Developing an RNAi Therapeutic for Liver Disease Associated with Alpha-1-Antitrypsin Deficiency

November 12, 2012
Alpha 1 Antitrypsin

**Alpha-1 anti-trypsin (AAT) is a serine proteinase inhibitor**
- Abundant plasma protein, largely synthesized by hepatocytes
- 52kDa glycoprotein, 418 aa
- Inhibits neutrophil elastase
- Inhibits trypsin, thrombin, chymotrypsin, factors XI and XIII, plasmin
- Characteristic secondary structure of beta sheets and alpha helices

**AAT deficiency**
- Autosomal Disease
- Mutation leading to lower secreted protein

**Liver Disease Associated with AAT deficiency**
- Z-AAT
- Accounts for 95% of AAT-deficient patient population
- Z allele: -Glu 342 Lys caused by GAG to AAG
**PIZZ: homozygous Z alleles**

- Autosomal disease (predicted homozygote frequency ~1 in 2000)
- Glu 342 Lys caused by GAG to AAG
- Accounts for 95% of AAT deficient patient population

*J Clin Invest, 2002, 110:1585–90*
http://flipper.diff.org/app/items/info/2843*
Liver Sections from PiZZ Patients

PAS Staining

Globules of AAT

EM

2400X

Inclusions in ER

20000X

Hepatology (2005): 41:160
Z-AAT Mediated Disease

- Inherited deficiency is associated with lung and liver disease.
- Wide individual variation
  - Lung: “Deficient” serum level → lungs susceptible to emphysema
  - Liver: Mutant misfolded Z protein accumulates in liver and injures hepatocytes, leading to liver fibrosis, cirrhosis and hepatocellular carcinoma (HCC)
  - ~ 15% of Protein is secreted

Conceptual Model for Z-AAT protein retention in ER

*Curr Gastro Reports; 8:14 (2006)*
Using RNA interference (RNAi) to Decrease Z-AAT Protein
RNA Interference

Natural Process of RNAi

Synthetic siRNA

dsRNA

dicer

Cleavage

Strand separation

Complementary pairing

RISC

mRNA

(A)ₙ

Targeted protein suppression

mRNA degradation

(A)ₙ
RNAi Lead Discovery

Work-flow

Starting Pool based on bioinformatics rules

Species Cross-reactivity
Off-target evaluation
Efficacy criteria

In vitro Efficacy

In vivo Efficacy
(select lead sequence)

Chemical modifications to increase stability

Lead Candidate

1
Lipid Nanoparticles (LNPs) for Systemic RNAi

Multi-component lipid formulation
- Amino lipid
- Structural lipid
- PEG lipid
- Cholesterol
- siRNA (Lead)

Highly efficient for liver delivery
- Hepatocyte-specific gene silencing achieved
  - In rodents
  - Non-human primates
  - Humans

- Low surface charge
- Small uniform size particle <100 nm
Preclinical Research Data

Testing LNP-AAT siRNA *invivo*
Z-AAT knock-down in Transgenic Animals
Collaboration with Teckman lab (St. Louis)

**Experiment Design**
- Transgenic mice expressing human Z-AAT
  - Exhibit liver disease
- Dose response with
  - AAT siRNA
  - Single IV dose in LNP, sac after 48 hours (n=3)
  - Collect liver and serum
- Analysis with human AAT specific reagents

**Liver hAAT Levels**

<table>
<thead>
<tr>
<th>Dose (mg/kg)</th>
<th>Liver hAAT/mGAPDH</th>
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<tbody>
<tr>
<td>PBS</td>
<td>1.0</td>
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<tr>
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<td>0.1</td>
<td>0.03</td>
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</table>

**Serum AAT ELISA**

<table>
<thead>
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<th>Dose (mg/kg)</th>
<th>Serum AAT (mg/L)</th>
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<td>PBS</td>
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![Graph showing liver hAAT levels and serum AAT ELISA](image)
Long Term Dosing I
Z-AAT Knock-down in Transgenic Animals

Experiment Design
- Transgenic male mice expressing Z-AAT (8-10 week old) n=6
- Multi-dose study, alternate weeks, ALN-AAT, 0.3mpk
- Analysis with human AAT-specific reagents

