Enhanced Pharmacologic Activity and Durability Demonstrated with an ESC GalNAc-siRNA Targeting Transthyretin

Oligonucleotide Therapeutics Society
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## Receptor Targeted siRNA Conjugates for Delivery to Hepatocytes

### Lead Selection & Optimization

<table>
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<tr>
<th>Selection</th>
<th>Optimization</th>
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</table>
| - siRNA sequence  
  » Specificity  
  » Crossreactivity  
  » Potency | - siRNA chemistry  
  » Stability  
  » Immuno-silence  
  » Potency |

Introduce chemical modifications for “drug-like” properties

### Subcutaneous Delivery

#### GaINAc-siRNA Conjugates

![GaINAc-siRNA Conjugates Diagram](image)

- ASGPR Receptor
  - Highly expressed on hepatocytes
  - Rapidly recycles
  - Conserved across species

### Chemistry, Manufacturing and Controls

- Small Scale
  - Gene walks
  - *In vitro* assays
- Medium Scale
  - *In vivo* biology
- Large Scale
  - GMP Production
  - Clinical trials
Optimization of siRNA Chemistry
Enhanced Stabilization Chemistry (ESC)

5'-S

Standard Template Chemistry

siRNA seq / chemistry design

In vivo efficacy / tissue exposure

Lead Optimization

siRNA synthesis

5'-AS

In vitro stability (lysosomal lysate)

Liver exposure (mouse)

Conjugate conc. in liver (ng/g)

AT3-STC

AT3-ESC

In vitro screening

Manoharan, TIDES, May 2014
ESC Significantly Enhances Efficacy and Duration
Reduction of AT Protein After Single SC Dose in NHP

Potent and durable silencing achieved after single SC dose

- >10-fold improvement in efficacy over standard template chemistry
- Substantially extended duration of effect
Selection of Transthyretin (TTR) ESC GalNAc-siRNAs

Lead ID → In vivo Screen → Rat Screening Tox → NHP Pharmacology → Development Candidate

Key Data

- In vitro activity of ESC TTR-GalNAc siRNAs
- Metabolic stability
- TTR knockdown in hTTR V30M transgenic mice
- Rat PK
- Assessment of MD rat tox
- Assessment of SD and MD knockdown in NHP

Graphs and charts showing data on % TTR mRNA Remaining, % Remaining mRNA, and % Remaining After 24h for different siRNA concentrations and Tristosomes.
Enhanced Activity of ESC GalNAc-siRNAs Targeting Transthyretin in hTTR V30M Tg Mouse
Activity of TTR ESC GalNAc-siRNAs in NHP
Single Dose SC

- Based on rodent and NHP PD, rat screening toxicity, and additional non-clinical data, ESC-2 advanced into development as ALN-TTRsc02
Robust and Durable TTR Knockdown in NHP ALN-TTRsc02 SD and QMx4 Regimens

- Sustained ≥ 95% TTR knockdown with QM dosing
Multi-Month Duration of TTR Knockdown in NHP
ALN-TTRsc02 QMx4 Regimen

- Sustained 90% TTR knockdown up to 4 months after last 3 mg/kg dose
Superior TTR Knockdown with ALN-TTRsc02
SD 1 mg/kg ALN-TTRsc02 Compared to MD 5 mg/kg Revusiran

![Graph showing serum TTR levels over days for ALN-TTRsc02 and Revusiran treatments.]

- 1 mg/kg ALN-TTRsc02
- 5 mg/kg Revusiran; QD x 5, QW x 4

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<tr>
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<th>Cumulative Dose (mg/kg)</th>
<th>eAUC (ug/mL*day)</th>
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<tbody>
<tr>
<td>Revusiran</td>
<td>45</td>
<td>19405 ± 1559</td>
</tr>
<tr>
<td>ALN-TTRsc02</td>
<td>1</td>
<td>8626 ± 1774</td>
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Favorable Clinical Translation of ESC GalNAc-siRNAs

- 1:1 or better translation from NHP to human with ESC GalNAc-siRNAs
- Extended durability in human vs. NHP due to attenuated nuclease environment

C5 KD Human vs NHP Correlation

Mean % C5 KD in Human vs Mean % C5 KD in NHP

\[ y = 29.9 + 0.78x \]

\[ r^2 = 0.83 \]

\[ P < 0.0001 \]

PCS KD Human vs NHP

Comparison of 3 mg/kg ALN-PCS in NHP to ~3.7 mg/kg ALN-PCS in human

Correlation analyses assumed 50 mg dose equivalent to 1 mg/kg and that 400 mg dose was equivalent to 5 mg/kg
Conclusions and Next Steps for ALN-TTRsc02

Conclusions

• Selected potent and durable ESC GalNAc-siRNA, ALN-TTRsc02
  ◦ ED50 < 1 mg/kg in both hTTR transgenic mice and NHP
  ◦ Durable suppression for > 30 days after a single SC 1 mg/kg dose in NHP
  ◦ Sustained ≥ 95% TTR knockdown with QM dosing at 1 mg/kg in NHP
  ◦ Clinically well tolerated in rats

• NHP to human translation of ESC GalNAc-siRNAs suggest potential for favorable profile of ALN-TTRsc02
  ◦ Expect low volume, once quarterly dosing

Next Steps

• IND planned in early 2016
• Initial Phase 1 results expected in late 2016
• Phase 3 start planned in 2017
Thank You