Delivery Roundtable
Current Status and Future Directions

June 25, 2009
Agenda

- RNAi and the Role for Delivery
  - Direct RNAi
  - Systemic RNAi
  - Future Strategies
RNA Interference

Synthetic siRNA

dsRNA

dicer

Cleavage

Natural Strand separation

Natural Process of RNAi

Targeted Gene Silencing

RISC

Complementary pairing

mRNA \((A)_n\)

mRNA degradation

\((A)_n\)
Making Drugs Out of siRNAs

The Challenge

Characteristics

- M.W 12,000-14,000
- Size: 2 turns of helix
- 40 negative charges
- Hydrophilic
- Hydrated heavily
- *ca.* 5.5 nm X 2 nm
- Biostability

Achieving RNAi as Therapy

• Introducing “drug-like” properties into siRNAs
  » Potency
  » Selectivity
  » Stability

• Achieving delivery to target tissues/cells
  » PK/PD/Biodistribution
  » Cellular uptake
Delivery of RNAi Therapeutics

**Key driver of success**
- Delivery
  - PK/PD/Biodistribution
  - Cellular uptake

**Major progress achieved**
- Robust *in vivo* efficacy
  - >25 Targets
    - Includes many “un-druggable”
  - >5 Organs
    - Includes lung, liver, and CNS
  - 6 Species
    - Includes humans

**Delivery Approaches**
- Conjugates
- Liposomal NPs
- Peptides
- Antibodies
RNAi Delivery Strategies

Direct RNAi

Respiratory
» RSV/Influenza
» Cystic fibrosis
» Asthma/COPD

Ocular
» AMD/Retinopathy
» Glaucoma

CNS
» Neuropathic pain
» Spinal cord injury
» Huntington’s disease
» Parkinson’s disease

Topical/Mucosal
» Infectious disease

Systemic RNAi

Multiple major diseases
» Metabolic
» Viral disease
» Cancer
» Inflammation
» Cardiovascular
RNAi Therapeutics Opportunity

Large Number of Undruggable Targets

New Drug Opportunities

- World of targets
- Accessible targets for small molecules/antibodies
- RNAi accessible targets
  - Undruggable targets
  - Potent, selective lead
  - Rapid; 3-6 month to lead
  - Cross species active
  - Multi-targeting
  - Allelic specificity
  - Mab-like PD

New targets and disease

Alnylam
## Alnylam Development Pipeline

### Key Programs

<table>
<thead>
<tr>
<th></th>
<th>Discovery</th>
<th>Development</th>
<th>Phase I</th>
<th>Phase II</th>
<th>Phase III</th>
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<td><strong>RSV Infection</strong></td>
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<td>PCSK9/Hypercholesterolemia</td>
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<td>TTR Amyloidosis</td>
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<td>Huntington’s Disease</td>
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- **Alnylam Proprietary Programs**
- **Co-development Programs**

### 3 programs in clinical trials in 2009
• RNAi and the Role for Delivery
  • Direct RNAi
• Systemic RNAi
• Future Strategies
Direct Delivery in CNS

Example: Cy3-Htt siRNA Distribution After Intrastriatal Infusion
Silencing of Rat CNPase

- Infusion Duration: ~3 d
- Infusion rate: 10 µl/hr

**p<0.001**
**p<0.01 vs PBS
Two way ANOVA

Silencing of Primate CNPase

- Relative CNPase/MBP mRNA (%)
- Injection Site
- Adjacent Site

Regeneration of Primate CNPase

- Injection Site
- Adjacent Site

Verifying by cloning and sequencing of amplified products (18/20 clones)

Querbes et al., *Oligonucleotides* (2008)
**Direct Delivery in CNS**

**Therapeutic Silencing of Huntingtin**

**Strong pre-clinical data support advancement toward clinic**

- RNAi therapeutic targeting huntingtin corrects disease in animal model
  - Silencing of huntingtin gene
  - Decreased disease pathology and behavioral changes

