Pacifichem 2010:
Development of RNAi Therapeutics
December 15, 2010
RNA Interference

Natural Process of RNAi

Targeted Gene Silencing

mRNA degradation

mRNA degradation
RNAi Therapeutics Opportunity
Large Number of Undruggable Targets

New Drug Opportunities

- World of targets

Accessible targets for small molecules/antibodies

RNAi accessible targets

- Transcriptome targeting
  - mRNA
  - miRNA
  - Other RNA

- "Undruggable" targets
- Cross species activity
- Multi-targeting
- Mutational, allelic, and splice variant specificity

New targets and disease
Agenda

- RNAi Lead Discovery
- RNAi Delivery
- Alnylam RNAi Therapeutic Programs
Alnylam RNAi Product Platform
Turning siRNAs into Drugs

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

Lead Optimization
- Stabilization
- Potency
- Selectivity

Delivery
- PK/PD
- Biodistribution
- Cellular uptake

Introduce chemical modifications for “drug-like” properties

Chemistry, Manufacturing and Controls
- Small Scale
- Gene walks
- In vitro assays

- Medium Scale
  - In vivo biology

- Large Scale
  - GMP Production
  - Clinical trials
Alnylam RNAi Product Platform
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## siRNA Design Architecture

<table>
<thead>
<tr>
<th></th>
<th>Natural, canonical</th>
<th>Unnatural, non-canonical</th>
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</thead>
<tbody>
<tr>
<td><strong>Design</strong></td>
<td><img src="image" alt="siRNA Diagram" /></td>
<td><img src="image" alt="shRNA Diagram" /></td>
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<tr>
<td><strong>siRNA</strong></td>
<td><img src="image" alt="siRNA Structure" /></td>
<td><img src="image" alt="shRNA Structure" /></td>
</tr>
<tr>
<td><strong>Length</strong></td>
<td>19-23</td>
<td>&gt;24</td>
</tr>
<tr>
<td><strong>Chemistry</strong></td>
<td>Yes/No; Lower COGS</td>
<td>Yes/No; Higher COGS</td>
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<tr>
<td><strong>Biology</strong></td>
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<tr>
<td>Potency ↑</td>
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<tr>
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<tr>
<td>Immunostimulation ↓</td>
<td><img src="image" alt="Green Dot" /></td>
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<td>miRNA pathway ↓</td>
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<tr>
<td># Publications <em>in vivo</em></td>
<td>100’s</td>
<td>&lt;10</td>
</tr>
<tr>
<td># Programs in clinic</td>
<td>10</td>
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</tbody>
</table>

Most to least positive pharmacologic properties

![Color Legend](image)
Canonical vs. “Dicer Substrate” siRNAs
Equipotent in Cell Assays

<table>
<thead>
<tr>
<th>Efficacy Screen (10 nM)</th>
<th>Range of Potencies of Leads</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Canonical (pM)</td>
</tr>
<tr>
<td>Gene 1</td>
<td>3-16</td>
</tr>
<tr>
<td>Gene 2</td>
<td>3-19</td>
</tr>
</tbody>
</table>
Active siRNAs are Not Evenly Distributed
Identifying High Potency siRNAs

*In Vitro* Screens

**Single dose screen**

Dose response curves of best candidates...versus a less efficient one

IC$_{50}$ 6.0 pM

IC$_{50}$ 3.2 pM

IC$_{50}$ 1.4 nM
In Vitro Potency Predicts In Vivo Potency
Data from Two Distinct Targets

\[ R^2 = 0.6 \]
Large datasets create “rules” for predicting efficacy
» >12,000 Data points

Rules direct siRNA modification when sets are limited

Rules continue to be refined as datasets grow
» >20,000 Data points to date
Key Chemical Modifications of siRNAs

Sugar

2'-Deoxy  Ribo  2'-O-Me  2'-O-MOE  2'-F  2'-araF (FANA)  4'-Thio  LNA  UNA

Backbone
(R = H, OH or 2'-modified)

Phosphate  (P=O)  Phosphorothioate  (P=S or PTO)
siRNA Stabilization
Critical Role for Chemistry

