12th US-Japan Symposium on Drug Delivery Systems

Advances in Systemic Delivery of RNAi Therapeutics

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December 18, 2013
RNA Interference (RNAi)
A New Class of Innovative Medicines

**RNAi Therapeutics**

- Harness natural pathway
  - Catalytic mechanism
  - Mediated by small interfering RNA or “siRNA”
- Treat disease with therapeutic gene silencing
  - Any gene in genome
  - Unique opportunities for innovative medicines
- Clinically validated platform
Outline

- GalNAc-siRNA Conjugate Platform
  - Ligand design for targeted delivery to hepatocytes
  - GalNAc-siRNA conjugates for subcutaneous administration
- PK and PD of GalNAc-siRNA Conjugates
- Translation Across Species
GalNAc-siRNA Conjugates
Targeted Delivery to Hepatocytes via ASGPR-Mediated Uptake

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

**GalNAc-siRNA**
- GalNAc ligand conjugated to chemically modified siRNA for targeted delivery to hepatocytes
- GalNAc carbohydrate cluster with high affinity for ASGPR (nM)
- Administered subcutaneously (SC)

Adapted from *Essentials of Glycobiology* (2009)
Carbohydrate Recognition by ASGPR
Structure and Carbohydrate Binding of H1-CRD*

Carbohydrate Recognition Domain of ASGPR Subunit H1

*Meier et al., J. Mol. Biol., 300:857-65 (200)
Design of High-Affinity Ligand for ASGPR

Adapted from Lee et al., Carbohydrates in Chemistry and Biology; 4:549 (2000)

Ligand Design

Impact of Carbohydrate Structure and Valency

~5-Fold decrease in binding affinity for siRNAs containing (GalNAc)2 or (Gal)3 (prim. mouse hepatocytes)

~2-Fold loss of in vivo efficacy (single dose efficacy screen in mouse)
Uptake of GalNAc-siRNA Conjugates

1º Mouse Hepatocytes

- Glucose conjugate does not mediate uptake
- GalNAc₃ BB & EGTA block uptake
- Substantially decreased uptake in ASGR2 KO cells
IV Administration of $^{125}$I-Labeled siRNA-GalNAc Conjugate

% of Injected Dose in Liver

Rapid uptake, receptor saturation and recycling
- Extended plasma exposure may help by engaging multiple rounds of receptor uptake
- Slow release after SC administration may improve receptor utilization

Collaboration with Dr Theo van Berkel
Mode of Administration Impacts Pharmacology
IV vs. SC Comparison of Efficacy and Drug Level in Mouse Liver

TTR mRNA KD in Liver

% mRNA Remaining
Relative to PBS

SC
IV
24 h

SiRNA in Liver

Drug level in Liver, g/g

SC
IV
24 h

GalNAc-siRNA conjugates efficiently target liver

- Achieve liver levels of >50% delivered dose
Synthesis of GalNAc Support and Conjugate

**Synthesis/Process**
- Developed at Alnylam
- Compatible with multiple solid supports
- Compatible with solid phase RNA synthesis and deprotection conditions

**GalNAc Solid Support**
- Convergent multi-step synthesis
- Scalable to multi-kilo scale

**siRNA-GalNAc Synthesis**
- Solid phase phosphoramidite chemistry
- Scalable to multi-kilo scale
GalNAc-siRNA Conjugates
Broad Platform for SC Delivery of RNAi Therapeutics

Potent, rapid, dose-dependent and durable target knockdown with SC administration

- Any hepatocyte target gene
- \( \text{ED}_{80} \) levels for target knockdown ranging from 0.5 – 5 mg/kg
  - Generally achieved with qw dosing regimen
  - More potent conjugates reflect benefit of continued platform improvements
- Doses of \( \leq 2.5 \) mg/kg enable SC injection at volumes \( \leq 1 \) mL

ALN-PCSsc targeting PCSK9 to lower cholesterol for the treatment of hypercholesterolemia

ALN-AT3 targeting AT to restore thrombin generation in in hemophilia and rare bleeding disorders
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PK/PD Study Design
ALN-AT3 Pre-clinical Studies in Wild Type Mice