**Behavior Improvement in Disease Model**

- Normal mice
- Control siRNA
- HTT siRNA

* p ≤ 0.01

**Improved Histopathology**

- Luc
- HTT siRNA

**Statistics**

- Mean number of foot slips
- Mean number of aggregates/2500 μm²

* PNAS (2007) 104, 17204-209
FISH Staining in Lung
4 Hours after Single 10 mg/kg Dry Powder or Liquid siRNA Installation

- Luc siRNA DP, 4 Hrs, x10
- No Treatment, x10
- Neg Ctrl siRNA DP, 4 Hrs, x10
- Luc siRNA D5W, 4 Hrs, x10
Direct Delivery in Lung

Example: Anti-viral Activity of RSV siRNAs

- siRNA i.n. (25-100ug) → 4 hrs → Virus (RSV/A2) i.n. (10^6 pfu) → 4 days → Viral Titer in Lung

Log10 PFU/g lung

- 1 mg/kg
- 2 mg/kg
- 4 mg/kg

P gene

N gene

L gene

P1, P2, P3, ALN-RSV01, N2, L1, L2, L3, P4, P4-MM

LLOD
ALN-RSV01 Phase II GEMINI Study
Human POC for RNAi Therapeutics

ALN-RSV01 shows statistically significant reduction in RSV infection
- Randomized, double-blind, placebo-controlled study (n=88)
- ~40% Relative reduction in infection rate (P<0.01)
- ~95% Increase in number of uninfected subjects (P<0.01)

Plaque Assay

1. Plaque Assay
2. Placebo
3. ALN-RSV01

Study Day
1 2 3 4 5 6 7 8 9 10 11

% Infected
0 10 20 30 40 50 60 70 80

P=0.0069

P<0.01

~40% Reduction

~95% Increase

Int'l Symp Res Vir Infect, Feb 2008
siRNA Conjugates Enhance Delivery

E.g., Cholesterol
- Promotes cellular uptake
  » Direct cell permeation

RNAi Activity \textit{in vitro} without transfection reagent

\begin{table}
\centering
\begin{tabular}{|c|c|}
\hline
siRNA dose (nM) & % Gene expression \\
\hline
500 & 100 ± 5 \quad 100 ± 5 \\
1000 & 80 ± 5 \quad 80 ± 5 \\
1500 & 60 ± 5 \quad 60 ± 5 \\
2000 & 40 ± 5 \quad 40 ± 5 \\
\hline
\end{tabular}
\end{table}

Direct Delivery to Mucosal Tissue

Intravaginal Application of Chol-siRNA against Nectin 1

Cell Host Microbe (2009) 5, 84-94
• RNAi and the Role for Delivery
• Direct RNAi
• Systemic RNAi
• Future Strategies
Systemic RNAi

- Parenteral administration increases access to multiple major diseases
  - Metabolic
  - Viral disease
  - Cancer
  - Inflammation
  - Cardiovascular

- Validated Systemic RNAi approaches
  - Conjugates
  - Liposomal nanoparticles

- Multiple approaches
  - Polymers
  - Small molecules
  - Peptides
  - Antibodies
Chol-siRNAs Silence apoB in Liver and Jejunum

* P<0.0001 compared to saline control animals

Lipophilic Conjugates
SAR for Gene Silencing In Vivo

Biodistribution of Chol-siRNAs is Lipoprotein-Mediated

Chol-siRNAs bind to circulating lipoproteins and traffic to tissue in a receptor-mediated fashion.
Lipoprotein-mediated uptake of chol-siRNAs into tissues of \textit{Ldlr}^{−/−} and wildtype mice

Stoffel, Keystone March 2008
Liposomal Nanoparticles for Systemic RNAi

- Multi-component lipid formulation
  - Cationic lipid
  - Fusogenic lipid
  - PEG lipid
  - Cholesterol

