**AIM:**

Despite treatment advances there remains a very high unmet medical need for therapies to treat cardiovascular and metabolic diseases. We have developed and validated in human trials a platform for identifying potent and specific subcutaneously delivered RNA interference molecules targeting genes expressed in the liver. The rapid, dose-dependent, consistent, and durable knockdown of serum TTR (ob/ob female mice) demonstrated knockdown in ob/ob female mice.

### RESULTS:

**Excellent correlation of human to non-human primate TTR knockdown on mg/kg basis**

- **ALN-TTRsc dose groups, mg/kg:**
  - 1.0
  - 2.5
  - 5.0 MAD (n=3)
  - Placebo MAD (n=4)

We have developed a robust and reliable platform for the delivery of RNAi therapeutics to the liver. This platform is administered as a small volume spanning cardiovascular and metabolic diseases such as diabetes, NASH and obesity.

![Figure 1. ALN-TTRsc Phase 1 study](image)

**Mean (SEM) % TTR KD in human**

- 1.4
  - 0.8
  - 0.4
  - 0.2
  - 0.0

**mANGPTL3 protein, relative to PBS=1**

![Figure 2. GalNAc-siRNA conjugates for systemic RNAi](image)

**Figure 4. Alnylam metabolic disease program**

- Reported AEs
  - Injection site reaction (ISRs)
  - Local erythema
  - Local bruise
  - Sept. 2013

**过程**

- Synthetic siRNA

**图示**

- Complementary pairing
- Cleavage
- RNAi occurs at the post-transcriptional level and is triggered during the recycling time ~15 minutes.

**图示**

- Process

**图示**

- Heart Failure Society of America,

**图示**

- Figure 2. GalNAc-siRNA conjugates for systemic RNAi

- Figure 4. Alnylam metabolic disease program

**图示**

- Process

<table>
<thead>
<tr>
<th>Single Dose</th>
<th>Total</th>
<th>Multiple Doses</th>
<th>Total</th>
</tr>
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<tbody>
<tr>
<td>1.25 mg/kg</td>
<td>10 mg/kg</td>
<td>TTRsc (n=3)</td>
<td>TTRsc (n=3)</td>
</tr>
<tr>
<td>2.5 mg/kg</td>
<td>10 mg/kg</td>
<td>TTRsc (n=3)</td>
<td>TTRsc (n=3)</td>
</tr>
<tr>
<td>5.0 mg/kg</td>
<td>10 mg/kg</td>
<td>TTRsc (n=3)</td>
<td>TTRsc (n=3)</td>
</tr>
<tr>
<td>10 mg/kg</td>
<td>10 mg/kg</td>
<td>TTRsc (n=3)</td>
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</table>

**图示**

- Reported AEs

- Injection site reaction (ISRs)

- Clinically mild, consisting of transient erythema associated in some cases with edema and/or pain; self-limiting, resolved within ~2 hours of onset.

**图示**

- Reported AEs

- Injection site reaction (ISRs)

- Local erythema

- Local bruise

- Sept. 2013

**图示**

- Reported AEs

- Injection site reaction (ISRs)

- Local erythema

- Local bruise

- Sept. 2013
We have developed a robust and reliable platform for the delivery of RNAi therapeutics to the liver. Significant lowering of total cholesterol, LDL-C and triglycerides. A dose response curve indicated that our current prototype molecule has a single dose ED50 of ~1mg/kg.

ALN-ANGsc was tested in two models of hyperlipidemia: the ob/ob mouse and the hCETP-ApoB mouse. In both model systems, treatment with ALN-ANGsc resulted in a substantial lowering remaining 60 days post the last dose given. ALN-PCSsc was selected as a development candidate and will be advanced towards an IND in 2014.

**RESULTS:**

**METHODS:** A set of chemically modified siRNAs were designed using bioinformatic algorithms and were synthesized and screened for potency by transfection in Hep3B cells. In this manner, highly active siRNA molecules were developed targeting either PCSK9 or ANGPTL3. The siRNAs were tested in either rodent models or in non-human primates (NHPs) for activity.

**RESULTS:** Highly potent and selective siRNAs were developed targeting both PCSK9 (ALN-PCScsc) and ANGPTL3 (ALN-ANGsc). In NHPs, ALN-PCScsc at a dose of 2mg/kg reduced circulating PCSK9 levels up to 94% with a subsequent lowering of LDL-C up to 67%. Moreover, the effect on both PCSK9 and LDL-C was durable; with substantial lowering remaining 60 days post the last dose given. ALN-PCScsc was selected as a development candidate and will be advanced towards an IND in 2014. ALN-ANGsc was tested in two models of hyperlipidemia: the ob/ob mouse and the hCETP-ApoB mouse. In both model systems, treatment with ALN-ANGsc resulted in a significant lowering of total cholesterol, LDL-C and triglycerides. A dose response curve indicated that our current prototype molecule has a single dose ED50 of ~1mg/kg.