**Male C57BL/6 mice (20-30 grams)**

**Organs:** Liver, spleen, kidney, blood (n=2 /time point/group) at specified time points.

**qPCR assay - LLOQ Plasma = 0.004 ng/mL & Liver = 0.4 ng/g**

**RISC-loaded siRNA levels were determined by Ago2 IP followed by RT-qPCR**

**Plasma and liver samples at each time point were analyzed for AT protein in Plasma and AT mRNA in liver**

<table>
<thead>
<tr>
<th>Group</th>
<th>siRNA</th>
<th>Dose (mg/kg)</th>
<th>Conc. (mg/mL)</th>
<th>Route</th>
<th>No. of Males</th>
<th>Blood and Tissues Collection Time Points</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>AT3</td>
<td>1</td>
<td>0.1</td>
<td>SC</td>
<td>44</td>
<td>0.083, 0.25, 0.5, 1, 2, 4, 8, 24, 48, 96, 120, 168, 240, 336, 408, 504, 576, 672, 744, 840, 912 and 1008 h post-dose</td>
</tr>
<tr>
<td>2</td>
<td>AT3</td>
<td>2.5</td>
<td>0.25</td>
<td>SC</td>
<td>44</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>AT3</td>
<td>5</td>
<td>0.5</td>
<td>SC</td>
<td>44</td>
<td></td>
</tr>
</tbody>
</table>
Time-Concentration Profiles in Plasma and Liver

ALN-AT3 Pre-clinical Studies

### AT3 Conjugate in Plasma

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>1.0</th>
<th>2.5</th>
<th>5.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apparent t1/2β (h)</td>
<td>0.46</td>
<td>0.38</td>
<td>0.61</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Cmax (µg/g)</td>
<td>0.117</td>
<td>0.420</td>
<td>0.686</td>
</tr>
<tr>
<td>AUC0-t (h·µg/g)</td>
<td>0.176</td>
<td>0.507</td>
<td>0.804</td>
</tr>
</tbody>
</table>

### AT3 Conjugate in Liver

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>1.0</th>
<th>2.5</th>
<th>5.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apparent t1/2β (h)</td>
<td>112</td>
<td>126</td>
<td>126</td>
</tr>
<tr>
<td>Tmax (h)</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Cmax (µg/g)</td>
<td>5.75</td>
<td>13.2</td>
<td>27.9</td>
</tr>
<tr>
<td>AUC0-t (h·µg/g)</td>
<td>302</td>
<td>1015</td>
<td>2274</td>
</tr>
</tbody>
</table>
Potent and durable silencing achieved after single s.c. dose of ALN-AT3

- Full recovery to baseline 40 days post-dose
- Comparable PD profiles for AT mRNA and plasma protein
Total vs. RISC-loaded siRNA in Liver
Silencing Correlates with RISC-Loaded siRNA

- Tmax of RISC-loaded siRNA shifted relative to total siRNA
- Rate of depletion of RISC-loaded siRNA slower than total siRNA
- Amount of RISC-loaded siRNA correlates well with silencing activity
PK/PD Relationship
Total ALN-AT3 in Liver

Target gene silencing achieved at low liver tissue exposure

- EC$_{50}$ at $\sim$0.1 $\mu$g/g tissue (>120 h post-dose)
  - Compares very favorably with other oligonucleotide platforms requiring >100 $\mu$g/g tissue levels$^1$

![Graph showing correlation between total siRNA in liver and percent AT mRNA silencing.](image)

$R^2 = 0.859$

$^1$Mipomersen FDA Advisory Committee Briefing Document, October 2012
Target gene silencing achieved at very low RISC-loaded siRNA concentrations

- EC$_{50}$ at ~1.5 ng/g tissue (~800 molecules/cell)
  - ~100x lower than EC$_{50}$ values obtained for total siRNA

\[ R^2 = 0.7111 \]
Weekly SC administration of ALN-AT3 in WT mice results in potent and consistent AT suppression

- Repeat dose ED<sub>50</sub> for AT knockdown <0.75 mg/kg
- Nadir reached at ~day 14

![Graph showing maintenance of AT knockdown with repeat dosing of ALN-AT3](image-url)

ALN-AT3 Pre-clinical Studies

100
80
60
40
20
0
-20

% Antithrombin Knockdown Normalized to Pre-Dose and PBS

ALN-AT3 (mg/kg)

- 0.75
- 1.50
- 3.00

Day

ALN-AT3, qw x5

WFH, July 2012
Drug Levels in Tissue During Chronic Dosing
ALN-AT3 Pre-clinical Studies

