RNAi therapeutics in clinical trials
  » Human proof of concept established
    – Alnylam GEMINI\(^1\), ALN-VSP\(^2\), and ALN-TTR\(^3\) studies
    – CalTech/Calando CALAA-01 study\(^4\)

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2 *ASCO*, Jun 2011
3 *Int'l Symp FAP*, Nov. 2011
Alnylam Approach: Turning siRNA into Drugs

**Lead Selection**
- siRNA design
- Selectivity screen
  - Off target effects

**Lead Optimization**
- Stabilization
- Potency
- Selectivity

**Delivery**
- PK/PD
- Biodistribution
- Cellular uptake

**Chemistry, Manufacturing and Controls**
- Small Scale
- Gene walks
- *In vitro* assays
- Medium Scale
  - *In vivo* biology
- Large Scale
  - GMP Production
  - Clinical trials

Introduce chemical modifications for “drug-like” properties
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
Mechanism of ApoE Mediated iLNP Delivery

1. Endogenous ApoE bind to LNPs

Lipoprotein particle
Apoe

Exchange of ApoE

pH 7.4

Blood Compartment

Fenestration

Space of Disse

Hepatocyte

Akinc et al., Mol Therapy, 18:1357-1364 (2010)
Mechanism of ApoE Mediated iLNP Delivery

2. LNPs traffic through fenestrated endothelium of liver; bind to LDL receptor

Akinc et al., Mol Therapy, 18:1357-1364 (2010)
Mechanism of ApoE Mediated iLNP Delivery

3. LNPs internalized and disrupt endosome; release siRNA in cytoplasm

As endosome acidifies, cationic charge on vesicle increases

Cationic lipid combines with anionic membrane lipids to disrupt endosomal membrane

siRNA cargo is released into cytoplasm where it can enter RISC

Akinc et al., Mol Therapy, 18:1357-1364 (2010)
Second Generation LNPs
Remarkable Potency Improvements with Novel Lipids

Novel LNPs set new benchmark for systemic RNAi with ~100-fold improved potency
- Efficacy in rodent models following single IV injection
- Each LNP comprised of distinct cationic lipid component
- Improved potency has resulted in single digit μg/kg ED$_{50}$
Efficacy in mouse with systemic RNAi after single i.v. injection

- Effects are rapid, potent, dose-dependent and durable
- ED₅₀ ~0.01 mg/kg with 2nd generation LNPs
# Alnylam Development Pipeline

<table>
<thead>
<tr>
<th>Condition</th>
<th>Discovery</th>
<th>Development</th>
<th>Phase I</th>
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- **Alnylam 5x15 Programs**
- **Partner Programs**
Human Translation with 1\textsuperscript{st} Generation LNP
Multiple Disease Targets

**Human Safety and PK**
- >500 Subjects/patients enrolled overall
- Systemic delivery in human trials
  - ~100 Patients dosed
  - >275 Doses administered
  - >18 Months of dosing
- RNAi therapeutics generally well tolerated
- Pharmacologically relevant human tissue levels achieved

**Human Proof of Concept**

![Graph showing % TTR Knockdown at Nadir](image)

* ALN-TTR01 Dose Cohorts (mg/kg)
  * p=0.02  
  0.01 - 0.2 (n=12)  
  0.4 (n=3)  
  0.7 (n=3)  
  1.0 (n=5)

**Human Proof of Mechanism**

<table>
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<tr>
<th>5'RACE on Biopsies</th>
<th>RNAi Cleavage</th>
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<td>Pre-</td>
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5' location along VEGF transcript 3'

Pre-Post

Positive,

\* p=0.02

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**2nd Generation LNPs**

**ALN-TTR02 Comparative Efficacy**

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

- ALN-TTR02 uses 2nd generation MC3 LNP formulation
- Single i.v. infusion; serum TTR levels post-dosing
- Potent, dose-dependent, durable TTR silencing
- Potential for q30-q60 day dosing

% Serum TTR Knockdown

Day Post Dose

ALN-TTR01 (1.0 mg/kg)

ALN-TTR02 (0.1 mg/kg)

ALN-TTR02 (0.3 mg/kg)

Control

Oligo Ther Soc., Sep 2011
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*Legend:* 
- **Blue** = Alnylam 5x15 Programs
- **Gray** = Partner Programs
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Hypercholesterolemia
Elevated LDL-C

- Cardiovascular disease (CVD) resulting from elevated low-density lipoprotein cholesterol (LDL-C) is leading cause of death worldwide

- Unmet need: >500,000 patients with severe hypercholesterolemia despite current therapies

