A Subcutaneously Administered Investigational RNAi Therapeutic (ALN-AT3) Targeting Antithrombin for Treatment of Hemophilia: Phase 1 Study Results in Subjects with Hemophilia A or B

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Antithrombin and ALN-AT3 Program

Antithrombin (AT) is genetically defined target
• AT is key natural anticoagulant
  ◦ Inactivates Factor Xa and thrombin
  ◦ Attenuates thrombin generation
• Human AT deficiency associated with increased thrombin generation
• Expressed in liver; circulates in plasma

Co-inheritance of thrombophilic traits in hemophilia\(^1\)
• Associated with milder bleeding, reduced factor requirements, fewer complications
• Includes heterozygous
  ◦ Antithrombin deficiency
  ◦ Factor V\(_{\text{Leiden}}\)
  ◦ Protein C deficiency
  ◦ Protein S deficiency

ALN-AT3 in clinical development
• Extensive pre-clinical efficacy and safety data in hemophilia models\(^2\)
• Orphan drug status in U.S./EU (HA/HB)
• Positive interim Phase 1 results
• Additional data late ’15


\(^2\)Seghal et al., Nat Med, doi:10.1038/nm.3847
ALN-AT3 Phase 1 Study
Dose-Escalation Study in Three Parts

Primary objectives
• Safety, tolerability

Secondary objectives
• AT knockdown, thrombin generation

Part A: Single-Ascending Dose (SAD)  |  Randomized 3:1, Single-blind, Placebo-controlled, Healthy volunteers
30 mcg/kg x 1 SC, N=4  

Part B: Multiple-Ascending Dose (MAD) – Weekly dosing  |  Open-label, Hemophilia A or B
15 mcg/kg qW x 3 SC, N=3  
45 mcg/kg qW x 3 SC, N=6  
75 mcg/kg qW x 3 SC, N=3  

Part C: Multiple-Ascending Dose (MAD) – Monthly dosing  |  Open-label, Hemophilia A or B
225 mcg/kg qM x 3 SC, N=3  
Ongoing

Presented January 2015

Up to 3 additional cohorts
## ALN-AT3 Phase 1 Study Part B (MAD)*
### Demographics & Baseline Characteristics

<table>
<thead>
<tr>
<th></th>
<th>Cohort 1 (N=3) 15 mcg/kg</th>
<th>Cohort 2 &amp; 3 (N=6) 45 mcg/kg</th>
<th>Cohort 4 (N=3) 75 mcg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, mean (SD)</strong></td>
<td>27 (9)</td>
<td>42 (14)</td>
<td>39 (4)</td>
</tr>
<tr>
<td><strong>Hemophilia A</strong></td>
<td>2</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td><strong>Hemophilia B</strong></td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td><strong>Severe</strong></td>
<td>3</td>
<td>6</td>
<td>3</td>
</tr>
<tr>
<td><strong>Moderate</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Historical Annualized Bleed Rate (ABR)</strong>, mean (SD)</td>
<td>18 (19.1)</td>
<td>16.3 (10.6)</td>
<td>6 (3.5)</td>
</tr>
<tr>
<td><strong>Weight (kg), mean (SD)</strong></td>
<td>76 (10.1)</td>
<td>80 (21.5)</td>
<td>82 (8.5)</td>
</tr>
</tbody>
</table>

*Data as of 2 June 2015

**Calculated as the reported number of bleeding events in past 6 months multiplied by 2
ALN-AT3 Phase 1 Study Part B (MAD)*
Safety/Tolerability; All TEAEs

- No serious adverse events or discontinuations
- No thromboembolic events or clinically significant D-dimer increases
- Normal physical exams, vital signs, and ECG
- No clinically significant changes in any laboratory parameter (LFTs, CBC, coagulation)
- Majority of adverse events were bleed events
  - All bleed events successfully managed with replacement factor administration
  - No adverse events associated with factor administration
- A total of 17 single non-bleed adverse events were observed, all mild/moderate and transient

<table>
<thead>
<tr>
<th>Cohort 1</th>
<th>Cohort 2 &amp; 3</th>
<th>Cohort 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>(N=3)</td>
<td>(N=6)</td>
<td>(N=3)</td>
</tr>
<tr>
<td>15 mcg/kg</td>
<td>45 mcg/kg</td>
<td>75 mcg/kg</td>
</tr>
</tbody>
</table>

- Subjects with at least 1 TEAE: 2 (67%), 4 (67%), 2 (67%)
- Total AEs: 5, 9, 3

List of AEs:
- Abdominal pain
- Arthralgia
- Costochondritis
- Dyspepsia
- Thermal burn
- Blood glucose increased
- Blood uric acid increased
- C-reactive protein increased
- Fall
- Injection site pain**,
- Muscle tightness
- Nasopharyngitis
- Rhinitis
- Urticaria
- Arthritis
- Headache**
- Respiratory tract infection

*Data as of 2 June 2015: Adverse event grouping based on verbatim terms, excluding bleed events.
**Possibly related/Related
#Mild pain lasting 2 minutes, resolved, no other associated symptoms
ALN-AT3 Phase 1 Study Part B (MAD)*
AT Knockdown