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

- Low surface charge
- Small uniform size particle < 100 nm
Liposomal siRNA Delivery to Liver Mouse

**Clearance from Plasma**

% Injected Dose vs. Time

**Detection of Cy3-siRNA in Liver**

Composite

Detection in Tissues

% Injected Dose

Liver, Spleen, Lungs, Kidney, Heart, Femur, Thymus, Small intestine, Large intestine, Muscle, Fat

Control, 10 mg/kg, 3 mg/kg, 1 mg/kg

30 mins
Dose-Dependent Silencing of Factor VII

Liver Factor VII mRNA

Plasma Factor VII Protein

Prothrombin Time

Durability

Repeat Silencing of Factor VII

Rat

Repeat dosing over 3 months highly effective

- Comparable potency and durability of silencing with repeat dosing
- No evidence for tachyphylaxis or immunogenicity

Molecular Therapy (2009) 17, 872-879.
Silencing apoB
Non-Human Primate

Efficacy in monkeys with Systemic RNAi after single IV injection
- Effects are rapid, potent, dose-dependent and durable
- RNAi effects are specific and lead to measurable therapeutic benefit
- RNAi mechanism proven in vivo

\[
\begin{array}{c|c|c}
\text{Day 11 Post-Dose (2.5 mg/kg)} & \text{Day 11} & \text{2 day} \\
\hline
\text{Cholesterol} & 34.1 & 14.2 \\
\text{LDL} & 109.8 & >85\% \text{ Inhibition} \\
\text{HDL} & 23.2 & >65\% \text{ Inhibition} \\
\end{array}
\]

* P < .05 ** P < .005

\[100, 80, 60, 40, 20, 0\]

\[\% \text{ Control}\]

\[100, 80, 60, 40, 20, 0\]

\[\% \text{ Control}\]

\(\text{Nature (2006) 441, 111-114}\)
Alnylam Systemic Programs
ALN-PCS, ALN-TTR, ALN-VSP

**ALN-PCS to treat hypercholesterolemia**
- Targets PCSK9
- IND candidate for 2009

**ALN-TTR to treat TTR amyloidosis**
- Targets liver expressed TTR
- IND candidate for 2009

**ALN-VSP to treat liver cancer**
- Target two key genes
  - KSP – critical for cell division
  - VEGF – critical for angiogenesis
- Phase I
RNAi to treat liver cancers

- Prevalent solid tumor and common site of metastatic disease
  - ~700,000/yr Incidence of HCC worldwide
  - ~500,000/yr Patients with liver metastasis

- ALN-VSP is dual-target product
  - Targeting 2 pathways increases potential therapeutic impact
    - Proliferation: Kinesin Spindle Protein (KSP)
    - Angiogenesis: VEGF
  - Liposomal nanoparticle formulation
    - With Tekmira Pharmaceuticals

- ALN-VSP in clinical development
  - Phase I liver cancer patient study
**RNAi-Mediated Cell Cycle Arrest**

**Murine Liver Cancer Model**

Orthotopic tumor model with intrahepatic Hep3B seeding in SCID mice

- Single IV bolus injection of ALN-VSP or control siRNA
- Mitotic arrest (monoasters) clearly detected in VSP-treated animals
- KSP and VEGF target mRNAs cleaved in tumors confirming RNAi mechanism
Orthotopic tumor model with intrahepatic Hep3B seeding in SCID mice

- ALN-VSP demonstrates clear anti-tumor activity compared with controls

Control siRNA, n=6

ALN-VSP, n=7

Keystone: RNAi, Feb 2009
ALN-VSP Anti-Tumor Activity

Comparative Efficacy vs. Sorafenib

Orthotopic tumor model with intrahepatic Hep3B seeding in SCID mice

- Significant survival benefit of ALN-VSP treatment vs. controls
- Superior survival advantage of ALN-VSP vs. sorafenib treatment

