Keystone Symposium: Advances in Biopharmaceuticals

Development of RNAi Therapeutics

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RNA Interference

Synthetic siRNA

dsRNA

dicer

Cleavage

Strand separation

Targeted Gene Silencing

mRNA degradation

RISC

Complementary pairing

mRNA (A)_n

Natural Process of RNAi

Cleavage

(A)_n
# Alnylam RNAi Product Platform

## Turning siRNAs into Drugs

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

### Lead Optimization
- Stabilization
- Potency
- Selectivity

- Introduce chemical modifications for “drug-like” properties

### 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
Lead Development and Selection

Starting Pool
1000’s

Design/Specificity/Crossreactivity
100’s
(Screening Set)

In vitro Activity Screen
100s → 30-50

Optimization/Stabilization/
Immunostimulation
30-50 → ~6

In vivo pharmacology
~6 → 1-2

Lead Candidate → 1
Achieving Delivery
Modification and Formulation

Key challenge
- PK/PD/Biodistribution
- Cellular uptake
- Key to broad application of RNAi

Broad and significant effort at Alnylam
- Internal and external collaborations

Major progress achieved
- Direct and Systemic delivery
- Robust \textit{in vivo} efficacy (Alnylam experience)
  - >25 Targets
    - Includes many “un-druggable”
  - >5 Organs
    - Includes lung, liver, gut, and CNS
  - 6 Species
    - Includes humans
1st Generation Lipid Nanoparticle (LNP) Systemic Delivery

In Vivo Silencing of apoB in Primates

Efficacy in primates with Systemic RNAi after single IV injection

- Rapid, potent, dose-dependent and durable effects
- RNAi specific and leads to measurable therapeutic benefit
- RNAi mechanism proven in vivo

\[ \begin{align*}
\text{Day 11 Post-Dose (2.5 mg/kg)} \\
\text{Cholesterol: 14.2} & \quad >85\% \text{ Inhibition} \\
\text{LDL: 34.1} & \quad >65\% \text{ Inhibition} \\
\text{HDL: 109.8} & \quad >65\% \text{ Inhibition}
\end{align*} \]

\[ \begin{align*}
\text{2 day} & \quad \begin{align*}
\text{Protein: 31.7} & \quad * P < .05 \\
\text{mRNA: 60.5} & \quad ** P < .005
\end{align*} \\
\text{11 day} & \quad \begin{align*}
\text{Protein: 8.9} & \quad * P < .05 \\
\text{mRNA: 11.7} & \quad ** P < .005
\end{align*}
\end{align*} \]

\[ \begin{align*}
\text{Nature, 441, 111-114, Mar 2006}
\end{align*} \]
Next Generation Lipid Nanoparticles (LNPs)
Improved Potency in Rodents

Novel LNPs set new benchmark for systemic delivery
- Each LNP comprised of distinct cationic lipid component
- Improvement in potency has resulted in $\text{ED}_{50} < 0.03 \text{ mg/kg}$

* Optimized formulation

![Graph showing % Residual Factor VII vs. siFVII Dose (mg/kg)]
Next Generation LNPs

*In Vivo* Silencing of Factor VII in Rodent

Efficacy in mouse with systemic RNAi after single IV injection

- Effects are rapid, potent, dose-dependent and durable
- ED$_{50}$ ~ 0.01 mg/kg

**mRNA Silencing**

**Protein Suppression**

**Pharmacological Effect**

**Durability**
Next Generation LNPs
Demonstrate Potent and Durable Silencing in Primates

Novel LNPs demonstrate enhanced potency in primates

- Single IV injection in primates
- Potent, rapid, and durable silencing of target genes; Example: transthyretin (TTR)
- Provides back-up LNPs for current development programs

![Graph showing serum TTR protein levels](image)

*ED$_{50} < 0.03$ mg/kg
ALN-PCS demonstrates potent efficacy in primates

- Employs 2\textsuperscript{nd} generation LNPs
- Rapid and durable dose-dependent reduction in PCSK9 protein
- PCSK9 silencing results in >50% reductions in LDLc
Gene Silencing Extended to Immune Cells

Novel KC2-based LNPs achieves silencing in immune cells

- CD45 and GFP protein suppression observed in peritoneal leukocytes 24 hrs after IV administration of LNP-KC2-siCD45 at 3 mg/kg
- Some silencing seen in dendritic cells in spleen and bone marrow

CD45 KD in Peritoneal Cavity

- CD45 siRNA compared to control siRNA
- Relative CD45 levels in macrophages and dendritic cells
- Some silencing observed in dendritic cells in spleen and bone marrow
Systemic Delivery to Liver Tumors
Efficacy in Mouse Orthotopic 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 ALN-VSP-treated animals
- KSP and VEGF target mRNAs cleaved in tumors confirming RNAi mechanism
Systemic Delivery to Liver Tumors
Efficacy in Mouse Orthotopic Liver Cancer Model

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
- C1R, C1R, C1G, C1G, C1B, C1B, C1W, C1W, C2R, C2R, C2G, C2G

Keystone: RNAi, Feb 2009
Mitotic arrest (monoasters) in metastatic colorectal tumors arising from intrahepatic HCT116 seeding in mice

- Multi-dose IV bolus of ALN-VSP or control siRNA (SNALP-siCont), 4 mg/kg (2x/week for 3 weeks)
- H&E histology of tumor bearing lymph nodes 48 hours post-last dose

