2015 Goring Coagulation Conference

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

Akin Akinc, PhD
January 12, 2015
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
- Associated with milder bleeding, reduced factor requirements, fewer complications
- Includes heterozygous
- Antithrombin deficiency
- Factor V Leiden
- Protein C deficiency
- Protein S deficiency

ALN-AT3 in clinical development
- Extensive pre-clinical efficacy and safety data in hemophilia models
- Positive initial Phase 1 results; study ongoing
- Orphan drug status in U.S./EU (HA/HB)
- Additional data mid- and late ’15

RNAi Therapeutics

A New Class of Innovative Medicines

• Harness natural pathway
  ◦ Catalytic mechanism
  ◦ Mediated by small interfering RNA or “siRNA”

• Therapeutic gene silencing
  ◦ Any gene in genome
  ◦ Distinct mechanism of action vs. other drug classes
  ◦ Unique opportunities for innovative medicines

• Clinically validated platform
  ◦ Human POC in multiple programs
    – Papers in *NEJM* and *Lancet*
ALN-AT3
SC-Administered GalNAc-Conjugated siRNA Targeting Antithrombin

• “Enhanced stabilization chemistry” (ESC) utilized for potency and durability of effect
• Weekly SC dosing results in potent and sustained suppression of AT levels

- Wild-type mice, N = 5
- Weekly (qw) dosing
ALN-AT3 Preclinical Proof-of-Concept

**Proof-of-concept in multiple preclinical settings**

- Increased thrombin generation in human hemophilia A and B plasma
- Correction of APTT in hemophilia mice
- Survival benefit in hemophilia mice
- Enhanced thrombus formation in microvessel laser injury model in hemophilia mice
- Improved hemostasis in saphenous vein bleeding model in hemophilia mice
- Increased thrombin generation in hemophilia A inhibitor model in NHPs

**Improved thrombin generation in NHP inhibitor hemophilia model**

<table>
<thead>
<tr>
<th>ALN-AT3 (mg/kg) qW</th>
<th>Normal</th>
<th>Induced HA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-dose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25</td>
<td>60% AT KD</td>
<td></td>
</tr>
<tr>
<td>0.50</td>
<td><strong>80% AT KD</strong></td>
<td><strong>(p&lt;0.01)</strong></td>
</tr>
</tbody>
</table>

**Peak Thrombin (nM)**

- 0
- 20
- 40
- 60
- 80
- 100
- 120
- 140
ALN-AT3 Survival Benefit in HA Mice
Results from 26-Week GLP Chronic Toxicity Study

Groups
- Animals: Hemophilia A (HA) mice (B6;129S4-F8\textsuperscript{tm1Kaz})
- Dosing: Saline; ALN-AT3: 10 mg/kg and 30 mg/kg (SC, weekly dosing)
- Group sizes: (N=70; 35 per sex) powered to account for spontaneous loss due to background sensitivity of HA mouse strain

Results
- No adverse clinical signs; no changes in body weight, hematology or clinical chemistry
- Survival benefit noted in treated animals (p <0.0001; Log-rank, Mantel-Cox test)
ALN-AT3 Phase 1 Study
Dose-Escalation Study in Two Parts

Primary objectives
• Safety, tolerability

Secondary objectives
• AT knockdown, thrombin generation

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

Part B
Multiple-Ascending Dose (MAD)
- 15 mcg/kg qW x 3 SC
- Open-label
- Hemophilia A or B
- N=3/cohort

- 45 mcg/kg qW x 3 SC
- TBD mcg/kg qW x 3 SC
- Up to 4 additional cohorts
## ALN-AT3 Phase 1 Study Part A (SAD)

### Demographics

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Age</th>
<th>Ethnicity</th>
<th>Follow up period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placebo</td>
<td>38</td>
<td>Black</td>
<td>65 days</td>
</tr>
<tr>
<td>30 mcg/kg x 1</td>
<td>21</td>
<td>Asian</td>
<td>94 days</td>
</tr>
<tr>
<td>30 mcg/kg x 1</td>
<td>29</td>
<td>Asian</td>
<td>70 days</td>
</tr>
<tr>
<td>30 mcg/kg x 1</td>
<td>38</td>
<td>White</td>
<td>71 days</td>
</tr>
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</table>
## ALN-AT3 Phase 1 Study Part A (SAD)
### Safety/Tolerability; All TEAEs*

