Digestive Disease Week
RNAi Therapeutics Ameliorate Liver Disease Associated with Alpha-1 Antitrypsin Deficiency

May 6, 2014
Alfica Sehgal
Alpha-1 Antitrypsin (AAT)

**AAT is a serine proteinase inhibitor (serpin)**
- Inhibits neutrophil elastase
- Inhibits trypsin, thrombin, chymotrypsin, factors XI and XIII, plasmin
- Abundant plasma protein

**AAT deficiency**
- Autosomal recessive disorder with multiple alleles (>80)
  - Nulls, PiMM, PiZZ, PiSS, PiMZ, PiSZ
  - Alleles described based on migration on IEF
  - Alleles have different impact on secretion
- PiZZ accounts for 95% of AAT-deficient patient population
  - Z allele point mutation Glu342Lys
  - Z-AAT not folded correctly in ER
    - Reduced secretion → lung disease
    - Forms polymers with aggregation → liver disease
Severe AAT Deficiency
Genotype-Phenotype Relationships

- Pi null
- PiZZ
- PiSZ
- PiMZ
- PiMM

Emphysema
(% Affected)

Liver Disease

Serum α1AT Level (µM)

Range of AAT in individuals

Z gene: Scandinavia, British Isles, France, Germany, Baltics
S allele: Spain and Portugal

- **Inherited deficiency** associated with lung and liver disease.
- **Wide individual variation**
  - **Lung**: “Deficient” serum level leaves tissues susceptible to damage by neutrophil proteases. In particular the lungs to smoke, leading to emphysema
  - **Liver**: Misfolded mutant Z protein accumulates in liver, → injures hepatocytes, → liver fibrosis, cirrhosis and hepatocellular carcinoma
    - ~15% of Protein is secreted
Accumulation of Mutant Z-AAT in Liver

PAS Staining

Globules of AAT

EM

2400X

Inclusions in ER

An et al., Hepatology; 41:160-7 (2005); Lomas et al., Nature; 357:605-7 (1992)
Using RNA interference (RNAi) to Knockdown Z-AAT Protein
Therapeutic Hypothesis for ALN-AAT

Z-AAT aggregates in liver leading to inflammation, fibrosis, cirrhosis, HCC

ALN-AAT

Reduction in mRNA in liver will reduce mutant protein

Decrease in Z-AAT polymers, reduces aggregate deposition in liver, yields less hepatocyte damage

Decrease in fibrosis and HCC
Z-AAT Knockdown in Transgenic Animals
Multi-Dose Pre-Clinical Efficacy with LNP-AAT

Z-AAT knockdown with LNP-AAT

- **Summary**
  - IV dosing Q2W x 7 at 0.3mg/kg
  - >90% Decrease in liver mRNA, serum protein
  - Decrease in proliferation index as measured by BrdU incorporation
  - Decrease in mitochondrial injury and fibrosis

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

**Relative Density Units**

**hAAT Protein Levels**

- **Control**
  - Polymer: 1.2
  - Monomer: 1.0

- **LNP-AAT**
  - Polymer: 0.4
  - Monomer: 0.2

* p=0.00001
# p=0.00226

**Decrease in PAS Positive Globules**

Sehgal, AASLD, Nov. 2012
Asialoglycoprotein Receptor (ASGPR)
- Clears serum glycoproteins via clathrin-mediated endocytosis
- Well suited for receptor-mediated, targeted delivery
  - Highly expressed in hepatocytes
    - 0.5-1 million copies/cell
  - High rate of uptake
  - Recycling time ~15 minutes
- Conserved across species

GalNAc-siRNA
- GalNAc ligand conjugated to chemically modified siRNA to mediate targeted delivery
- Trivalent GalNAc carbohydrate cluster has nM affinity for ASGPR
- Administered subcutaneously (SC)
- Enhanced Stabilization Chemistry (ESC) GalNAc-siRNA conjugates achieve improved potency and duration

Adapted from Essentials of Glycobiology (2009)
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?