Prevention of exonuclease degradation
- phosphorothioate

Prevention of endonuclease degradation
- 2'-OMe
- 2'-F

Unmodified siRNA
Modifed siRNA

Full Length Product (%)
Time, hours

Chemically-modified siRNAs

Unmodified siRNA
Maximizing RNAi Selectivity

**RISC/miRNA “seed”-mediated**
- Potential to target homologous sequences in non-target genes
- **Solutions**
  - IC\textsubscript{50} specificity analysis toward “nearest” homologs

**Interferon/TNF-mediated**
- Innate immunity can be activated by dsRNAs
  - Specific motifs appear to activate via Toll-like receptors and RIG-1
    - Collaborations with Drs. Gunther Hartmann, (U. Bonn) and Bryan Williams, (Monash)
- **Solutions**
  - Screen for interferon and TNF-induction using human primary blood mononuclear cells (PBMCs)
  - Confirm *in vivo* during pharmacology studies
Off-Targeting Not Observed \textit{In Vitro} With Selection Rules

\section*{On-target mRNA}

5' - TTGCGAAUCGUGCAUGCAGUGACCAG - 3'
3' - CUUACGACGUACGUCACU - 5'

\textbf{siRNA AS strand}

\section*{Off-target 1}

5' - NNNNGACUGCAUGCAUGACNNN - 3'
3' - CUUACGACGUACGUCACU - 5'

\section*{Off-target 2}

5' - NNNNGAAUCGCAUGCAUGACNNN - 3'
3' - CUUACGACGUACGUCACU - 5'

\section*{Off-target 3}

5' - TTGCGAAUCGCAUGCAUGCAGCG - 3'
3' - CUUACGACGUACGUCACU - 5'

\section*{Off-target 4}

5' - TTGCGAAUCGCAUGCAUGCAGCG - 3'
3' - CUUACGACGUACGUCACU - 5'

\section*{Off-target 5}

5' - TTGCGAAUCGCAUGCAUGCAGCG - 3'
3' - CUUACGACGUACGUCACU - 5'

\section*{Off-target 6}

5' - TTGCGAAUCGCAUGCAUGCAGCG - 3'
3' - CUUACGACGUACGUCACU - 5'

\section*{Off-target 7}

5' - TTGCGAAUCGCAUGCAUGCAGCG - 3'
3' - CUUACGACGUACGUCACU - 5'

\section*{Off-target 8}

5' - TTGCGAAUCGCAUGCAUGCAGCG - 3'
3' - CUUACGACGUACGUCACU - 5'
Selecting “Immunosilent” siRNAs

**In Vitro PBMC Assay**

**IFNα**

**TNFα**

**In Vivo Serum Cytokines**

i.v. bolus 4 mg/kg siRNA in liposomal formulation

<table>
<thead>
<tr>
<th>Cytokine (pg/mL)</th>
<th>Saline</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
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<tbody>
<tr>
<td>IFNα</td>
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<td></td>
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</table>

Tides, May 2008
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  » Introduce chemical modifications for “drug-like” properties

Delivery
- conjugation
- formulation
- 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
Achieving Delivery
Modification and Formulation

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

Broad and significant effort at Alnylam
- Alnylam delivery platform leads across industry
- Internal and external collaborations
  - MIT, MP, UBC, Tekmira, AlCana, Isis

Major progress achieved
- Direct and Systemic delivery
- Robust *in vivo* efficacy (Alnylam experience)
  - >75 Targets silenced in vivo
    - Includes many “un-druggable”
  - >5 Organs
    - Includes lung, liver, gut, and CNS
    - Multiple tumor models (xenograft, orthotopic, and transgenic); hepatic, extra-hepatic, and subcutaneous
    - New data with immune cells and endothelial cells
  - 6 Species
    - Includes humans
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
1st Generation 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*

**Graph 1**
- 2 day and 11 day post-dose comparisons
- mRNA and Protein percentage control
- Dose: 1 mg/kg, 2.5 mg/kg
- 100% control

**Graph 2**
- Day 11 post-dose (2.5 mg/kg)
- Cholesterol: 34.1, >65% inhibition
- LDL: 14.2, >85% inhibition
- HDL: 109.8