- Recent clinical trials suggest further health benefits with even lower LDL-C levels in patients with CVD (<70 mg/dL)

- Real-world studies indicate that many patients (up to 50%) do not meet LDL-C goals even on statins
  - Poor adherence
  - Incomplete response to statins
  - Intolerance to statins

1 World Health Organization (2004)
2 Ridker et al., NEJM; 359: 2195-207 (2008)
PCSK9 is Ideal Target for Lowering LDL-C

- Member of subtilisin protease family, predominantly expressed in liver
- Genetically validated: GOF mutations associated with hypercholesterolemia and premature CHD and LOF mutations associated with hypocholesterolemia and decreased CHD risk
- Animal models: PCSK9 lowering results in increased LDLR and decreased serum LDL-C
- RNAi provides a therapeutic modality for specific knockdown of PCSK9
- Statins and ezetimibe upregulate PCSK9 expression, which can attenuate their effectiveness in lowering LDL-C

Rashid et al., PNAS; 102: 5374-9 (2005)
Ason et al., J. Lipid Res; 52:679 (2011)
PCSK9 Loss of Function Human Mutations

**Heterozygous Mutations**
- ~40 mg/dl shift in LDL-C
- 88% CHD risk reduction over 15 yrs

**Homozygous Mutations**
- 32 Year-old woman
- Healthy, fertile
- No detectable PCSK9 levels

**Plasma LDL Cholesterol in Black Subjects (mg/dl)**

<table>
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<th>PCSK9 null person</th>
<th>Number mg/dL</th>
<th>% Normal</th>
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<td>HDL Cholesterol</td>
<td>65</td>
<td>118</td>
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<tr>
<td>Triglycerides</td>
<td>119</td>
<td>108</td>
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<tr>
<td>LDL Cholesterol</td>
<td>14</td>
<td>13</td>
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<tr>
<td>Total Cholesterol</td>
<td>96</td>
<td>55</td>
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PCSK9 Levels

Impact of Statin Treatment in Man and Mouse

Rashid et al., PNAS; 102:5374-5379 (2005)
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PCSK9 siRNA Screening Summary

Screened: 166
- 16 Human-NHP-Rodent
- ~150 Human-NHP

Lead molecule and surrogate identified
- Surrogate siRNA (less active but cross-reactive in all species) useful tool for rodent studies (possible tox-surrogate)
- Lead siRNA (human-NHP specific) for NHP studies and clinic

IC50 ~10pM

PCSK9 Conference, March 2010
**RNAi Silencing of PCSK9**

**Rat**

Liver PCSK9 mRNA and serum Tc levels

![Bar chart showing PCSK9 mRNA and serum Tc levels for different treatments.](chart1)

Serum Tc level over time

![Line graph showing serum Tc levels over days post-injection.](chart2)

5’RACE (Liver)

Lane | LDLR | TFR | PBS | LNP-Ctrl | LNP-PCS-A2 | LNP-PCS-A2 | LNP-Ctrl | PBS
---|---|---|---|---|---|---|---|---
1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9
10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18

![ Gel electrophoresis image showing 5’RACE results.](gel1)

Predicted PCR band Seq. Confirmed

ALN-PCS Drug Product

PCSK9 siRNA targeting intracellular and extracellular PCSK9
- Targets 3’UTR
- Highly specific, >2 nt mismatches with every known human gene
- Chemically modified to increase stability and reduce risk of immunostimulation
- $\text{IC}_{50} = 10\text{pM}$

Second generation lipid nanoparticle (LNP) formulation
- Ionizable lipid (MC3)
- Fusogenic lipid
- PEG lipid
- Cholesterol
ALN-PCS Pre-Clinical Efficacy Phenocopies Human Genetics

ALN-PCS demonstrates potent efficacy after single dose in NHPs
- Rapid and dose-dependent reduction in PCSK9 protein
- PCSK9 silencing results in >50% reduction in LDL-C
- Durable efficacy lasting >30 days
- No decrease in HDL-C levels

PCSK9 Conference: From Gene to Therapeutic, Mar 2010
Multi-Dose Studies of ALN-PCS
Non-Human Primate

Multi-dose regimen achieves sustained PCSK9 and LDL-C lowering with once-monthly dosing

- 1.0 mg/kg initial dose followed by monthly dosing at 0.3mg/kg
- Significant effects achieved with 4 doses over 4 month period
- No effects observed on HDL-C
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ALN-PCS Phase I Study

