Pre-Clinical Evaluation of ALN-AAT to Ameliorate Liver Disease Associated with Alpha-1 Antitrypsin Deficiency

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May 17, 2015

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Alpha-1 Antitrypsin (AAT)

**AAT is a serine proteinase inhibitor (serpin)**
- Inhibits neutrophil elastase, trypsin, thrombin, chymotrypsin, etc
- Abundant plasma protein, primarily synthesized in hepatocytes

**AAT deficiency**
- PiZZ accounts for 95% of AAT-deficient patient population
  - Z allele has point mutation Glu342Lys
    - Slows rate of protein folding
    - Accumulation of an intermediate that polymerizes
    - Reduced secretion
    - Polymer formation
      - Plasma deficiency “Loss of function”
      - Accumulation in hepatocytes “Toxic gain of function”

Lung Disease
Liver Disease
Accumulation of Mutant Z-AAT in the Liver Causes Disease

PAS Staining

Immunostaining

EM

Globules of AAT

Normal

Inclusions in ER

Hepatology (2005): 41:160
GalNAc-siRNA Conjugates
Subcutaneous Delivery of RNAi Therapeutics

**Asialoglycoprotein Receptor (ASGPR)**
- Highly expressed in hepatocytes
- High rate of uptake
- Recycling time ~15 minutes
- Conserved across species

**GalNAc-siRNA Conjugates**
- siRNA conjugated to N-acetylgalactosamine (GalNAc) ligand
- Efficient delivery to hepatocytes following subcutaneous administration
- “Enhanced stabilization chemistry” (ESC) used with ALN-AT3, ALN-PCSsc, ALN-CC5, ALN-AS1, ALN-AAT, and future programs
ASGPR is Qualitatively Similar Between Normal and PiZZ Patients

ASGPR Staining (red)

- Normal
- PiZZ Patient
- Fibrotic Area

ASGPR1

ASGPR2

- ASGPR1 or ASGPR2 Immunostaining
- Blue: DAPI/Nuclear Stain
- Human liver tissues
Using RNA interference (RNAi) to Knockdown Z-AAT Protein
Effective Z-AAT Knockdown in Fibrotic Livers in Tg-PiZ Mice

Experiment Hypothesis

- Transgenic human Z-AAT expressing mice develop liver tumors with age
- Can chronic dosing in aged mice with fibrotic livers decrease the tumor incidence?

6-11 month old animals

<table>
<thead>
<tr>
<th>Day 0</th>
<th>14</th>
<th>28</th>
<th>42</th>
<th>56</th>
<th>70</th>
<th>84</th>
<th>98</th>
<th>112</th>
<th>126</th>
<th>132</th>
</tr>
</thead>
<tbody>
<tr>
<td>First SC Dose</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

PAS Globule Staining

PBS vs. AAT-siRNA:

![](PAS_Globule_Staining.png)

Globule Area

- Relative Globule Area
  - PBS
  - AAT siRNA

![Globule Area Graph](Globule_Area_Graph.png)
Z-AAT Knockdown Improves Liver Histology and Decreases Tumor Burden in Tg-PiZ Mice

**Proliferating Cells**

<table>
<thead>
<tr>
<th></th>
<th>PBS Treated Animal</th>
<th>AAT-siRNA Treated Animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nucleus</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Tumor Incidence**

<table>
<thead>
<tr>
<th></th>
<th>PBS Treated Animal</th>
<th>AAT-siRNA Treated Animal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Incidence</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Number of Tumors**

<table>
<thead>
<tr>
<th>Count</th>
<th>PBS Treated Animal</th>
<th>AAT-siRNA Treated Animal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

**Tumor Size**

<table>
<thead>
<tr>
<th>Size in mm</th>
<th>PBS Treated Animal</th>
<th>AAT-siRNA Treated Animal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</table>

**Relative BrdU Count**

<table>
<thead>
<tr>
<th></th>
<th>PBS Treated Animal</th>
<th>AAT-siRNA Treated Animal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tbody>
</table>

*p = 0.02*
ALN-AAT Leads to Dose-dependent, Durable, Reduction of Z-AAT in Transgenic Mice

Dose Response

Experiment Design
- Tg-PIZ mice age at dosing = 11±1.6 wks
- N=4, single subcutaneous dose on Day 0
- Weekly bleeds to measure human serum AAT

Results
- Durable, dose dependent silencing
- ED$_{50}$ ~0.5 mg/kg
Cumulative, Dose-Dependent Knock-down of AAT
Ongoing Study with Weekly Administration of ALN-AAT

ALN-AAT in Transgenic Mice

Results
• Durable, dose dependent, sustained silencing
• Similar levels of knockdown in serum and mRNA
• Cumulative knockdown seen after repeat dose administration
  • SD 1mg/kg : 60% KD; Q1W x 3: >90% KD
ALN-AAT in Non Human Primates (NHP)
Experimental Design