<table>
<thead>
<tr>
<th>Adverse event</th>
<th>ALN-AT3 30 mcg/kg N=3</th>
<th>Relationship to Study Drug</th>
</tr>
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<tbody>
<tr>
<td>Atypical chest pain</td>
<td>1</td>
<td>Unlikely related</td>
</tr>
<tr>
<td>Coryza</td>
<td>1</td>
<td>Not related</td>
</tr>
<tr>
<td>Headache</td>
<td>1</td>
<td>Possibly related</td>
</tr>
<tr>
<td>Panic like symptoms</td>
<td>1</td>
<td>Unlikely related</td>
</tr>
<tr>
<td>Upper Respiratory Tract Infection</td>
<td>1</td>
<td>Not related</td>
</tr>
</tbody>
</table>

- No serious adverse events
- All adverse events mild
- No discontinuations
- No injection site reactions
- No thromboembolic events or clinically significant D-dimer increases
- Normal physical exams, vital signs, and ECG
- No laboratory AEs (LFTs, CBC, coagulation parameters)

*Data as of Nov. 24, 2014; adverse event grouping based on verbatim terms*
AT knockdown after single dose in human volunteers

- Maximum AT knockdown relative to baseline up to 28%
- Mean maximum AT knockdown of 19 ± 4.4% (mean ± SEM)
  - Placebo vs. treated, ANOVA p < 0.01
- AT knockdown durable for over 60 days
ALN-AT3 Phase 1 Study Part A (SAD)
Pharmacodynamics and Clinical Activity

**Increase in thrombin generation with AT knockdown**
- Significant association between AT knockdown and peak thrombin generation
- Up to 152% increase in peak thrombin generation
- Mean maximum increase of peak thrombin $138 \pm 8.9\%$ (mean $\pm$ SEM)

![Graph showing correlation between AT knockdown and peak thrombin generation](image)

$r = 0.44, p = 0.004$
ALN-AT3 Phase 1 Study Part B (MAD)
Cohort 1 and 2 Demographics*

<table>
<thead>
<tr>
<th>Cohort 1</th>
<th></th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td>Hemophilia</td>
<td>ALN-AT3 Treatment</td>
<td>Age</td>
<td>Ethnicity</td>
<td>Follow up period</td>
</tr>
<tr>
<td>A, Severe</td>
<td>15 mcg/kg qW x 3</td>
<td>27</td>
<td>Black</td>
<td>88 days</td>
</tr>
<tr>
<td>B, Severe</td>
<td>15 mcg/kg qW x 3</td>
<td>36</td>
<td>White</td>
<td>75 days</td>
</tr>
<tr>
<td>A, Severe</td>
<td>15 mcg/kg qW x 3</td>
<td>19</td>
<td>White</td>
<td>70 days</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Cohort 2</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemophilia</td>
<td>ALN-AT3 Treatment</td>
<td>Age</td>
<td>Ethnicity</td>
<td>Follow up period</td>
</tr>
<tr>
<td>A, Severe</td>
<td>45 mcg/kg qW x 3</td>
<td>61</td>
<td>White</td>
<td>49 days</td>
</tr>
<tr>
<td>A, Severe</td>
<td>45 mcg/kg qW x 3</td>
<td>36</td>
<td>White</td>
<td>19 days</td>
</tr>
<tr>
<td>A, Severe</td>
<td>45 mcg/kg qW x 3</td>
<td>33</td>
<td>White</td>
<td>21 days</td>
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</tbody>
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*Data as of Jan. 6, 2015*
ALN-AT3 Phase 1 Study Part B (MAD) Cohort 1 and 2 Interim Safety/Tolerability; All TEAEs*