Study Design
- Randomized, single-blind, placebo-controlled, single-dose escalation study
  - 3:1 Randomization
  - 4 Subjects/cohort
  - 5 Dose levels completed to date
- Up to 32 healthy volunteer subjects with elevated baseline LDL-C (>116mg/dL)
  - Conducted at 2 centers in the UK
  - Trial performed in absence of statins or other lipid lowering therapy

Primary objective
- Safety and tolerability

Secondary objectives
- Characterization of pharmacokinetics
- Assess preliminary pharmacodynamic and clinical activity
  - Plasma PCSK9 protein and LDL-C levels

Study Status
- Additional dose escalation planned
**ALN-PCS Phase I Study Design**

**Dose levels and dosing schedule**
- 0.015, 0.045, 0.090, 0.150, 0.250 mg/kg; additional dose escalation planned
  - Option of enrolling up to 3 additional cohorts within specified dose range to further assess safety, PK and PD/clinical activity
- Single 60-min i.v. infusion
- Pre-medication including corticosteroid, H1/H2 blocker and acetaminophen
ALN-PCS Phase I Study Preliminary Results
Baseline Characteristics

- Total Subjects N=20
  - ALN-PCS: Placebo = 15:5
- Median Age: 45 years (range 25-61)
- Subjects all males
- Mean BMI (kg/m\(^2\)): 29.2 (range 23.4-33.9)
- Mean baseline PCSK9 levels: 1052 ng/mL (range 476-1821)
- Mean screening LDL levels: 148.5 mg/dL (range 116.4-187.2)
## ALN-PCS Phase I Study Preliminary Results

TEAEs ≥ 10%

<table>
<thead>
<tr>
<th>Adverse Events</th>
<th>ALN-PCS Dose Groups</th>
<th>Overall ALN-PCS (N=15)</th>
<th>Placebo (N=5)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0.015 (n=3)</td>
<td>0.045 (n=3)</td>
<td>0.090 (n=3)</td>
</tr>
<tr>
<td>Headache</td>
<td>1 (33.3)</td>
<td>1 (33.3)</td>
<td>2 (66.7)</td>
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<tr>
<td>Rash</td>
<td>1 (33.3)</td>
<td>1 (33.3)</td>
<td>0 (00.0)</td>
</tr>
<tr>
<td>Feeling hot</td>
<td>2 (66.7)</td>
<td>0 (00.0)</td>
<td>0 (00.0)</td>
</tr>
<tr>
<td>Hiccups</td>
<td>2 (66.7)</td>
<td>0 (00.0)</td>
<td>0 (00.0)</td>
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<tr>
<td>Arthralgia</td>
<td>0 (00.0)</td>
<td>0 (00.0)</td>
<td>0 (00.0)</td>
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<tr>
<td>Dyspepsia</td>
<td>0 (00.0)</td>
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- All TEAEs mild-moderate in severity
- No SAEs related to study drug administration
  - One subject at 0.045 mg/kg dose level was diagnosed with bilateral PEs and DVTs; determined by PI to be unrelated to study drug given subject’s prior history and symptoms prior to study drug administration
- No significant increases in LFTs

Preliminary, Study Ongoing

Conclusions: The preliminary results from the ALN-PCS Phase I Study indicate that the drug is tolerable with the majority of adverse events being mild to moderate in severity. There were no serious adverse events related to the study drug administration. One subject at the 0.045 mg/kg dose level was diagnosed with bilateral pulmonary embolisms (PEs) and deep vein thromboses (DVTs), which were determined to be unrelated to the study drug given their prior history and symptoms prior to drug administration. No significant increases in liver function tests (LFTs) were observed.
ALN-PCS Phase I Study Preliminary Results
Pharmacokinetic Data

- siRNA exposure observed for at least 7 days and at higher doses up to 21 days
- Cmax increases with increase in dose in linear and approximately dose-proportional manner
- AUC increases with increase in dose
  » Preliminary AUC data trend suggest, in general, AUC was dose-proportional at higher doses but not captured at lower doses due to assay sensitivity