Results
- >90% Decrease in Liver mRNA, serum protein
- Decrease in pathogenic protein polymers

![Graph showing hAAT Protein Levels](image)

- First IV dose
- Day 0 14 28 42 56 70 84
- 8-10 week olds
- Sac-Day 86

![Images of Monomer and Polymer bands](image)
Decreased Polymers Lead to Reduced Globules
PAS-Globule Staining in Liver

**Pink Globules visible after PAS Staining → indicate AAT aggregates**

**Average Globule Area**
- Luc: 91% decrease, p=0.000000082
- AAT: 65% decrease, p=0.0000235

**Average Globule Size**
- Luc: 65% decrease, p=0.0000235
- AAT: 65% decrease, p=0.0000235

Littermates are in same Column
Long Term Dosing-III
Effect of AAT Knock-down at Ultra-structural Level

- AAT treated animals show:
  » Smaller and fewer globules
  » Less ER dilation
  » Fewer autophagic vacuoles
  » Less mitochondrial Injury
Long Term Dosing IV
AAT Knock-down Improves Liver Physiology

- Treatment ALN-AAT decreased BrdU incorporation
- Normal livers have low proliferative index
- Treatment with ALN-AAT reduced collagen levels in PiZ mouse to WT levels
- Col1a1 and Col1a2 showed same pattern
LNP Mediated Delivery to Fibrotic Livers
Measured by Target Knock-down

**Experiment Design**

- 10-12 month old PiZZ mice
- PiZZ animals have severe disease with significant fibrosis
- Test Delivery in diseased livers
  - Single IV dose in LNP, sac after 48 hours (n=5)
  - LNP-AAT, LNP-Control, PBS
  - Collect liver and serum
- Analysis with human AAT specific reagents

**Liver mRNA Levels**

- hAAT mRNA Levels (normalised to GAPDH) Relative to PBS
- PBS, Control, AAT

**Serum hAAT Levels**

- AAT Levels on Day 2 relative to Day 0
- PBS, Control, AAT
Effective Z-AAT silencing obtained in vivo
- Identified specific and highly potent siRNA against AAT
- ED50 = 0.1-0.03mpk
- 3 weekly doses start to show decrease in polymer and globules

Chronic Dosing Study
- Starting with 10-12 week old transgenic animals
  - ALN-AAT dosing on alternate weeks is very effective in decreasing disease phenotype
    - Data from 13 weeks, 7 doses
    - Decrease in Liver protein and mRNA
    - Decrease in PAS staining: correlates nicely with decrease in liver polymer
    - Decrease in proliferative index: less liver damage, so less number of dividing hepatocytes
    - Decrease in fibrosis markers
    - Clear benefit of treatment at the ultra structural levels by EM

- Dosing in Fibrotic Livers
  - Achieved robust silencing in 10-12 month old PiZZ mice
  - ALN-AAT can be delivered to diseased livers
ALN-AAT Summary

- AAT deficiency is genetic cause of both lung and liver disease

- Liver disease caused by deposition of mutant misfolded Z allele protein product

- ALN-AAT demonstrates efficacy in transgenic mouse model
  » Reduces liver globules and polymers
  » Improves liver physiology
Acknowledgments

Alnylam Pharmaceuticals

- David Bumcrot
- Amy Simon
- Akshay Vaishnaw
- Oved Amitay
- Brian Bettencourt
- Satya Kuchimanchi & team
- Stuart Milstein & team
- Klaus Charisse & team
- Formulation team
- Tim Racie
- June Qin

St. Louis University

- Jeff Teckman
- Keith Blomenkamp
Liver Z-AAT Analysis
Monomer Analysis by Semi-quantitative Western Blot

Western blot with antibodies specific for human AAT
- Monomer: soluble fraction of liver homogenate
  - Show a decrease at 48 hours
  - Closely follow the liver mRNA
- Polymer: insoluble fraction

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- Dose
- 2d
- Serum

Monomer Density

RDU

0
1000
2000
3000
4000
5000
6000
7000
8000
9000

0
1000
2000
3000
4000
5000
6000
7000
8000
9000

PBS | 1.0 | 1.0 | 0.3 | 0.1 | 0.03

Monomer density graph shows the decrease in monomer density with increasing AAT concentrations.