AsiaTIDES 2012:
Formulation and Delivery of Peptides and Oligonucleotides
Strategies for Delivery of RNAi Therapeutics

March 1, 2012
Akin Akinc, PhD
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
- RISC loading

**Chemistry, Manufacturing and Controls**
- Small Scale
- Gene walks
- *In vitro* assays
- Medium Scale
  - *In vivo* biology
- Large Scale
  - GMP Production
  - Clinical trials
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siRNA Conjugates: An Emerging Approach to Delivery

- Lipophilic molecules
- Vitamins
- Carbohydrates
- Peptides
- Antibodies

siRNA

GalNAc-siRNA

Free Uptake and Silencing by GalNAc-siRNA

Graph showing % Remaining Message vs uM siRNA for TTR-GalNAc 1, TTR-GalNAc 2, and TTR-GalNAc 3.
**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
Features of ASGPR Support Dose Optimization

- Fast uptake, saturation and recycling point towards optimizing dosing regimen
  - Delayed release via SC dosing may help by engaging multiple rounds of receptor uptake
  - Multi-dose SC administration as one approach to optimize receptor utilization
SC Administration of TTR-GalNAc Leads to Robust Gene Silencing

Efficacy via SC greater than via IV administration
- ~55% bioavailability from SC space
- Greater liver exposure after SC dosing
  - $C_{\text{max}}$ and AUC ~3-fold higher compared to IV dosing
ALN-TTRsc achieves sustained silencing in pre-clinical animal model

- Multi-dose subQ regimen; once weekly
- Sustained TTR silencing achieved over multi-week period
- Product differentiation across ATTR patient populations; planned IND in H2 ’12

Subcutaneous Dose Optimization

Summary

• Receptor is available for multiple rounds of uptake
  » As early as 10 minutes post-initial dose (IV)

• SC administration enhances liver exposure of GalNAc-siRNA

• SC split dosing at lower doses demonstrates equivalent silencing to single high dose
  » Multiple schedules possible for maintenance of silencing at clinically feasible dose levels
siRNA-GalNAc Conjugates are Well Tolerated *In Vivo* Rat Tolerability

**Non-GLP Single Dose Tolerability**
- Animals: S-D rats
- Dose levels: 100, 250, 500 & 750mg/kg SC
- Clinical chemistry at 24 and 72 h
- Necropsy at 72 hours, histopathology of kidneys, spleen, liver, heart, testes, thymus, and injection site

**Results**
- No test article-related clinical signs of toxicity or alterations in clinical chemistry
- No histopathology in kidneys, spleen, liver, heart, testes, and thymus
- Non-adverse, test article-related (≥ 250 mg/kg) minimal histiocytic inflammation at injection site
- Single-dose of up to 750 mg/kg is well tolerated in rats
- ~150X safety margin based on ED50 ~ 5mg/kg

![ALT vs TTR-GalNAc](image-url)
siRNA-GalNAc Conjugates are Well Tolerated

Cytokine Assessment

**Whole Blood Assay**
- ≤ 2 fold change in cytokines in whole blood assay (13 cytokine panel)

**In vivo**
- No IL-1β, IFN-γ, TNF-α, IL1-RA or IL6 induction at 4 or 24hrs in NHP dosed at 100mg/kg SC
- No cytokine induction in CD-1 mice dosed at 125mg/kg SC at multiple time points (22 cytokine panel)
- No erythema or edema observed at injection sites in rats or NHP
TTR Protein Suppression in NHP SC Single vs. Multi-Dose Regimen

5X 5mg/kg vs 25mg/kg siRNA

- PBS
- 5x5 mg/kg Control
- 5x5 mg/kg siRNA
- 25mg/kg siRNA

Normalized Serum TTR (Fraction Pre-dose)

Study Day

-15 -10 -5 0 5 10 15 20 25 30

Normalized Serum TTR (Fraction Pre-dose) vs Study Day
Subcutaneous siRNA Conjugate Program Summary

Technology
- Liver specific silencing via ligand-mediated uptake
- Simple formulation – siRNA-GalNAc in buffer

Dosing, Efficacy
- Single dose ED50 ≤ 5 mg/kg
- Low dose multi-dosing enables ED80 silencing

Tolerability
- Non-GLP tolerability assessment in rats consistent with large safety margin
- Well tolerated with single dose NOAEL > 250mg/kg in rats

Overall Goal: Human ALN-TTRsc IND submission in H2’12
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
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$_{50}$
LNP Intracellular Delivery Still Relatively Inefficient Despite Significant Potency Advances

• *in vitro*: HeLa cells transfected at 20 nM siRNA
• *in vivo*: Hepatocytes of treated mice dosed at 0.2 mg/kg siRNA

EM analysis of intracellular localization of gold-siRNA both *in vitro* and *in vivo* indicates that only 1-2% of total cellular gold is in cytoplasm.

Collaboration with Dr. Marino Zerial, Max-Planck
Majority of LNPs Accumulate in Lysosomes

- Significant co-localization with LAMP1, a marker of late endosomes/lysosomes
- Similar results obtained in vivo via imaging of hepatocytes

20nM-24h

Collaboration with Dr Marino Zerial, Max-Planck
Stable Ionizable Lipids Are Persistent *In Vivo*

Formulation: SNALP (DLinDMA)
Dose: 1 mg/kg (siRNA) via 15 min IV infusion
Animals: SD rats

- Stable ionizable lipids have long elimination half-lives in plasma and tissues
Higher Doses of Persistent LNPs Result in Cytotoxicity in Target Tissues