<table>
<thead>
<tr>
<th>ALN-PCS Doses*</th>
<th>Cmax (Mean ± SD)</th>
<th>AUC₀-last (Mean ± SD)</th>
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<tr>
<td>mg/kg</td>
<td>µg/mL</td>
<td>µg·hr/mL</td>
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<tr>
<td>0.015</td>
<td>0.14 ± 0.08</td>
<td>1.30 ± 1.18</td>
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<tr>
<td>0.045</td>
<td>0.71 ± 0.18</td>
<td>5.70 ± 0.87</td>
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<tr>
<td>0.090</td>
<td>1.81 ± 0.47</td>
<td>28.94 ± 4.86</td>
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<tr>
<td>0.150</td>
<td>2.54 ± 0.61</td>
<td>50.70 ± 37.18</td>
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<tr>
<td>0.250</td>
<td>4.11 ± 0.35</td>
<td>Pending*</td>
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*Human PK data from the 0.25 mg/kg dose is only up to Day-7 plasma ALN-PCS concentrations, sample analysis is ongoing
ALN-PCS Phase I Study Preliminary Results
Effects of Steroid Pre-Medication on PCSK9 and LDL-C

• Steroid pre-medication associated with transient increases in PCSK9 and decreases in LDL-C
  » 65% Increase on day 2 in PCSK9 serum levels in placebo subjects
  » 25% Decrease on day 3 in LDL-C in placebo subjects

• Non-specific effects of steroid pre-medication addressed in data analysis

• Future studies with ALN-PCS planned without steroid pre-medication
ALN-PCS Phase I Study Preliminary Results

Dose-Dependent Knockdown of PCSK9 Serum Levels

Rapid, dose-dependent, and durable PCSK9 knockdown after single dose

- Up to 66% decrease at current top dose at day 4
- 60% mean reduction at current top dose (p<0.001) at day 4
ALN-PCS Phase I Study Preliminary Results
PCSK9 Knockdown Relative to Baseline and Placebo

ALN-PCS dose group (n=3)
- 0.015 mg/kg
- 0.045 mg/kg
- 0.090 mg/kg
- 0.150 mg/kg
- 0.250 mg/kg

Preliminary, Study Ongoing
Clinical efficacy for ALN-PCS as measured by LDL-C
- Up to >50% reduction in LDL-C and 39% mean reduction at current top dose at day 4 (p<0.05)
- Significant reductions through at least day 14
- No significant or dose-dependent changes in HDL-C

**Day 4**
Cohorts 1-5

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<th>Placebo</th>
<th>0.015</th>
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<th>0.09</th>
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<td>Percent Change in LDL-C Relative to Baseline</td>
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ANOVA: \( p = 0.045 \)

**Day 14**
Cohorts 1-5

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</tr>
</thead>
<tbody>
<tr>
<td>Percent Change in LDL-C Relative to Baseline</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

ANOVA: \( p = 0.0098 \)
Clinical efficacy for ALN-PCS in achieving LDL-C “target” levels

- 100% subjects at top two doses achieved LDL-C <100 mg/dL compared with 21.4% subjects in other groups

<table>
<thead>
<tr>
<th>Treatment Group (mg/kg)</th>
<th>Hit Target n</th>
<th>Missed Target n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>0.015</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>0.045</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>0.090</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>0.150</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>0.250</td>
<td>3</td>
<td>0</td>
</tr>
</tbody>
</table>

*Fisher’s exact test: p=0.04*
Agenda

- RNAi Background and Systemic Delivery with LNPs
- Rationale for Targeting PCSK9
- ALN-PCS Program: Pre-clinical Data
- ALN-PCS Phase I Trial
  - Study Design
  - Safety and Pharmacokinetic Data
  - Pharmacodynamic Data: Proof of Concept
- Summary and Next Steps
ALN-PCS Phase I Preliminary Data Summary

Key findings for ALN-PCS in current study results

- ALN-PCS is safe and well tolerated
  - No drug-related discontinuations and no liver enzyme elevations

- Rapid, dose-dependent, and durable silencing of PCSK9
  - Up to 66% PCSK9 knockdown relative to baseline and mean 60% PCSK9 knockdown at Day 4 at 0.25 mg/kg (p<0.001)
  - Durable silencing out to 28 days following single dose supports once monthly dosing

- Rapid, dose-dependent and durable reductions in LDL-C
  - Up to >50% reduction in LDL-C relative to baseline and mean 39% LDL-C reduction at day 4 at 0.25 mg/kg (p<0.05)
  - 100% (6/6) of subjects at two highest dose groups achieved target LDL-C compared with 21% (3/14) in other dose groups (p<0.05)

- Significant milestone in advancement of RNAi therapeutics
  - First ever demonstration of efficacy for an RNAi therapeutic toward a clinically validated endpoint (LDL-C)
  - First demonstration of improved potency in human with second generation LNP delivery
ALN-PCS Program

Next Steps

- Continue Phase I study with planned further dose escalation
- Additional study results to be reported in H1 2012
- Continued clinical development of ALN-PCS for severe hypercholesterolemia
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