- Exploring dose responses, dosing regimens, and repeat dosing
- Part I: Single subcutaneous dose
- Part II: Repeat subcutaneous dosing

<table>
<thead>
<tr>
<th>Group Number</th>
<th>N- males</th>
<th>Dose level (mg/kg)</th>
<th>Dosing Frequency</th>
<th>Number of Doses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3</td>
<td>1</td>
<td>q1w</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>3</td>
<td>1</td>
<td>q4w</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>3</td>
<td>3</td>
<td>q4w</td>
<td>3</td>
</tr>
</tbody>
</table>

- Subcutaneous single site injection for each dosing
- Weekly sample collection for measuring serum AAT levels
ALN-AAT in Non Human Primates (NHP) Single Dose Data

- Single 1mg/kg dose leads to 60% KD and it takes 75 days for serum AAT to return to baseline
- AAT Levels return to baseline and are maintained there
- Nadir after single dose is between day 20-30
- ED$_{50}$ NHP ~0.6mg/kg
ALN-AAT in Non Human Primates (NHP) Pre-Clinical Results

- 1mg/kg q1w x 12 and 3mg/kg q1m show similar knock-down
- AAT levels maintained for ~50 days post last dose

相对AAT水平（Prebleed = 1）

Days Post First Dose

- Last dose administered
ALN-AAT in Non Human Primates (NHP)
Pre-Clinical Results

- 1mg/kg q1w x 12 and 3mg/kg q1m show similar knock-down
- AAT levels maintained for ~50 days post last dose

Last dose administered
## Nonclinical Safety and Metabolism

### CTA-Enabling Studies for Start of Phase I Clinical Trial

<table>
<thead>
<tr>
<th>Exploratory Studies</th>
<th>Cytokine screening in vitro (hWBA) and in vivo (mouse)</th>
<th>No acute pro-inflammatory effects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dose range-finding toxicity studies in mouse, rat, NHP (repeat dose, 3-5 weeks in duration)</td>
<td>Establish dose levels for GLP studies</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>GLP-Compliant Safety Studies</th>
<th>Safety pharmacology (cardiovascular and respiratory) in telemetered NHPs</th>
<th>NOAEL ≥150 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Genetic toxicology in vitro (Ames, chromosomal aberrations in hPBL) and in vivo (rat BM micronucleus)</td>
<td>All negative up to limit doses</td>
</tr>
<tr>
<td></td>
<td>General Toxicology studies in rat and NHP • 13 week studies + 8 week recovery • Toxicokinetics and CNS evaluation (NHP)</td>
<td>Rat NOAEL ≥ 50 mg/kg NHP NOAEL ≥ 150 mg/kg</td>
</tr>
</tbody>
</table>

| Absorption, Distribution, Metabolism, Excretion (ADME) Studies | PK/PD in rat, NHP • Toxicokinetics for all Tox studies (plasma, liver, kidney) • Metabolic profiling in rat, NHP (plasma, liver) • Plasma protein binding (mouse, rat, NHP, human) • In vitro CYP studies |

**Safety margins of >160x (rat) and >500x (NHP), based on respective NOAELs and mg/kg translation**

**Filed CTA for treatment of AAT deficiency associated liver disease**
ALN-AAT Phase 1/2 Study
Dose-Escalation Study Including PiZZ Patients

**PART A**

**Study Design**
- Randomized, single-blind, placebo-controlled SAD study in healthy volunteers (up to N=24)

**Primary Objective**
- Safety and tolerability of single dose in healthy volunteers

**Secondary Objectives**
- PK/PD and clinical activity
  - AAT levels in serum

**PART B**

**Study Design**
- Randomized, single-blind, placebo-controlled MAD study in healthy volunteers (up to N=24)

**Primary Objective**
- Safety and tolerability of multi-dose in healthy volunteers

**Secondary Objectives**
- PK/PD and clinical activity
  - AAT levels in serum

**PART C**

**Study Design**
- Randomized, single-blind MD study in PiZZ patients (up to N=18)

**Primary Objective**
- Safety and tolerability of multi-dose in PiZZ patients

**Secondary Objectives**
- PK/PD and clinical activity
  - AAT levels in serum
  - Z-AAT levels in liver
Nonclinical Development Summary

Conclusions

• Nonclinical pharmacology data have shown that ALN-AAT can consistently and durably, reduce serum and liver AAT levels
• In the Tg-PiZ mouse model, AAT siRNA led to:
  ◦ Reduction in Z-AAT globules
  ◦ Lower liver tumor burden
• Chronic dosing leads to cumulative, sustained, dose-dependent knock-down of Z-AAT
• Results demonstrate efficient and robust delivery to fibrotic livers
• Monthly dosing in NHP at 3mg/kg leads to ~90% reduction in serum AAT
  ◦ Maximal levels of knock-down are maintained for at least 30 days after the last dose
• Safety Pharmacology and toxicology studies support proposed Phase 1/2 clinical program
• Filed CTA for treatment of liver disease associated with AAT deficiency
Thank You