**Decrease in Liver mRNA**

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<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>
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**Serum AAT After First Injection**

- PBS Female
- PBS Male
- AAT-Female
- AAT-Male
Z-AAT Knockdown Improves Liver Physiology

PBS Treated Animal

AAT-siRNA Treated Animal

Decrease in Fibrosis

Decrease in Immune Cells

Relative Col1a2 mRNA Levels

Relative PTPRC mRNA Levels

$p=0.04$

$p=0.002$
Z-AAT Knockdown Reduces Tumor Formation

PAS Globule Staining

Tumor Incidence

- 4/6 PBS animals had liver tumors
- 1/6 AAT treated animals had liver tumor

<table>
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<th>Ttmt</th>
<th>An #</th>
<th>Observation (p=0.045)</th>
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<tr>
<td>A</td>
<td>No macroscopic tumor</td>
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<tr>
<td>B</td>
<td>large tumor in left lateral lobe, ~5mm diameter</td>
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<tr>
<td>C</td>
<td>2mm tumor in caudate lobe, many lesions in 2nd aux lobe</td>
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<tr>
<td>D</td>
<td>1.5mm tumor in caudate lobe, 1mm lesion in right medial lobe, multiple 1mm lesions in 1st aux lobe</td>
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<tr>
<td>E</td>
<td>3mm tumor in left lateral lobe</td>
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</tr>
<tr>
<td>F</td>
<td>No macroscopic tumor</td>
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</table>

AAT Globules

- Relative Globule Area
- PBS: 1.2, AAT-siRNA: 0.8, p=0.02

Proliferating Cells

- Relative BrdU Count
- PBS: 1.2, AAT-siRNA: 0.8, p=0.02

PBS

AAT-siRNA

- No macroscopic tumor
- 3mm tumor in caudate lobe
Therapeutic Hypothesis Holds True
Summary

- Suppressing Z-AAT leads to
  - Reduction in PAS globules and AAT polymers
  - Reduction of hepatic fibrosis markers
  - Decrease in proliferative index
  - Decrease in hepatic tumor incidence
- Demonstrates efficient and robust delivery to fibrotic livers of aged animals
- Chronic dosing can decrease disease burden
**ALN-AAT Development Candidate in Mice**
Duration, Dose Response and Repeat Dosing

**Screening AAT-siRNA Candidates**

- Relative Serum hAAT (prebleed=1)
- Days: -4, 3, 7, 10, 14, 21
- ALN-AAT Dev Candidate

**Single Dose Efficacy**

- ED50 ~ 0.5mg/kg

**Single Dose: Dose Response and Duration**

- Relative Serum hAAT (prebleed=1)
- Days: -5, 5, 10, 15, 20
- ALN-AAT Dev Candidate

**Multi-Dose at 0.5mg/kg, BIW**

- Relative serum AAT (prebleed=1)
- Days: 10, 30, 50, 70
- ALN-AAT Dev Candidate 0.5mg/kg biw x 4
ALN-AAT Development Candidate in NHP
Initial Pre-Clinical Results in Single Dose Study

Ongoing Study in NHP

- Single dose at 1.0 and 3.0 mg/kg
  - N=3, males
  - Serum AAT by ELISA
- Well tolerated
  - No safety findings
  - No change in cytokines
  - No injection site reactions
- Rapid, potent AAT knockdown
  - Single dose ED$_{50}$ <3 mg/kg
  - Comparable single dose potency with other ESC-GalNAc-siRNA conjugates
    - E.g., ALN-PCSsc and ALN-AT3 (pre-clinical)
    - Expect multi-dose ED$_{50}$ <1 mg/kg
Summary

• Identified potent RNAi Therapeutic Development Candidate, ALN-AAT, for treatment of liver disease associated with AAT deficiency

• Pre-clinical data with ALN-AAT demonstrated
  » Dose-dependent and durable lowering of AAT in liver after SC dosing in mice and NHP
  » Efficacy and tolerability was maintained in mice with liver disease
  » Chronic siRNA dosing leads to sustained knockdown of AAT → improved liver physiology

Next Steps

• Plan for IND filing in mid-2015
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