* P < .05 ** P < .005

*Nature*, 441, 111-114, Mar 2006
Recent LNP Delivery Publications

Key Publications

- Lipid-like materials for low-dose \textit{in vivo} gene silencing

- Rational design of cationic lipids for siRNA delivery

- Targeted delivery of RNAi therapeutics with endogenous and exogenous ligand-based mechanisms
  » Akinc \textit{et al.}, \textit{Molecular Therapy}, 18:1357-1364 (July 2010)
Combinatorial Chemistry Approach for Novel Materials

Library Components

Synthetic Scheme

Rational Design Approach for Novel Materials
Alnylam-UBC/AlCana Collaboration

Cylindrical shape supports bilayer structure

Cone shape disrupts bilayer structure

Bilayer

Hexagonal H_{II}

DLinDMA

Headgroup

Linker

Hydrocarbon Chains
Next Generation LNPs
Remarkable Potency Improvements with Novel Lipids

Novel LNPs set new benchmark for systemic RNAi with ~100 fold improved potency
- Efficacy in pre-clinical models following single IV injection
- Each LNP comprised of distinct cationic lipid component
- Improvement in potency has resulted in ED$_{50}<$0.01 mg/kg

**Efficacy in mouse with systemic RNAi after single IV injection**

- Effects are rapid, potent, dose-dependent and durable
- \( ED_{50} \approx 0.01 \text{ mg/kg} \)
PK and PK/PD Properties of LNPs in Rat

Plasma PK

Liver PK

PK/PD Correlation in Liver

Tissue siRNA levels of ~1 ng/g at ED₅₀ for mRNA suppression demonstrates potency of natural RNAi pathway in vivo
Next-Generation LNPs
Demonstrate Highly Potent and Durable Silencing in NHPs

Single i.v. infusion into NHP and measurement of liver TTR mRNA 48 hrs later

ED50 <0.03 mg/kg

Keystone: Advance in Biopharm., Jan 2010
Evolution and Mechanistic Insights into LNP Delivery
Two Classes of Formulations

**Ionizable LNP (iLNP)**
Alnylam, Tekmira, AlCana, UBC

**DLinDMA**
- Neutral surface charge at physiological pH
- Becomes positively charged at low endosomal pH
- Enters via clathrin-mediated endocytosis
- ApoE-dependent

**In vivo Hepatocyte Silencing**

**EC50**

**Cationic LNP (cLNP)**
MIT

**TETA-5-LAP**
- Slightly positively charged
- Enters via macropinocytosis
- ApoE-independent

**DLin-KC2-DMA**

**DLin-MC3-DMA**

**C12-200**
Cellular uptake of siRNA requires ApoE for ionizable LNPs (iLNPs) but not cationic LNPs (cLNPs)
- Primary mouse hepatocytes exposed to 20 nM cLNP- or iLNP- AF647-siRNA
- Cultures fixed and stained with DAPI after 4 hours

Molecular Therapy, 18:1357-1364, July 2010
Mechanism of Silencing in Liver with iLNPs
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
- FVII liver mRNA and serum protein levels measured 48 hours post-dose

WildType

<table>
<thead>
<tr>
<th>PBS</th>
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<th>ApoE 0.1 mg/kg</th>
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<tbody>
<tr>
<td>LNP-siFVII (0.2 mg/kg)</td>
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ApoE -/-

<table>
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</table>

Relative to control

Molecular Therapy, 18:1357-1364, July 2010
Mechanism of ApoE Mediated iLNP Delivery

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

- **Exchange of ApoE**
- **Fenestration**
- **Blood Compartment**
- **Space of Disse**
- **Hepatocyte**

- **pH 7.4**

- **ApoE-binding cell surface receptor**
- **Cationic lipid combines with anionic membrane lipids to disrupt endosomal membrane**
- **As endosome acidifies, cationic charge on vesicle increases**
- **siRNA cargo is released into cytoplasm where it can enter RISC**

*Molecular Therapy, 18:1357-1364, July 2010*
Translating Delivery Beyond Liver

Current status

- Additional cells/tissues for LNP delivery
  - Liver tumors
  - Liver stellate cells
  - Extra-hepatic tumors
    - Subcutaneous xenografts
    - Lymph node metastases
  - Immune cells
  - Endothelial cells
    - Multiple vascular beds, including heart, muscle, kidney

