DMD Clinical Gene Therapy Trial

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AAVrh74.MHCK7.Micro-dystrophin

- MHCK7 promoter enables robust dystrophin expression in heart and skeletal muscle
- AAVrh74 provides broad distribution to all muscle types, including the heart and diaphragm
Strong Profile for AAVrh74

• Demonstrated efficacy
  — Ideal systemic biodistribution vs other vectors in preclinical testing
  — Widespread high-level gene expression after IV infusion in preclinical animal models
  — Gene expression in Phase 1 trials across multiple diseases by IM and IV delivery*

• Demonstrated safety
  — *No observed adverse effect level (NOAEL)* in primates and mice
  — *14 human subjects* dosed (IM, n=4; IV, n=10) without vector-related adverse effects
  — *6 approved INDs*

GLP, good laboratory practice; ILP, isolated limb perfusion; IM, intramuscular; IND, investigational new drug; IV, intravenous.

*Unpublished data based on screening of approximately 70 patients with Limb girdle muscular dystrophy (LGMD) or DMD.*
Preclinical IV Study Design for the Delivery of AAVrh74.MHCK7.Micro-dystrophin

Assess efficacy and safety 12 weeks to 6 months after gene delivery

- Transgene expression and biodistribution
- Western blot and qPCR
- Histological analysis
- Functional benefit
- Safety and toxicity
Gene Expression-Systemic Delivery

Expression exceeds 75% in all muscles at clinical dose levels 2e14vg/kg
Reassembly of Dystrophin-associated protein complex with Micro-dystrophin

Skeletal Muscle  Heart  Diaphragm

Wildtype

LR in WT/MDX

MDX-LR

2e14 vg/kg

Mid Dose

MDX Ascending dose

BSG
Phase I/II Trial in DMD

Nationwide Children’s Hospital
Open-label Trial Design

• 12 subjects with DMD – open trial
  – Cohort A: 6 subjects; 3 months-3 years of age
  – Cohort B: 6 subjects; 4-7 years of age

• Inclusion criteria
  – Confirmed *DMD* mutation between exons 18-58, inclusive
  – AAVrh74 antibodies <1:50 titer
Cohort B (4-7 years of age): Endpoints

• **Primary endpoint:**
  — Safety

• **Secondary endpoints:**
  — Change in micro-dystrophin expression pre- vs post-therapy
  — Decrease in CK
  — 100-meter timed test (100m)
  — North Star Ambulatory Assessment (NSAA; 10-meter timed test included)
  — Timed Up and Go (TUG)
  — Ascend and descend 4 steps
  — Hand-held Dynamometry (HHD)
  — Cardiac MRI (at 1 year)
## Subject Demographics at Baseline

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age (years)</th>
<th>CK Levels at Baseline (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5</td>
<td>20,691</td>
</tr>
<tr>
<td>2</td>
<td>4</td>
<td>23,414</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>34,942</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>29,210</td>
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</tbody>
</table>
CK Levels Are Dramatically Reduced with Micro-dystrophin Therapy

CK Levels (U/L)

Days After Gene Therapy

CK Values Over Time*

*Normal CK values are <175 U/L.
†Based on a hypothetical DMD patient starting with a CK value of 35,000 U/L and decreasing at an annual rate of 8%.1
Robust Micro-dystrophin Expression in Muscle Fibers from the Gastrocnemius

<table>
<thead>
<tr>
<th>Normal Control</th>
<th>Subject 1</th>
<th>Subject 2</th>
<th>Subject 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-Treatment</td>
<td><img src="image1" alt="Image" /></td>
<td><img src="image2" alt="Image" /></td>
<td><img src="image3" alt="Image" /></td>
</tr>
<tr>
<td>Post-Treatment</td>
<td><img src="image4" alt="Image" /></td>
<td><img src="image5" alt="Image" /></td>
<td><img src="image6" alt="Image" /></td>
</tr>
</tbody>
</table>

Micro-dystrophin expression (IHC)
Robust Micro-dystrophin Expression in Muscle Fibers from the Gastrocnemius

<table>
<thead>
<tr>
<th>Subject</th>
<th>Mean Intensity</th>
<th>Percentage of Dystrophin-Positive Fibers</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>82.0 %</td>
<td>78.0 %</td>
</tr>
<tr>
<td>2</td>
<td>59.0 %</td>
<td>73.5 %</td>
</tr>
<tr>
<td>3</td>
<td>83.0 %</td>
<td>77.0 %</td>
</tr>
<tr>
<td>Mean</td>
<td>74.5 %</td>
<td>76.2 %</td>
</tr>
</tbody>
</table>
Detection of Micro-dystrophin Expression by Western Blot Post-Treatment

Western Quantitation Method | Mean Micro-dystrophin expression compared vs normal
--- | ---
Sarepta | 36.5% (not adjusted for fat and fibrotic tissue)
Nationwide | 53.7% (adjusted for fat and fibrotic tissue)
Vector Genome Copy Number Is >1 Copy Per Nucleus, Consistent With Micro-dystrophin Expression Levels

<table>
<thead>
<tr>
<th>Subject</th>
<th>Vector Copies/μg DNA</th>
<th>Copies per Nucleus*</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>$&gt;10^5$</td>
<td>1.7</td>
</tr>
<tr>
<td>2</td>
<td>$&gt;10^5$</td>
<td>1.3</td>
</tr>
<tr>
<td>3</td>
<td>$&gt;10^5$</td>
<td>1.9</td>
</tr>
</tbody>
</table>

*1 vector copy per nuclei translates to ~50% micro-dystrophin positive fibers
Micro-dystrophin Gene Therapy Upregulates DAPC Proteins

Expression of DAPC Proteins in Muscle Fibers from the Gastrocnemius of Subject 2 (IHC)

<table>
<thead>
<tr>
<th>Normal Control</th>
<th>Pre-treatment</th>
<th>Post-treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-Sarcoglycan</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-Sarcoglycan</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Post Micro-Dys Gene Therapy

No Toxicity
Early Outcome Testing
2 Month Post GT

1) Improved 10m walk
2) Stair Climbing
3) North Star
   Ambulatory Assess
4) CK reduced
   20,691 to 2444 U/L
Subject 3 Home Video: Stair Climbing

2 days post gene delivery

60 days post gene delivery
Safety

• No Serious Adverse Events (SAEs) in this study

• 3 subjects had elevated GGT within first 2 weeks except patient 4 normal by week three and increased following pred taper

• No other clinically significant laboratory findings

• 3 Patients had transient nausea generally within the first week coincident with increased steroid dosing
  — Did not correlate with liver enzyme elevations or any other abnormality

GGT, gamma-glutamyl transpeptidase.
Summary

Preliminary Clinical Results

• Consistent with preclinical results
• Widespread micro-dystrophin expression
• Upregulation of the DAPC complex
• Reduction in CK
• Vector genome copy levels (>1 copy/nucleus) are consistent with robust micro-dystrophin protein expression
• Use of the MHCK7 promoter will potentially alter DMD disease natural history related to cardiac expression