AT knockdown after multi-dose in hemophilia subjects

<table>
<thead>
<tr>
<th>Cohort</th>
<th>AT Knockdown (Mean ± SEM)</th>
<th>Max AT Knockdown</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 mcg/kg (N=3)</td>
<td>29 ± 12%</td>
<td>53%</td>
<td></td>
</tr>
<tr>
<td>45 mcg/kg (N=6)</td>
<td>54 ± 9%*</td>
<td>86%</td>
<td>*&lt; 0.05, relative to baseline</td>
</tr>
<tr>
<td>75 mcg/kg (N=3)</td>
<td>59 ± 7%*</td>
<td>70%</td>
<td></td>
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*Data as of 2 June 2015
ALN-AT3 Phase 1 Study Parts A and B*  
Thrombin Generation

Post hoc analysis of thrombin generation by AT knockdown tertiles

<table>
<thead>
<tr>
<th></th>
<th>AT KD &lt;33%</th>
<th>AT KD 33-66%</th>
<th>AT KD &gt;66%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak Thrombin Generation (nM, Mean ± SD)</td>
<td>18 ± 8</td>
<td>35 ± 24</td>
<td>120 ± 81*</td>
</tr>
<tr>
<td>% Increase in Peak Thrombin Generation (Mean ± SD)</td>
<td>25 ± 72%</td>
<td>69 ± 92%</td>
<td>350 ± 239%*</td>
</tr>
</tbody>
</table>

*p < 0.05, compared with AT knockdown less than 33%

*Data as of 2 June 2015
Whole Blood Clot Formation
Materials and Methods

ROTEM® Thromboelastometry
• Evaluates viscoelastic changes in blood following physiologic coagulation stimulus
• CTI stabilized citrate whole blood; diluted tissue factor (Innovin®); CaCl₂

Materials and Methods
Hemophilia characterized by defect in propagation of whole blood clot formation; measured by Clot Formation Time (CFT)
Whole Blood Clot Formation
Results in All 3 Subjects with ROTEM Data

**Improvement of whole blood clot formation; shortening of clot formation time**
- Day 1 mean CFT was $1166 \pm 614$ sec; Day 35 mean CFT was $323 \pm 46$ sec (p <0.05)

<table>
<thead>
<tr>
<th></th>
<th>101-009 45 mcg/kg</th>
<th>101-013 75 mcg/kg</th>
<th>101-016 75 mcg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT KD (%)</td>
<td>CFT (sec) ± SEM</td>
<td>AT KD (%)</td>
<td>CFT (sec) ± SEM</td>
</tr>
<tr>
<td>Day 1</td>
<td>1</td>
<td>1441 ± 394</td>
<td>14</td>
</tr>
<tr>
<td>Day 8</td>
<td>30</td>
<td>625 ± 43</td>
<td>8</td>
</tr>
<tr>
<td>Day 21</td>
<td>57</td>
<td>289 ± 5</td>
<td>41</td>
</tr>
<tr>
<td>Day 35</td>
<td>56</td>
<td>333 ± 64</td>
<td>46</td>
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</tbody>
</table>

AT KD (%) relative to baseline
**Exploratory Analysis of Bleed Events**

**Annualized Bleed Rate (ABR)**

**Post hoc analysis of bleed events by AT knockdown tertiles**

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<th>AT KD 33-66%</th>
<th>AT KD &gt;66%</th>
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<tbody>
<tr>
<td>Patients#</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>Cumulative Days</td>
<td>509</td>
<td>414</td>
</tr>
<tr>
<td>Cumulative Bleeds</td>
<td>33</td>
<td>18</td>
</tr>
<tr>
<td>ABR##, Mean (SEM)</td>
<td>22 ± 5</td>
<td>14 ± 5</td>
</tr>
</tbody>
</table>

*Data as of 2 June 2015

*Number of patients with time spent in tertile

## For each subject, the ABR in each tertile is calculated by 365.24*(number of bleed events/number of days in tertile).

**Based on negative binomial regression model**

**p<0.001**
Hemophilia Subject 400-002*

Bleed-free period of 114 days correlates with AT KD and increase in thrombin generation, with no increase in D-dimer

100-150 nM peak thrombin represents >25% factor VIII in this subject

*Subject 400-002 has severe hemophilia A and has a self-reported ABR of 22; enrolled in 45 mcg/kg dose cohort
ALN-AT3 Phase 1 Study
Summary and Next Steps

• ALN-AT3 represents novel investigational approach for potential treatment of hemophilia and rare bleeding disorders
• In ongoing Phase 1 study in healthy volunteers (n=3) and subjects with hemophilia (n=12), single- and multi-dose administration of ALN-AT3 generally well tolerated
  ◦ Data cutoff date of June 2, 2015
  ◦ No SAEs; all AEs mild or moderate, and transient; no discontinuations
  ◦ No clinically significant increases in D-dimer
• Initial evidence for clinical activity and re-balancing of hemostasis in severe hemophilia
  ◦ Up to 86% AT knockdown, with mean maximum AT knockdown of 59 ± 7% at 75 mcg/kg
  ◦ Durable knockdown supportive of a once-monthly subcutaneous dose regimen
  ◦ Increase of 350% in mean thrombin generation in highest AT knockdown tertile
    – Represents a normalization of thrombin generation in severe hemophilia subjects
  ◦ Marked improvement in whole blood clot formation with an over 3-fold shortening of clot formation time
  ◦ In exploratory post hoc analysis, reduced estimated ABR associated with increased AT knockdown
    – No bleeds in highest AT knockdown tertile
    – Subject with highest degree of AT knockdown remained bleed-free for 114 days
• Additional results expected to be presented in late-2015
• Plan to advance to pivotal study in mid-2016
## Acknowledgements

### Patients

### Investigators

<table>
<thead>
<tr>
<th>Country</th>
<th>PI Name</th>
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<tbody>
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Thank You