**Results**

- SNALP-siCont: 2 monoasters
- ALN-VSP: 15 monoasters
- SNALP-siCont: 6 simple mitotic figures
- ALN-VSP: 4 simple mitotic figures

**Graph**

- Lymph node
- ALN-VSP vs. SNALP-siCont
PK/PD Relationship for Liver Silencing with LNPs

Tissue siRNA levels of ~1 ng/g at ED\textsubscript{50} for mRNA suppression demonstrates potency of natural RNAi pathway in vivo

- Single IV bolus of LNP-siFVII at 0.0625, 0.125 or 0.25 mg/kg
- Liver mRNA and siRNA levels measured on days 1, 3, 7 and 10 post-dose
Mechanism of Liver Silencing with LNPs
ApoE Dependence in Cultured Primary Hepatocytes

Cellular uptake of siRNA requires ApoE for ionizable LNPs but not cationic LNPs
- Primary mouse hepatocytes exposed to 20 nM cLNP- or iLNP- AF647-siRNA
- Cultures fixed and stained with DAPI after 4 hours

Control + ApoE

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cLNP
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![Images of cellular uptake with control and ApoE treatment for cLNP and iLNP](Image)
Mechanism of Silencing in Liver with LNPs
ApoE Dependence In Vivo

iLNP silencing of FVII is absent in ApoE KO mice but restored by premixing with ApoE
- Single IV bolus of iLNP-siFVII at 0.2 mg/kg, with or without rhApoE at 0.0003 - 0.1 mg/kg
- FVII liver mRNA and serum protein levels measured 48 hours post-dose
Mechanism for LNP Delivery Solved
Delivery is ApoE Mediated

1. Endogenous ApoE bind to LNPs

Lipoprotein particle

ApoE

Exchange of ApoE

pH 7.4

Blood Compartment

Fenestration

Space of Disse

Hepatocyte
Mechanism for LNP Delivery Solved
Delivery is ApoE Mediated

2. LNPs traffic through fenestrated endothelium of liver; bind to LDL receptor
Mechanism for LNP Delivery Solved
Delivery is ApoE Mediated

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

Lipoprotein particle
ApoE
Exchange of ApoE
pH 7.4
Blood Compartment
Fenestration
Space of Disse
Hepatocyte

ApoE-binding cell surface receptor

pH ~ 5
Asialoglycoprotein Receptor (ASGR) Targeting

- Tissue specific
  - High receptor expression on liver hepatocytes
- Readily endocytosed (high turnover)
- Well-studied design requirements for high affinity ligands
- Well conserved across species
  - Mouse and human are 89% and 80% identical to rat (HL-1)
- Proven delivery of cargo to liver
  - E.g., recombinant proteins with incomplete sialylation

Asialoglycoprotein receptor (ASGR)
Impact on rate of clearance
ASGR Targeting by GalNAc LNPs

iLNP silencing of FVII in ApoE KO mice can be rescued by incorporation of GalNAc3

- Single IV bolus of iLNP-siFVII at 0.2 mg/kg, with or without 0.005 – 0.5% GalNAc3 incorporated
- FVII serum protein levels measured 48 hours post-dose
Delivery of RNAi with LNPs
Summary of Research Progress

• Delivery extended from lung, liver, liver tumor and CNS to immune cells and extrahepatic tumors
• Systemic delivery to liver has advanced
  » Improved potency with LNPs by ~ 100-fold over prototype
  » Mechanistic understanding of LNPs beginning to be elucidated
    – PK/PD relationship defined for LNPs
    – ApoE mechanism demonstrated
    – Proof-of-concept for ASGR-targeted LNPs demonstrated with GalNAc
• Mechanistic understanding of systemic delivery with LNPs provides basis for design of next generation formulations
Delivery of RNAi with LNPs
Clinical Programs

ALN-VSP to treat solid tumors with liver involvement
- Prevalent solid tumor and common site of metastatic disease
  - ~630,000/yr Incidence of HCC worldwide
  - ~515,000/yr Patients with liver metastasis
- ALN-VSP is a dual-target product, formulated in LNP for tumor delivery
  - Targeting 2 pathways increases potential therapeutic impact
    - Proliferation: Kinesin Spindle Protein (KSP)
    - Angiogenesis: VEGF
  - Phase I liver cancer study enrolling; Preliminary data mid-2010

ALN-TTR to treat transthyretin (TTR)-mediated amyloidosis (ATTR)
- ATTR caused by mutation in TTR gene
  - Amyloid deposits in nerves and heart
    - Familial Amyloid Polyneuropathy (FAP) - loss of autonomic function, painful neuropathy
    - Familial Amyloid Cardiomyopathy (FAC) - congestive heart failure
  - ~50,000 patients with significant morbidity and mortality
- Clinical pathology
  - Typical onset ~40 to >60 yr
  - Fatal within 5-15 years
- ALN-TTR comprises an siRNA targeting all mutant forms of TTR as well as wild-type TTR, formulated in LNP for liver delivery
- Phase I start in H1, ’10
# Alnylam Development Pipeline

## Key Programs

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<th>Disease Area</th>
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<th>Development</th>
<th>Phase I</th>
<th>Phase II</th>
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<td>Huntington’s Disease</td>
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- **Alnylam Proprietary Programs**
- **Co-development Programs**
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