LNP GLP Toxicology Summary

- 4-Dose rat and NHP GLP studies
- Major clinically-significant target organs: liver and spleen
  - Liver primary and spleen secondary in rat; spleen primary and liver secondary in NHP
  - Liver findings: necrosis, incidence and severity increasing with dose; correlates with ALT/AST increases
  - Spleen findings: vacuolation, histocytic infiltration, lymphoid depletion, incidence and severity increasing with dose
- NOAEL rat = 1 mg/kg (based on liver findings), NHP = 1-3 mg/kg (based on spleen findings)
- MTD rat = 3 mg/kg, NHP = 6 mg/kg
- Safety pharmacology generally unremarkable and mutagenicity studies negative
A New Class of Lipids For Rapid Eliminated LNPs (reLNPs)

Rationale

- Stable ionizable lipid components of LNPs are persistent in cells and tissues—may result in accumulation over time and may be responsible for cytotoxicity
- Novel, rapidly eliminated lipid components are less likely to accumulate with chronic dosing and should be better tolerated

Approach

- Design functional ionizable lipids that are biodegradable in vivo
  - Ideally enzymatically degradable for rapid degradation in vivo, but chemically stable for long shelf life
  - Lipid degradation products should be easily metabolized or eliminated
Design of Degradable Linkages Within Lipid Chains

Expected Initial Metabolic Products

**Internal Chain Ester Lipid**

- **Mono-acid (mono-ester):**

  - **Di-acid:**

  - **Hydrolase / H2O**

**Terminal Chain Ester Lipid**

- **Mono-acid (mono-ester):**

  - **Di-acid:**

  - **Hydrolase / H2O**

Expected Initial Metabolic Products include mono-acids and di-acids resulting from the degradation of lipid chains.
Design of Degradable Linkages Within Lipid Chains
Metabolites as Potential Substrates for $\beta$-Oxidation Pathway of Fatty Acids

Lipid Chain Modifications

Additional enzymes 2,4-dienoyl CoA reductase and cis-$\Delta^3$-enoyl isomerase used to oxidize unsaturated fatty acids
Novel Biodegradable Lipids Are Rapidly Eliminated From Plasma \textit{In Vivo}

- reLipid 1 and reLipid 2 undetectable in plasma after 8 hours

![Graph showing the elimination of lipids over time.](image)
Novel Biodegradable Lipids Are Rapidly Eliminated From Tissues In Vivo

- reLipid 1 undetectable in liver and spleen after 8 h and 24 h, respectively
- reLipid 2 undetectable in liver and spleen after 2 h and 8 h, respectively

0.3 mg/kg (siRNA) dose via IV in mice
Rapidly Eliminated LNPs (reLNPs) Are Well-Tolerated In Vivo

Single Dose Toxicology
- Animals: S-D rats
- Dose levels: 1, 3, 5, and 10 mg/kg
- Serum chemistry at 24 and 72 h
- Necropsy at 72 hours, histopathology of liver and spleen

Results
- No adverse clinical signs noted
- No significant alterations in serum chemistry at 24 or 72 h
- No significant histopathology findings (liver and spleen only)
- NOAEL > 10 mg/kg
reLNPs Are Efficacious in Rodents and NHPs

- reLNPs maintain potency gains of 2\textsuperscript{nd} Generation LNPs

**Efficacy in Mice (FVII)**

<table>
<thead>
<tr>
<th>reLNP (mg/kg)</th>
<th>Relative FVII Protein Level</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBS</td>
<td>1.0</td>
</tr>
<tr>
<td>0.1</td>
<td>0.6</td>
</tr>
<tr>
<td>0.03</td>
<td>0.8</td>
</tr>
<tr>
<td>0.01</td>
<td>1.0</td>
</tr>
<tr>
<td>0.003</td>
<td>1.4</td>
</tr>
</tbody>
</table>

**Efficacy in NHPs (TTR)**

<table>
<thead>
<tr>
<th>reLNP siTTR (mg/kg)</th>
<th>Relative Liver TTR mRNA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Naive</td>
<td>1.0</td>
</tr>
<tr>
<td>0.3</td>
<td>0.2</td>
</tr>
</tbody>
</table>
reLNPs Represent 3\textsuperscript{rd} Generation LNPs
With Significantly Improved Therapeutic Index

- Derived from LNP research efforts focusing on both mechanistic understanding and lipid chemistry
- Equivalent potency and \textit{substantially} improved tolerability relative to second generation LNPs
- Formulation optimization and in vivo testing on-going

**Improvement of Therapeutic Index from 1\textsuperscript{st} to 3\textsuperscript{rd} Generation LNP**

<table>
<thead>
<tr>
<th>Rat</th>
<th>0.01 mg/kg</th>
<th>0.1 mg/kg</th>
<th>1.0 mg/kg</th>
<th>10 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>2\textsuperscript{nd} Gen.</td>
<td>3\textsuperscript{rd} Gen.</td>
<td>1\textsuperscript{st} Gen.</td>
<td>3\textsuperscript{rd} Gen.</td>
<td></td>
</tr>
<tr>
<td>Efficacy:</td>
<td>~ 30x</td>
<td>~ 300x</td>
<td>~ 5x</td>
<td></td>
</tr>
</tbody>
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Key: Efficacy

NOAEL Efficacy based on FVII protein reduction
NOAEL based on single dose rat tox.
Systemic RNAi Delivery

Summary

Conjugates
- Efficacy in liver via SC route in rodents and NHP
- Simple formulation
- Promising preliminary toxicology
- Plan to enter clinical testing in H1 2013

Lipid nanoparticles (LNPs)
- Clinically validated approach for delivering RNAi therapeutics to liver (POC established for 1st and 2nd generation LNPs)
- Platform continues to advance rapidly
  - ~100-fold potency improvement over 1st generation
  - 3rd generation LNPs emerging, suggest substantially improved therapeutic index
Thank you