Follistatin Gene Therapy for sIBM and Becker Muscular Dystrophy

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The Clinical Problem

• Quadriceps muscle weakness
  - Becker muscular dystrophy
  - Inclusion body myositis

• Frequent falls
  - Limb fractures
  - Loss of ambulation

• Improving quadriceps muscle strength would result in a “clinically meaningful outcome”
Resistant to Approaches

- Weight training
- Electrical Stimulation
- Anabolic Steroids
Myostatin Circulates Propeptide Complex

Activated Protease Cleavage

INHIBIT BINDING
Gene mutation
Antibody Peptides

Myostatin Regulates Muscle Growth

MYOSTATIN REGULATION OF MUSCLE SIZE
Myostatin Gene Mutation

• Targeted disruption of the myostatin gene: increases muscle size and body weight

- “Mighty” Mouse (Mstn KO)
- Double-muscled cow (Mstn Het)
Wyeth sponsored 11 Center Trial (10 USA; 1 GB) Using antibody to myostatin

- No Clinical benefit
- High dose cohorts developed skin hypersensitivity reactions
Follistatin Gene Therapy

Circulating complex Propeptide-myostatin

Propeptide cleavage

M M

FOLLISTATIN

AAV-FOLLISTATIN

Activin Receptor

INJECT AAV INTO MUSCLE
Follistatin Gene

1) Alternative Splicing

2) Translation

3) Cleavage of Signal Peptide (29aa)

FS 288  Tissue bound isoform

FS 315  Circulating isoform
Treatment of *mdx* with AAV1.FS344

**AAV1-GFP  AAV1-FS**

Q and TA Bilateral

1e11  1e10
Tibialis Anterior

5 mo post gene transfer

> 1 year
Hind Limb Grip Strength

3 months post gene transfer

![Graph showing CK levels for different groups: GFP, FS, C57Bl.](a)

![Graph showing Hind Limb Grip Strength over Age (Days) with Injection marker.](b)
Can the Mouse Studies Predict Safety and Efficacy in a Clinical Trial?

Moving to Non-Human Primates to Simulate Clinical Trial
FS344 Gene Transfer to Monkey
AAV1-FS

Control

5 MO POST GENE TRANSFER

Control  MCK-FS  CMV-FS
## Functional Improvement

<table>
<thead>
<tr>
<th>Promoter</th>
<th>Twitch Force</th>
<th>Tetanic Force</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Untreated Leg</td>
<td>FS-Treated</td>
</tr>
<tr>
<td>MCK</td>
<td>17.0</td>
<td>19.0 (11.8%)</td>
</tr>
<tr>
<td>CMV</td>
<td>19.0</td>
<td>24.0 (26.3%)</td>
</tr>
</tbody>
</table>
No Cardiotoxicity**
5 and 15 months
Clinical Chemistries
Monkeys used in Pre-clinical Studies

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Baseline</th>
<th>5 months</th>
<th>15 months</th>
<th>Baseline</th>
<th>5 months</th>
<th>15 months</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Post Injection</td>
<td>Post Injection</td>
<td></td>
<td>Post Injection</td>
<td>Post Injection</td>
</tr>
<tr>
<td>Hgb (mg/dL)</td>
<td>11.7 ± 1.2</td>
<td>12.3 ± 0.7</td>
<td>13.5 ± 0.6</td>
<td>12.9 ± 0.9</td>
<td>12.9 ± 0.3</td>
<td>12.6 ± 0.8</td>
</tr>
<tr>
<td>WBC (K/cu mm)</td>
<td>9.4 ± 3.6</td>
<td>11.0 ± 1.8</td>
<td>7.5 ± 1.7</td>
<td>13.2 ± 1.7</td>
<td>10.8 ± 2.8</td>
<td>15.5 ± 8.9</td>
</tr>
<tr>
<td>Platelets (K/cu mm)</td>
<td>444.7 ± 78.6</td>
<td>473.7 ± 101.5</td>
<td>448.5 ± 34.6</td>
<td>475.3 ± 21.2</td>
<td>470.0 ± 10.8</td>
<td>432.0 ± 39.6</td>
</tr>
<tr>
<td>CK (U/L)</td>
<td>282.3 ± 123.3</td>
<td>103.3 ± 34.0</td>
<td>261.0 ± 97.6</td>
<td>315.1 ± 436.8</td>
<td>–</td>
<td>141.0 ± 5.7</td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>29.7 ± 12.9</td>
<td>19.7 ± 2.1</td>
<td>31.5 ± 2.1</td>
<td>28.7 ± 10.3</td>
<td>21.7 ± 4.6</td>
<td>29.5 ± 6.4</td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>35.3 ± 3.51</td>
<td>34.7 ± 9.9</td>
<td>37.5 ± 6.4</td>
<td>44.3 ± 11.4</td>
<td>31.7 ± 6.0</td>
<td>35.5 ± 4.9</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>19.0 ± 1.0</td>
<td>12.3 ± 1.5</td>
<td>16.0 ± 1.4</td>
<td>16.3 ± 4.9</td>
<td>16.0 ± 4.4</td>
<td>18.5 ± 7.8</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.5 ± 0.1</td>
<td>0.8 ± 0.1</td>
<td>0.7 ± 0.1</td>
<td>0.9 ± 0.2</td>
<td>0.9 ± 0.1</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>72.0 ± 28.8</td>
<td>92.0 ± 38.7</td>
<td>77.5 ± 51.6</td>
<td>77.0 ± 20.7</td>
<td>71.3 ± 18.2</td>
<td>75.0 ± 9.8</td>
</tr>
</tbody>
</table>
Necropsies of NHP used in Pre-Clinical Studies

• Full necropsy on all monkeys
  - slides on each organ evaluated by a board certified veterinary pathologist blinded to treatment group (control vs FS)

• No treatment-related abnormalities found in heart, liver, lung, spleen, kidney, testis, ovary and uterus (5 & 15 months)
Taking this to Clinical Trial

Prepare for Clinical Trial

Develop Outcome Measures
Final Step Before Clinical Trial
Submit the IND
AAV1. Follistatin Clinical Trial

- 18 subjects (9 sIBM /9 Becker muscular dystrophy patients)
- Dose escalation study injection of AAV1.CMV.FS344 into quadriceps
- Outcome: 6MWT and Quantitative myometry of Knee extensors
- Muscle biopsies at 3 months and 6 months
- Patients will be followed for 2 years
Circumventing Barriers to Negative Gene Therapy Trial

• **Avoid pre-existing immunity to AAV**
  - Some individuals have been exposed to virus and should be excluded from the trial

• **Avoid immune immune response to follistatin**
  - Less likely but possible and patients should be checked
Three Patients have been treated to date and no adverse events encountered.

Hopefully Making Progress For Challenging Conditions!
SUCCESS IS A TEAM EFFORT!

Cellular Immune
Christopher Walker
Katie Campbell

Gene Therapy Center
Brian Kaspar  PRE-CLINICAL EFFORTS
K Reed Clark  VECTOR MANUFACTURING

Chris Shilling  PROGRAM MANAGER

Xiomara Rosales  FDA LIAISON