Adeno-associated virus vectors: Clinical Development issues

T.R. Flotte, M.D.
University of Florida
Long-term Goals

• Identify and Manage Long-term risks of Gene Therapy with Recombinant Adeno-Associated Virus (rAAV) Vectors
  – Insertional Mutagenesis
  – Inadvertent Germline Transmission
  – Immune Responses to capsids and transgene products

• Construct Safe and Effective Vector to treat Alpha-1 Antitrypsin (AAT) Deficient Patients
Adeno-associated Virus (AAV)

- Small virus, lives in symbiotic relationship with humans
- Simple DNA structure and protein capsid
- Used for long-duration transfer of genes
- Present in numerous serotypes in humans and non-human primates
Insertional Mutagenesis: Data supporting Episomal rAAV

- **Lung** *(Afione, Engelhardt)*
- **HSC** *(Niehuis, Walsh)*
- **Muscle** *(Clark, Johnson)*
- **Liver** *(Kay, Song)*

![Insertional Mutagenesis Diagram]
Alpha 1-antitrypsin Deficiency (Genetic Emphysema)

- Mutations in AAT gene (esp. PiZ)
  - Defective secretion
  - Lack of antiprotease defense from this 52kD serpin
- Lung disease: unopposed action of NE and other white blood cell products on interstitial elastin
  - 800\(\mu\)g/ml is protective
- Liver disease: only 10% of cases, due to clumping of PiZ in liver cells
Skeletal muscle as a Platform for Delivery of secreted proteins

Long term secretion of hAAT from murine muscle transduced with C-AT

Song, et al., PNAS 1998
Song, et al., PNAS 2001
AAT made in muscle is functional

Binding to Neutrophil Elastase
Song, et al., 2005 submitted
Specific Aims

• To assess the safety of IM administration of rAAV2-CB-hAAT in adult AAT-deficient patients

• To determine the dose of rAAV2-CB-hAAT required to achieve a detectable level of normal M-variant AAT in AAT-deficient adults
**cGMP Manufacturing at UF:**
Production Scheme and Lot Release

![Diagram](image)

**Test** | **Method** | **Specification**
---|---|---
**Sterility** | Direct Inoculation | No growth
**Endotoxin** | LAL | < 50 EU/mL
**Titer** | | |
| Infectious Titer | ICA | >1X10^11 IU/ml
| Vector Genome Titer | Dot Blot | >1X10^13 vector genomes/ml
| Capsid Titer | ELISA | Report Results
| Infectivity Ratio | ICA/Dot Blot | Report Results

**Purity**

<table>
<thead>
<tr>
<th>Test</th>
<th>Method</th>
<th>Specification</th>
</tr>
</thead>
</table>
| Protein purity | PAGE and coomassie blue stain | >90% pure
| 293 Cell contaminating DNA | for protein Hybrization | < 100 ng per dose
| Benzonase residual | ELISA | Report Results
| rcAAV | Infectious Center assay | <1 in 1X10^8 IU rAAV

*Snyder and Flotte, 2004*
Phase I protocol

- Single site (UF)
- Open label
- Single dose
- Dose escalation between subjects
- Intramuscular administration with ultrasound guidance to avoid vascular structures
- N = 12 (4 cohorts of 3 subjects)

<table>
<thead>
<tr>
<th>Cohort #</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
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<tbody>
<tr>
<td>Dose, vg</td>
<td>2.1x10^{12}</td>
<td>7.0x10^{12}</td>
<td>2.1x10^{13}</td>
<td>7.0x10^{13}</td>
</tr>
<tr>
<td>N</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
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</tbody>
</table>
Subject Selection

• Inclusion criteria
  – Male or female ≥ 18
  – Diag of AAT def by level (< 11µM) and genotype (Z)
  – FEV1 >30% pred
  – Willing to discontinue AAT replacement 4 weeks prior and resume 11 after injection

• Exclusion criteria
  – Recent (15d) IV antibiotics
  – LFTs > 2x ULN
  – CK > 3xULN
  – Other investigational drug
  – Pregnant or nursing
  – Fertile and not using contraception
  – Cig smoker or substance abuse
  – Immune response to AAT replacement
  – Other conditions at discretion of PI
Outcome Measures