<table>
<thead>
<tr>
<th>Preferred Term</th>
<th>Cohort 1 (N=3) 15 mcg/kg n (%)</th>
<th>Cohort 2 (N=3) 45 mcg/kg n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemorrhage</td>
<td>3 (100.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>1 (33.3%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Epistaxis</td>
<td>1 (33.3%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Mouth haemorrhage</td>
<td>0 (0.0%)</td>
<td>1 (33.3%)</td>
</tr>
<tr>
<td>Muscle haemorrhage</td>
<td>1 (33.3%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Myalgia</td>
<td>1 (33.3%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Myalgia - Right Thigh Bleed</td>
<td>1 (33.3%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Post procedural haemorrhage</td>
<td>1 (33.3%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Skin haemorrhage</td>
<td>0 (0.0%)</td>
<td>1 (33.3%)</td>
</tr>
<tr>
<td>Thermal burn</td>
<td>1 (33.3%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Tongue haematoma</td>
<td>0 (0.0%)</td>
<td>1 (33.3%)</td>
</tr>
<tr>
<td>Traumatic haematoma</td>
<td>0 (0.0%)</td>
<td>1 (33.3%)</td>
</tr>
<tr>
<td>Vessel puncture site haematoma</td>
<td>0 (0.0%)</td>
<td>1 (33.3%)</td>
</tr>
</tbody>
</table>

• No serious adverse events
• All adverse events mild/moderate
• No discontinuations
• No injection site reactions
• No thromboembolic events or clinically significant D-dimer increases
• Normal physical exams, vital signs, and ECG
• No laboratory AEs (LFTs, CBC, coagulation parameters)

*Data as of Jan. 6, 2015
ALN-AT3 Phase 1 Study Part B (MAD)
Cohort 1 and 2 Interim Pharmacodynamics and Clinical Activity*

Initial data on AT knockdown after multi-dose in hemophilia subjects

- **Cohort 1 (n=3) results**
  - Mean maximum AT knockdown of 29 ± 12% (n=3; mean ± SEM)
  - Maximum AT knockdown up to 53%

- **Cohort 2 (n=3) results**
  - Mean AT knockdown of 44 ± 6.5% (n=3, mean ± SEM; p=0.02) on Day 16
  - Mean AT knockdown of 64 ± 6.8% (n=2; mean ± SEM) on Day 21
  - Maximum AT knockdown up to 70%

*Data as of Jan. 6, 2015*
Increased thrombin generation in hemophilia subjects

- Up to 334% increase (relative to baseline) in thrombin generation in hemophilia subjects
- Mean increase in thrombin generation of 112 ± 38% (p<0.05, relative to baseline) at AT KD ≥50%
- Maximum increase in peak thrombin at low end of range for thrombin generation in normal subjects

*Data as of Jan. 6, 2015
Analysis excludes data points influenced by replacement factor
Most Advanced Hemophilia Subject in Cohort 2 101-009

- Severe Hemophilia A, FVIII < 1%
- Age 61
- Weight: 93.7 kg
- Medical history
  - Factor prophylaxis therapy prior to study entry
  - Diagnosis of hepatitis C in 1980
  - Orthopedic surgeries
    - Left elbow
    - Left knee
    - Bilateral total hip replacement
  - Estimated Annualized Bleed Rate (per treating physician)
    - During prophylaxis therapy: 0-2
    - During on-demand therapy: 10-12
Hemophilia Subject 101-009
Interim Pharmacodynamics and Clinical Activity*

Initial data on AT knockdown in hemophilia subject
• Maximum AT knockdown of 60%; nadir on Day 28
• Durable pharmacodynamic effect, 54% knockdown on Day 42 (28 days post last dose)

*Data as of Jan. 6, 2015
Hemophilia Subject 101-009
Interim Pharmacodynamics and Thrombin Generation*

Increase in thrombin generation with AT knockdown
- Association between AT knockdown and peak thrombin generation
- Maximum increase in peak thrombin of 121%

*Data as of Jan. 6, 2015
Analysis excludes data points influenced by replacement factor
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

