XXIV Congress of the ISTH

An RNAi Therapeutic Targeting Antithrombin Increases Thrombin Generation and Improves Hemostasis

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July 2, 2013
Therapeutic Hypothesis
Targeting Antithrombin to Rebalance the Coagulation System

Intrinsic system
- Hemophilia B
  - FIX
  - FIXa
- Hemophilia A
  - FXIII
  - FVIIIa

Extrinsic system
- FVIIa
- FVII
- FX
- FXa
- AT
- FVa
- FV
- Prothrombin
- Thrombin
- Fibrinogen
- Fibrin
- Blood clot
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**
Weekly SC dosing results in potent and sustained suppression of antithrombin levels.

- Wild-type mice, N = 5
- Weekly (qw) dosing
Thrombin Generation
A Global Hemostasis Assay

- Thrombin generation assay is sensitive to both pro-coagulants and anticoagulants in coagulation cascade
- Thrombin generation capacity correlates with severity of hemophilia

\[\text{Relative Thrombin Generation (Normal = 1.0)}\]

1Dargaud et al., Thromb Haemost; 93, 475-480 (2005)
ALN-AT3 Activity in Hemophilia B Mice
Treatment Normalizes Thrombin Generation

- N = 3, hemophilia B (FIX^{-/-}) mice or wild-type mice
- Single subcutaneous injection
- Blood collected 8 days post-dose in sodium citrate with CTI

In collaboration with Dr. Yesim Dargaud and Dr. Claude Negrier
ALN-AT3 Improves Hemostasis in Microvessel Laser Injury Model

- Cremaster muscle microvessel laser injury model (Camire, CHOP)
- HB mice treated with single dose 30 mg/kg ALN-AT3, injury induced 8 days post treatment
- 2 HB mice, 3 injury sites each (6 total injuries)
- 6/6 injury sites demonstrate platelet accumulation (red) and fibrin deposition (green)

In collaboration with Dr. Lacra Ivanciou and Dr. Rodney Camire
ALN-AT3 Improves Hemostasis in Microvessel Laser Injury Model (HA Mice)

In collaboration with Dr. Lacra Ivanciou and Dr. Rodney Camire
ALN-AT3 Repeat-Dose Pharmacology in NHP
Potent, Titratable, and Reversible Effects
Objective: Demonstrate increased thrombin generation in large animal Hemophilia A inhibitor model

- Hemophilia A inhibitor model transiently induced via administration of a polyclonal anti-FVIII Ab

<table>
<thead>
<tr>
<th>Test Article</th>
<th>Group Number</th>
<th>N</th>
<th>Dose Level (mg/kg)</th>
<th>Route and Regimen</th>
<th>Number of Doses</th>
<th>Dose of Anti-FVIII Ab</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>1</td>
<td>4</td>
<td>--</td>
<td></td>
<td>--</td>
<td>20,000 BU/kg</td>
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<tr>
<td>ALN-AT3</td>
<td>2</td>
<td>4</td>
<td>0.25</td>
<td>SC, qw</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>4</td>
<td>0.50</td>
<td></td>
<td>6</td>
<td></td>
</tr>
</tbody>
</table>

*Dose of anti-FVIII Ab titrated in Group 1 animals to achieve <1% FVIII at 4 hours post Ab administration*
ALN-AT3 Treatment Normalizes Thrombin Generation in Hemophilic NHPs with Inhibitors

**Induction of Hemophilia A**

<table>
<thead>
<tr>
<th>ALN-AT3 (mg/kg) qw</th>
<th>Relative FVIII Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>1.0</td>
</tr>
<tr>
<td>0.25</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>0.50</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

**Normalization of Thrombin Generation†**

<table>
<thead>
<tr>
<th>ALN-AT3 (mg/kg) qw</th>
<th>Peak Thrombin (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-dose</td>
<td>100</td>
</tr>
<tr>
<td>Saline</td>
<td>60% AT reduction</td>
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<tr>
<td>0.25</td>
<td>60% AT reduction</td>
</tr>
<tr>
<td>0.50</td>
<td>80% AT reduction</td>
</tr>
</tbody>
</table>
Expanded Therapeutic Index in Hemophilia Setting

- Toxicity in WT animals is attributable to exaggerated on-target pharmacology of ALN-AT3 and expected pro-coagulant effects of prolonged AT suppression at >90%
- In contrast, highly exaggerated doses of ALN-AT3 (resulting in >95% reduction in AT) are well tolerated in a hemophilia setting

Exaggerated Pharmacology in WT Mice

Exaggerated Pharmacology in HA Mice
Summary

Coagulation rebalancing approach utilizing ALN-AT3 represents potentially new prophylaxis therapy option in persons with hemophilia, including those with inhibitors

- Weekly SC administration of ALN-AT3 results in potent inhibition of AT in mice and NHPs
- Proof-of-concept achieved in experimental settings with AT reduction
  - Normalization of thrombin generation in hemophilia mouse
  - Enhanced hemostatic plug formation in microvessel laser injury model in hemophilia mice
  - Normalization of thrombin generation in a hemophilia A inhibitor model in NHPs

- Data in hemophilia models demonstrate substantially greater tolerance for AT reduction, suggesting potentially wide therapeutic index in hemophilia patients
- We plan to initiate Phase I study in late 2013
Acknowledgements

Alnylam Pharmaceuticals
- Alfica Sehgal, PhD
- Scott Barros, PhD
- June Qin
- Tim Racie
- Harsha Prabhala
- Yongfeng Jiang
- Julia Hettinger
- Mary Carioto

Edouard Herriot University Hospital, Université Claude Bernard, Lyon, France
- Yesim Dargaud, MD, PhD
- Claude Negrier, MD, PhD

Children’s Hospital of Philadelphia, USA
- Lacramioara Ivanciu, PhD
- Rodney Camire, PhD