• General Safety
  – Physical findings
  – CBC, PT, PTT
  – Serum chemistry, UA

• Injection Site Safety
  – Physical findings
  – Arm Circumference
  – CK levels

• Biologic Effect
  – Total AAT
  – M-Specific IEF/Western
  – M-Specific ELISA

• Biodistribution
  – Blood Taqman
  – Semen Taqman

• Immune Response
  – Anti-AAV capsid Ab
  – Anti-AAT Ab
  – Lymphocyte prolif (ASR) to AAV
  – Lymphocyte prolif (ASR) to AAT
Time Line: Phase I rAAV2-AAT

- **Protein Therapy**
- **Vector Injection**
- **Blood and Semen PCR**
- **Immune response profile to AAV and hAAT**
- **AAT expression studies done throughout up to 180d**
- **General safety studies done throughout up to 365d**
Subject 101 Injection site
Ultrasound localization

Injection Site
Doppler Flow During Injection
First human use of rAAV2-AAT

- 59 year old Male
- Caucasian
- Protein replacement prior to study entry

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>Day -1</th>
<th>Day 0 Dosing</th>
<th>Day 3</th>
<th>Day 14</th>
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<tr>
<td>hAAT uM/ IEF</td>
<td>10.1/ M1Z</td>
<td>3.67/ ZZ</td>
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<td>3.56/ ZZ</td>
<td>3.34/ ZZ</td>
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<tr>
<td>FEV1 %</td>
<td>34.8</td>
<td>33.3</td>
<td></td>
<td>31.7</td>
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<tr>
<td>CK U/L</td>
<td>63</td>
<td>69</td>
<td></td>
<td></td>
<td>82</td>
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<tr>
<td>GGT</td>
<td>19</td>
<td>17</td>
<td></td>
<td>19</td>
<td>17</td>
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</tbody>
</table>
Note: all patients with high levels of M-AAT before injection coincide with those that were on Prolastin. Pt. 101 went back on Prolastin therapy at D90.
Antigen-Specific Lymphocyte Proliferation vs. AAV

Stimulation Index (SI)

Time, days

Tests:
102
103
201
202
203
204
205
301
302

Antigen-Specific Lymphocyte Proliferation vs. AAV
Antigen-specific Lymphocyte Proliferation vs. AAT

Stimulation Index (SI)

Time, days

-40 -20 0 20 40 60 80 100

0 1 2 3 4 5

102 103 201 202 203 204 205 301 302
Preliminary Findings for first 3 cohorts

- Eight (8) patients safely treated
- No serious vector-related adverse effects
- No antibodies to AAT protein
- Have antibodies to capsid proteins
- Waiting on levels of M-AAT protein
- Subjects to participate in Long-term follow-up
Future Clinical Vector Candidates: rAAV1-hAAT in muscle

- Pseudotypes: same rAAV-hAAT cassette (AAV2 ITRs, diff caps)
- Advantage of type 1 in muscle; type 8 in liver
- rAAV1-hAAT-IM
  - GLP tox data generated in mice
  - OBA/RAC application\}
Investigators

- Terence R. Flotte, M.D.
- Mark L. Brantly, M.D.
- L. Terry Spencer, M.D.
- Barry J. Byrne, M.D., Ph.D.
- Carolyn T. Spencer, M.D.
- Margaret Humphries, R.N.
- Richard O. Snyder, Ph.D.
Contributors to the AAT Project

• Flotte Lab
  – Sihong Song
  – Michael Morgan
  – Thomas Conlon
  – Pedro Cruz
  – Amy Poirier
  – Lynn Combee
  – Ashley Martino
  – Sato Klein
  – Scott Loiler
  – Qiushi Tang
  – Fu-Sheng Wei
  – Kevin Foust
• Mark Brantly
• Barry Byrne
• Nick Muzyczka
• Bryon Petersen
  – Rafael Wital
• Mark Atkinson

• GMP Production/Vector Core/Regulatory
  – Richard Snyder
  – Kye Chesnut
  – Mark Potter
  – The rest of the crew
  – Joyce Francis
  – Aleta Crawford

• Pathology Core
  – Jim Crawford
  – Martha Campbell-Thompson
  – Marda Jorgensen

• AGTC
  – Sue Washer

• Alpha One Foundation
• NHLBI