**CONCLUSION:** We have developed a robust and reliable platform for the delivery of RNAi therapeutics to the liver in vivo. This platform is administered as a small volume subcutaneous dose and has been shown to be safe and effective in a recently reported Phase I trial in healthy human volunteers. We have extended this platform with two targets of high interest in the cardiovascular field, PCSK9 and ANGPTL3. This platform is modular and enables targeting of any gene that is expressed in the liver spanning cardiovascular and metabolic diseases such as diabetes, NASH and obesity.
We have developed a robust and reliable platform for the delivery of RNAi therapeutics to the liver.

**CONCLUSION:**
- ALN-PCSsc was selected as a development candidate and will be advanced towards an IND in 2014.
- GalNAc-siRNA conjugates for systemic RNAi.

**A** Rapid, dose-dependent, consistent, and durable knockdown of serum TTR
- Statistically significant knockdown of serum TTR at all doses evaluated (p<0.01)
- Consistent level of TTR knockdown with weekly dosing; durable effects lasting weeks after last dose
- Up to 94% TTR knockdown; mean TTR knockdown of 87.5% and 92.4% at 5.0 and 10.0 mg/kg, respectively
- Excellent correlation of human to non-human primate TTR knockdown on mg/kg basis
  - Confirmation of human translation of GalNAc-siRNA conjugate platform

**B** Safety and tolerability

**Reported AEs**

<table>
<thead>
<tr>
<th>Dose Level</th>
<th>Injection site reaction (ISRs)</th>
<th>Headache</th>
<th>Local erythema</th>
<th>Local bruise</th>
<th>Local tenderness</th>
<th>Abdominal tenderness</th>
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<tbody>
<tr>
<td>1.25 mg/kg</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>2.5 mg/kg</td>
<td>0 (0%)</td>
<td>3 (33%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (33%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>5.0 mg/kg</td>
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<td>0 (0%)</td>
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<td>1 (100%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

**Status**
- Dosing completed; analysis ongoing

**Figure 3. ALN-TTRsc Phase I study results**

- **A** Rapid, dose-dependent, consistent, and durable knockdown of serum TTR
- **B** Safety and tolerability

**Additional information**
- All TEAEs mild or moderate in severity
- ISRs: clinically mild, consisting of transient erythema associated in some cases with edema and/or pain; self-limiting, resolved within ~2 hours of onset
- No study discontinuations, flu-like symptoms, or changes in cytokines, CRP, liver function tests, renal function and hematologic parameters
- No significant adverse events associated with drug at doses through 10.0 mg/kg

**Figure 4. Alnylam metabolic disease program**

- Alnylam PCSK9 Phase I POC and TTRsc Phase I POC (with GalNAc-siRNA conjugate platform) opens significant opportunities in metabolic disease to address validated but undruggable, liver-expressed targets
- E.g., ANGPTL3 for mixed hyperlipidemia, ApoC3 for hypertriglyceridemia, SCAP for Statoxis

**Figure 5. ALN-PCSsc lowers PCSK9 and LDL-C (in NHPs)**

- ALN-PCSsc, 2 mg/kg qd x5; qw x3"
ALN-ANGsc was tested in two models of hyperlipidemia: the ob/ob mouse and the hCETP-ApoB mouse. In both model systems, treatment with ALN-ANGsc resulted in a 2 mg/kg reduced circulating PCSK9 levels up to 94% with a subsequent lowering of LDL-C up to 67%. Moreover, the effect on both PCSK9 and LDL-C was durable, with a persistence over time.

**RESULTS:**

In human primates (NHPs) for activity, highly active siRNA molecules were developed targeting either PCSK9 or ANGPLT3. The siRNAs were tested in either rodent models or in non-human primates. This natural, endogenous mechanism may be utilized to down-modulate any protein of interest.

**METHODS:**

- **AIM:**
  - Develop lead conjugate
  - Validate directionality of genetics
  - Silence all genes in candidate loci

- **Study design:**
  - Dosing completed; analysis ongoing

- **Figure 6. ALN-ANG:**
  - Demonstrated knockdown in ob/ob female mice

- **Figure 4. Alnylam metabolic disease program**
  - Rapid, dose-dependent, consistent, and durable knockdown of serum TTR
  - Confirmation of human translation of GalNAc-siRNA conjugate platform

- **Figure 5. ALN-PCSsc conjugate lowers PCSK9 and LDL-C in NHPs.**

**Conclusions**

- We have developed a robust and reliable platform for the delivery of RNAi therapeutics to the liver in vivo.
- This platform is administered as a small volume subcutaneous dose and has been shown to be safe and effective in a recently reported Phase I trial in healthy human volunteers.
- We have extended this platform to two targets of high interest in the cardiovascular field, PCSK9 and ANGPLT3.
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