Expect that this will continue to expand
**ALN-VSP Anti-Tumor Activity**

**Orthotopic Tumor Model**

**RNAi-Mediated Cell Cycle Arrest**
- Single IV injection of ALN-VSP or control siRNA
- Mitotic arrest (monoasters) in VSP-treated animals
- KSP and VEGF mRNAs cleaved, confirming RNAi mechanism

**Potent Anti-Tumor Efficacy**
- ALN-VSP demonstrates anti-tumor activity compared with controls
- Anti-tumor activity associated with improved survival

Keystone RNAi., Jan 2008
Tumor Delivery Extended to Extra-Hepatic Tumors

Mitotic arrest (monoasters) in metastatic colorectal tumors arising from intrahepatic HCT116 seeding in mice

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

AACR., April 2010
Gene Silencing Extended to Immune Cells

LNPs achieves silencing in immune cells

- Silencing observed with multiple targets
  - CD45, GFP, β1 integrin, CD11b, TNFα
- Silencing seen in monocyte/macrophage and dendritic cells
  - Peritoneal cavity > spleen > bone marrow

**CD45 Silencing in Myeloid Cells**

**β1 Integrin Silencing in CD11b+ Myeloid Cells**
Gene Silencing Extended to Endothelial Cells

Dose = 2.5 mg/kg, N= 5 mice/group
siRNA Conjugates for Delivery

- Lipophilic molecules
- Vitamins
- Carbohydrates
- Peptides
- Antibodies

**Ligand**
- Ligand display will affect (receptor) binding and uptake
  - Site of attachment
  - Mono- vs. multivalent
  - Ligand spacing
siRNA Conjugates for Systemic Delivery

- Cholesterol
  - Promotes cellular uptake
  - Improves plasma PK

**RNAi activity in vitro without transfection reagent**

Chol-siRNA Silences ApoB in Liver and Jejunum

* p<0.0001 compared to saline control animals

Asialoglycoprotein Receptor (ASGPR)

**ASGPR**
- Highly expressed in hepatocytes
  - 0.5-1 million copies/cell
- Clears serum glycoproteins via clathrin-mediated endocytosis
- High rate of uptake
- Recycling time ~15 minutes
- Conserved across species

Lee, *JBC*, 1982, **257**, 939
Cummings *et al* *Essentials of Glycobiology* 2008, Park *et al* *PNAS* 2005
Design of GalNAc Ligand for siRNA Conjugation

- Site of Conjugation

- Alnylam Design
  - Spacing: 20 Å

- Ligand
  - Mouse Hepatocyte Ki

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<thead>
<tr>
<th>Ligand</th>
<th>Mouse Hepatocyte Ki</th>
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<tbody>
<tr>
<td>GalNAc$_2$</td>
<td>~24 nM</td>
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<tr>
<td>GalNAc$_3$</td>
<td>2.7 nM</td>
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AASLD Annual Meeting, Oct 2010
Silencing via I.V. Dosing
Single Dose, 48h, Mouse Liver mRNA

Relative ApoB mRNA (% PBS Treated)

ApoB siRNA – Chol/GalNAc₃ (mg/kg)

PBS
100
75
50
25

AASLD Annual Meeting, Oct 2010
Optimizing Dose Regimen with GalNAc-siRNA Conjugates

Fast uptake and receptor recycling points towards exploring dosing regimen

Once Daily Dosing For 3 Consecutive Days

125I labeled 3’ Chol/GalNAc₃

ApoB-Chol-GalNAc

PBS  3x25mg/kg  3x10mg/kg  3x5mg/kg

% PBS Control (mApoB/mGapdh)
Robust Silencing with TTR-GalNAc Conjugates
S.C. Single Dose, 72h, Mouse

Major advance in RNAi delivery: *in vivo* efficacy with subcutaneous dosing
- Single SC dose; Transthyretin target
- ED$_{50}$ ~5 mg/kg

![Graph showing relative TTR mRNA levels for different doses of TTR-GalNAc.](image)

- **Control**: 0
- **30 mg/kg**: 46
- **15 mg/kg**: 30
- **7.5 mg/kg**: 20
- **3.5 mg/kg**: 15
- **1.75 mg/kg**: 10
- **0.5 mg/kg**: 5
Direct RNAi
• Successfully applied for both lung and CNS applications