Survival (%)

log-rank Control Soraf Soraf/Cntrl Soraf/VSP Soraf/VSP
NS NS NS NS NS
p=0.002 p=0.012 p=0.020 VSP p=0.0006 p=0.003 p=0.025 NS
PCSK9/Hypercholesterolemia Program
ALN-PCS

**RNAi to treat hypercholesterolemia**

- Significant unmet medical need
  - >500,000 Patients with severe hypercholesterolemia
    - Inadequately managed by statins and other drugs
- PCSK9 is regulator of LDL metabolism
  - Controls expression of LDL receptor
  - Validated in human genetics
- Attractive opportunity for Systemic RNAi
  - PCSK9 expressed in liver
  - Early clinical markers of activity possible
- Collaboration with UT Southwestern
  - Horton, Hobbs, Brown, and Goldstein
RNAi Silencing of PCSK9 Protein and LDLc in Non-Human Primates

Efficacy of PCSK9 silencing in non-human primates

- PCSK9 plasma levels reduced by up to 70% of pre-dose levels
- Rapid reductions in LDL cholesterol levels by 40-60%
- Durable effects after single injection

**PCSK9 protein**

<table>
<thead>
<tr>
<th></th>
<th>Day 4</th>
<th>Day 7</th>
<th>Day 14</th>
<th>Day 21</th>
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<tbody>
<tr>
<td>PBS</td>
<td>1.6</td>
<td>1.4</td>
<td>1.3</td>
<td>1.2</td>
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<tr>
<td>Control siRNA</td>
<td>1.4</td>
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<tr>
<td>PCSK9 siRNA</td>
<td>1.0</td>
<td>0.8</td>
<td>0.6</td>
<td>0.4</td>
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</table>

* * p ≤ 0.05

**LDL Cholesterol**

<table>
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<tr>
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<td>0.6</td>
<td>0.4</td>
<td>0.3</td>
<td>0.2</td>
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* * p ≤ 0.05

* * *
RNAi to treat significant orphan disease

- Transthyretin (TTR) Amyloidosis
  - Caused by mutation in TTR gene
  - Amyloid deposits in nerves and heart
    - Familial Amyloid Polyneuropathy (FAP)
    - Familial Amyloid Cardiomyopathy (FAC)
  - ~10,000 patients WW with FAP

- Clinical pathology
  - Typical onset ~30-50 yr
  - Fatal within 5-15 years
  - Severe pain/loss of autonomic nervous function

- TTR is well-validated target
  - Human and mouse genetics
    - No pathology in knock-out mouse
  - Produced almost exclusively in liver (95%)

- Liver transplant current standard of care
RNAi Silencing of Mutant and Wild-Type TTR
Mouse and Non-Human Primate

Efficacy in transgenic mouse model and non-human primates

- Reduced mutant V30M-TTR plasma levels and liver mRNA >90% in transgenic mice
- Reduced liver TTR mRNA levels ~80% in non-human primates

**hTTR transgenic mouse**

<table>
<thead>
<tr>
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<th>1 mg/kg</th>
<th>3 mg/kg</th>
<th>6 mg/kg</th>
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<td>PBS</td>
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<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>TTR siRNA</td>
<td>0.9</td>
<td>0.8</td>
<td>0.7</td>
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**Non-human primate**

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<th>0.3 mg/kg</th>
<th>1 mg/kg</th>
<th>3 mg/kg</th>
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<tr>
<td>Control</td>
<td>1.6</td>
<td>1.4</td>
<td>1.0</td>
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<tr>
<td>TTR siRNA</td>
<td>0.4</td>
<td>0.3</td>
<td>0.2</td>
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*Keystone: RNAi, Feb 2009

*Lipidoid formulation, w/ MIT; **SNALP formulation, Tekmira
- RNAi and the Role for Delivery
- Direct RNAi
- Systemic RNAi
- Future Strategies
Future Strategies