Drug Levels in Mouse Liver
Actual Data

- ALN-AT3, dosing (qw x 14)
- Tissue collection:
  - post-dose (4 h)
  - Pre- and post-dose (4 h)
  - Pre- and post-dose (4 h, 3 days)
  - Pre- and post-dose (4 h)
  - Pre- and post-dose (4 h)

- Day

- siRNA in liver (ng/g)
- 10000
- 1000
- 100
- 10

- 0.2 mg/kg qw
- 0.5 mg/kg qw
No evidence for drug accumulation during chronic dosing (after 3rd weekly dose)

Mean steady state liver drug load of 400 and 1100 ng/g at 0.2 and 0.5 mg/kg qw, respectively

Mean liver drug load proportional to dose
Long Term Conjugate Pharmacology
Example: Ongoing Chronic Dosing Study in Mice w/ TTR GalNAc-siRNA

Steady knockdown maintained with chronic dosing
- Sustained knockdown at both ED50 (1 mg/kg) and ED80 (2.5 mg/kg) dose levels
  - Rodent orthologue with improved potency compared with ALN-TTRsc
- Demonstrated absence of tachyphylaxis or sensitization
- No changes in serum TTR levels in PBS control group

![Graph showing % Knockdown Normalized Serum TTR (Fraction Pre-dose) over time for PBS, 1.0 mg/kg, and 2.5 mg/kg doses.](image)
● GalNAc-siRNA Conjugate Platform
  » Ligand design for targeted delivery to hepatocytes
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● PK and PD of GalNAc-siRNA Conjugates

● Translation Across Species
ALN-AT3 Pre-Clinical Efficacy
Potent and Durable Pharmacologic Effect in NHP

ALN-AT3 achieves potent AT knockdown and fully corrects thrombin generation in NHP

- Weekly SC doses result in potent, dose-dependent, and durable AT knockdown over >5 mos
- In NHP hemophilia “inhibitor” model, ALN-AT3 fully restores thrombin generation to normal levels

** ISTH, July 2013 **
ALN-PCSsc Pre-Clinical Efficacy
Potent PCSK9 Knockdown and LDL-C Lowering in NHP

ALN-PCSsc achieves potent PCSK9 knockdown and LDL-C lowering with SC dosing
- Up to 95% PCSK9 knockdown and up to 67% lowering of LDL-C in absence of statins in NHP
- Durability >50 days after last dose; supports q2w dosing, possibly q4w
- Development Candidate selected; IND in late ’14
ALN-TTRsc Phase I Study Results
TTR Knockdown in Multi-Dose Cohorts

Randomized, double-blind, placebo-controlled SAD and MAD study in healthy volunteers

- Rapid, dose-dependent, consistent, and durable knockdown of serum TTR
  - Statistically significant knockdown of serum TTR at all doses evaluated (p<0.01)
  - Up to 94% TTR knockdown; Mean TTR knockdown of 87.5% and 92.4% at 5.0 and 10.0 mg/kg, respectively

- Generally safe and well tolerated
  - Only AEs associated with drug were generally mild ISRs, resolving within ~2 hours of onset

- Excellent correlation of human to non-human primate TTR knockdown on mg/kg basis
  - Confirmation of human translation of GalNAc-siRNA conjugate platform

Heart Failure Society of America, Sept. 2013
GalNAc-siRNA Conjugates

Summary

- Broad therapeutic platform for any hepatocyte target gene utilizing high capacity receptor-ligand system enabling convenient multi-dosing paradigm
  - Sub-cutaneous administration
  - Potent and durable target knockdown demonstrated for multiple targets
  - No evidence for drug accumulation in target tissue during chronic dosing
  - Sustained target knockdown without tachyphylaxis or sensitization during long term chronic dosing

- Rational design and efficient synthesis of trivalent GalNAc ligand
  - High receptor binding affinity enabling efficient in vivo delivery to hepatocytes
  - Successful large scale manufacture of siRNA-conjugates

- PK/PD evaluation confirms potency of RNAi
  - $EC_{50}$ for RISC-loaded conjugate: $\sim 1.6 \text{ ng/g (} \sim 800 \text{ molecules/cell)}$
  - $EC_{50}$ for total conjugate in liver: $\sim 0.1 \text{ ug/g}$

- Wide therapeutic index with favorable safety profile

- Excellent translation to higher species with potent and durable target knockdown in non-human primates and in human
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