Systemic RNAi
• Lipid nanoparticles (LNPs)
  » Systemic delivery to liver greatly advanced
    – ~100-Fold potency improvement over 1st generation
    – \(ED_{50}\) at very low doses <10 \(\mu\)g/kg
  » Mechanistic understanding of LNPs elucidated
  » LNP delivery extended beyond hepatocytes
    – Includes hepatic tumors, extra-hepatic tumors, immune cells, endothelial cells, and hepatic stellate cells
    – Significantly broadens opportunities for RNAi therapeutic medicines

• Conjugates
  » Efficacy in liver via both i.v. and s.c. routes
    » \(ED_{50}\) at single digit mg/kg doses
  » Additional ligands possible for targeted delivery
Agenda

- RNAi Lead Discovery
- RNAi Delivery
- Alnylam RNAi Therapeutic Programs
Clinical Development of RNAi Therapeutics
Mapping Alnylam’s Progress

- 8 Completed or ongoing clinical trials
- >400 Subjects/patients enrolled
- 1st Human proof-of-concept 2008
- 1st Systemic delivery program 2009
- By end-2010, studies conducted in 10 countries
  > US, Canada, UK, Germany, France, NL, Portugal, Sweden, Austria, Australia, Spain
# Alnylam Development Pipeline

## Key Programs

<table>
<thead>
<tr>
<th>Condition</th>
<th>Phase I</th>
<th>Phase II</th>
<th>Phase III</th>
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</thead>
<tbody>
<tr>
<td><strong>RSV Infection</strong></td>
<td>ALN-RSV01 (Adult)</td>
<td>ALN-RSV02 (Pediatric)</td>
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</tr>
<tr>
<td><strong>Liver Cancers</strong></td>
<td>ALN-VSP02</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>TTR-Mediated Amyloidosis</strong></td>
<td>ALN-TTR01</td>
<td>ALN-TTR02</td>
<td></td>
</tr>
<tr>
<td><strong>PCSK9/Hypercholesterolemia</strong></td>
<td>ALN-PCS</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Huntington’s Disease</strong></td>
<td>ALN-HTT</td>
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</tr>
</tbody>
</table>

**Legend:**
- Alnylam Proprietary Programs
- Co-development Programs
Respiratory Syncytial Virus (RSV) Program
ALN-RSV

Harness RNAi for a major infectious disease

- Significant unmet medical need
  - >125,000 Pediatric hospitalizations/yr in US
  - >170,000 Adult hospitalizations/yr in US
- No effective therapies to treat RSV infection
  - Synagis used for prevention
- ALN-RSV01 in clinical development
  - Human POC demonstrated in GEMINI Phase II study
  - Preliminary Phase IIa adult lung transplant data
  - Phase IIb adult lung transplant study planned
    - Enrollment start in 1Q’10
- ALN-RSV02 for pediatric indication
- 50-50 Partnership with Cubist
- Partnered with Kyowa Hakko Kirin in Asia
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 experimental infection study (n=88)
- ~40% Relative reduction in infection rate (P<0.01)
- ~95% Increase in number of uninfected subjects (P<0.01)

Int'l Symp Res Vir Infect, Feb 2008
Liver Cancer Program
ALN-VSP02

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-VSP02 is dual-target product
  - Targeting 2 pathways increases potential therapeutic impact
    - Proliferation: Kinesin Spindle Protein (KSP)
    - Angiogenesis: VEGF
  - Lipid nanoparticle formulation
    - With Tekmira Pharmaceuticals

- ALN-VSP02 in clinical development
  - Phase I liver cancer study enrolling
  - ASCO 2010: Encouraging preliminary data
  - Dose escalation ongoing
ALN-VSP Phase I Study
Study Design and Objectives

Study Design
- Multicenter, open label, dose escalation study
- ~55 Patients with advanced solid tumors with liver involvement
  - Failed to respond or progressed after standard treatment

Primary Objective
- Safety, tolerability, and pharmacokinetics
  - Demonstration of maximum tolerated dose

Secondary Objectives
- Tumor response
- Tumor blood flow/vascular permeability
  - Measured by DCE-MRI
- Plasma biomarkers of angiogenesis
- Pharmacodynamic effects of ALN-VSP on tumors
  - Patients volunteering for pre- and post-treatment biopsies
ALN-VSP02 Phase I Study Results
Preliminary Data at ASCO 2010 and Chemotherapy Foundation Symposium