- Optimizing LNPs
  - New formulations of lipids
  - New lipids
  - Tailoring PK/PD and biodistribution
- Targeting ligands
  - New conjugates
  - Targeted liposomes
- ssRNAi
  - New physical properties
Liposome Formulation Development Progress

Efficacy Improvement Over Time

% Residual Factor VII vs FVII siRNA Dose (mg/kg)

- LNP01
- LNP A
- LNP B
- LNP C
- LNP D

ED50

Int'l Symp Athero, June 2009
“Lipidoid” Library for Novel Nanoparticles

Alnylam-MIT Collaboration

Library Components

In Vitro Screen

Synthetic Scheme

Factors That Impact LNP Pharmacology

Stability of PEGylation

Particle Size

Molecular Therapy (2009) 17, 872-879.
PK/Biodistribution of LNPs Can Be Modified

**A**

<table>
<thead>
<tr>
<th>Tumor volume (mm$^3$)</th>
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<tbody>
<tr>
<td>Control</td>
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<tr>
<td>PLK FEG-cDMA</td>
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<tr>
<td>PLK FEG-cDSA</td>
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**B**

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<th>hPLK1/hGAPDH ratio</th>
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<tr>
<td>PLK</td>
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<td>PEG-DM</td>
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<tr>
<td>PEG-DSA</td>
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**C**

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<th>Tumor volume (mm$^3$)</th>
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<tr>
<td>LUC 3 mg/kg</td>
</tr>
<tr>
<td>PLK 0.5 mg/kg</td>
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<tr>
<td>PLK 1 mg/kg</td>
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<tr>
<td>PLK 3 mg/kg</td>
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Time after seeding (d)

Initial Dosing and Lower Maintenance Dose
Multiple Injections

LNP formulated PCS-A2 (mg/kg)

Total Cholesterol Relative to PBS=1

Days post initial bolus

Initial 3mg/kg bolus
Maintenance: 1 x wk
Rats were bled one day prior to repeated dosing
Targeted Delivery

• The use of targeting ligands may substantially develop delivery solutions to increase
  » Efficiency
  » Access to other tissues
• Applicable to both conjugate and LNP strategies
• Targeting approaches
  » Small molecules
  » Peptides
  » Monoclonal antibodies
Alnylam Delivery Strategy

• Two key systemic delivery strategies at Alnylam
  » Lipid nanoparticles
    – Including targeting with ligands
  » Conjugates
    – Including targeting with ligands

• A third delivery strategy
  » Single stranded siRNAs
    – An early an alternate approach to delivery of siRNAs
    – Agreement with Isis
Single-Stranded and Duplex si-RNAs

Physicochemical Properties

**Single-stranded siRNA**
- MW ~ 7000
- 19 formal negative charges
- Flexible with ~ 1 nm width
- Hydrophobic surfaces accessible for protein interactions
  » Aromatic bases (green) are exposed

**Double-stranded siRNA**
- MW ~ 13,000
- Two molecules (one strand pink)
- 40 formal negative charges
- Rigid with ~ 2 nm diameter
- Very little exposed hydrophobic surface
  » Aromatic bases (green) are paired and buried in duplex

These different physical/chemical properties will likely result in marked differences in pharmacokinetic profiles.
Importance of Mechanistic Insights
Multiple Ways into the Cell
Collaboration with Merino Zerial

Keystone: RNAi, Feb 2009
Alnylam has broad and long-term commitment to improving and expanding delivery technology

- Continue development of direct and systemic approaches
  - Many technologies under evaluation
    - Direct RNAi
    - Systemic RNAi
      - New Liposomes and lipidoids
      - New conjugates including peptides and antibodies
      - Single strand siRNA

- Basic research
  - Marino Zerial

- Alnylam approached pro-actively by academics and companies
  - >25 evaluations ongoing with external groups at any one time
visit our newly launched

www.alnylam.com