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**Parameters of ROTEM® Analysis**

- Alpha-angle
- Lambéda-angle (Lysis rate)
- Maximum clot firmness (MCF)
- Maximum lysis (%)
- Lysis on set time (LOT)

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**Materials and Methods**

- Hemophilia
- Normal
Hemophilia Subject 101-009
Whole Blood Clot Formation

Day 1          Day 8          Day 15          Day 21
Replicate 1
Replicate 2
Replicate 3
Hemophilia Subject 101-009
Whole Blood Clot Formation and Bleeding*

Last bleed (Day 2)

Time (sec) [Mean +/- SEM]

Clot formation time
Clotting time

Days

*Data as of Jan. 6, 2015
Hemophilia Subject 101-009

Summary

- Dose of 45 mcg/kg qW x 3 generally well tolerated
- No new bleeding events since Day 2
  - Day 2: Oral mucosal bleed
    - Controlled with single dose 2000 IU FVIII
  - Currently 47 days bleed free (as of Jan 6, 2015)
- Increased peak thrombin generation
- Markedly improved whole blood clot formation
- Improvements in thrombin generation and whole blood clot formation quantitatively and temporally associated with AT knockdown
ALN-AT3 Phase 1 Study
Summary and Next Steps

- ALN-AT3 represents novel approach for treatment of hemophilia and RBD
- In ongoing Phase 1 in healthy volunteers (n=3) and subjects with hemophilia (n=6), single- and multi-dose administration of ALN-AT3 generally well tolerated
  - No SAEs; all AEs mild or moderate, and transient; no discontinuations
- In Part A in healthy volunteers, single 30 mcg/kg dose of ALN-AT3 resulted in an up to 28% knockdown in AT with durable effect lasting >60 days
- In Part B in hemophilia subjects, ALN-AT3 resulted in up to 70% knockdown in AT
  - Most advanced hemophilia subject receiving 45 mcg/kg demonstrates a durable effect, with 54% AT KD at Day 42 (28 days post last dose)
- Initial evidence for potential correction of hemophilia phenotype observed
  - At AT KD >50%, mean peak thrombin generation increase of 112 ± 38%, with max peak thrombin increase of 334%
  - Marked and durable improvement in whole blood clot formation as measured by ROTEM®
    - Most advanced subject receiving 45 mcg/kg remains bleed free for 47 days (as of Jan 6, 2015)
- Additional results expected to be presented in mid- and late ’15
### Acknowledgements

## Trial Participants

### Investigators

<table>
<thead>
<tr>
<th>Country</th>
<th>PI Name</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
<td>United Kingdom</td>
<td>Steve Austin</td>
<td>London – St. George’s Healthcare NHS Trust Haemophilia Centre</td>
</tr>
<tr>
<td></td>
<td>David Bevan</td>
<td>London – The Centre for Haemostasis and Thrombosis Guy’s and St. Thomas’ Hospital</td>
</tr>
<tr>
<td></td>
<td>Desmond Creagh</td>
<td>Truro – Royal Cornwall Hospital</td>
</tr>
<tr>
<td></td>
<td>Charles Hay</td>
<td>Manchester – Manchester Royal Infirmary</td>
</tr>
<tr>
<td></td>
<td>Tim Mant</td>
<td>London – Quintiles Drug Research Unit</td>
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<tr>
<td></td>
<td>John Pasi</td>
<td>London – The Royal London Haemophilia Centre</td>
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<tr>
<td></td>
<td>Savita Rangarajan</td>
<td>Basingstoke – North Hampshire Haemophilia Centre</td>
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<tr>
<td>Bulgaria</td>
<td>Pencho Georgiev</td>
<td>Plovdiv – University Multiprofile Hospital for Active Treatment “Sveti Georgi’</td>
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<tr>
<td>Switzerland</td>
<td>Brigitte Brand-Staufer</td>
<td>Zurich – Universitatsspital Zurich, Klinik fur Hamatologie</td>
</tr>
</tbody>
</table>
Thank You