Presented Data Demonstrate Tolerability, PK and PD Effects

- Trial continuing in dose escalation
  - 28 Patients enrolled; 127 Total doses administered; Range of 2-13 doses/patient
  - MTD not yet achieved
- ALN-VSP02 well tolerated in most patients
  - No dose-dependent trends in clinical/laboratory adverse effects, including changes in LFTs
- 17 Biopsy samples obtained for molecular analysis; Ongoing
- While preliminary, some encouraging pharmacodynamic data
  - >40% decline in Ktrans in 62% of evaluable liver tumors
- Several patients at higher dose groups with stable disease, enrolled in extension study

ASCOC, June 2010; Chemotherapy Foundation Symposium, November 2010
KSP and VEGF siRNA Mean Plasma Concentrations
Time Profiles and Parameter Estimates by Dose Level

ALN-VSP Dose 1 (mg/kg) | 0.1 | 0.2 | 0.4 | 0.7
---|---|---|---|---
C<sub>max</sub> (µg/mL) | 0.76 ± 0.36 | 2.3 ± 0.54 | 3.2 ± 1.2 | 9.8 ± 4.1
<sup>t</sup>max (min) | 18.3 ± 5.8 | 16.7 ± 2.9 | 17 ± 3 | 20 ± 5
AUC<sub>0-last</sub> (µg·min/mL) | 30.9 ± 21.1 | 130.7 ± 44.9 | 201.3 ± 38.6 | 501.2 ± 203.9

ALN-VSP Dose 3 (mg/kg) | 0.1 | 0.2 | 0.4 | 0.7
---|---|---|---|---
C<sub>max</sub> (µg/mL) | 0.93 | 2.2 ± 0.40 | 4.8 | 9.3
<sup>t</sup>max (min) | 15 | 16.7 ± 2.9 | 18 | 15
AUC<sub>0-last</sub> (µg·min/mL) | 37.3 | 115.5 ± 63.3 | 252.3 | 579.3

**KSP siRNA**

**VEGF siRNA**

Dose 1

- 0.1 mg/kg
- 0.4 mg/kg
- 0.2 mg/kg
- 0.7 mg/kg

Dose 3

- 0.1 mg/kg
- 0.4 mg/kg
- 0.2 mg/kg
- 0.7 mg/kg

Standard Deviation was calculated for n≥3
### Human PK Correlation with Pre-Clinical PK Data

<table>
<thead>
<tr>
<th>ALN-VSP02 Phase I Doses (mg/kg)</th>
<th>VEGF siRNA Phase I Doses (mg/kg)</th>
<th>VEGF siRNA Predicted Exposure in Human (AUC) (µg•min/mL)</th>
<th>Observed Human VEGF siRNA Exposure Mean AUC (range) (µg•min/mL)</th>
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</thead>
<tbody>
<tr>
<td>0.1</td>
<td>0.05</td>
<td>41.2</td>
<td>31.7 (13.7 to 50.4)</td>
</tr>
<tr>
<td>0.2</td>
<td>0.1</td>
<td>82.4</td>
<td>115.2 (84.5 to 131.6)</td>
</tr>
<tr>
<td>0.4</td>
<td>0.2</td>
<td>164.8</td>
<td>149.6 (133.2 to 160.0)</td>
</tr>
<tr>
<td>0.7</td>
<td>0.35</td>
<td>288.4</td>
<td>395.4 (263.4 to 482.2)</td>
</tr>
</tbody>
</table>

- Observed human siRNA exposure data show dose proportionality
- Exposure in humans in terms of plasma exposure within 1-2 fold of preclinical modeling
Alnylam RNAi Therapeutic Programs
Summary

- Alnylam is leading translation of RNAi therapeutics in development
  - 3 RNAi therapeutic programs in clinical trials
  - Expect at least 2 additional clinical programs in 2011
  - Advancing programs with both local delivery and systemic delivery
- Human PK/PD correlates with pre-clinical experience
- Initial human POC established
  - ALN-RSV01 GEMINI study
  - More to emerge in upcoming months and years
    - Includes data from systemic delivery programs: VSP and TTR
- Significant discovery pipeline across broad range of disease indications