Systemic Gene Transfer with rAAVrh74.MHCK7.micro-dystrophin in Patients with Duchenne Muscular Dystrophy

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BACKGROUND AND OBJECTIVE
- Duchenne muscular dystrophy (DMD) is a rare, X-linked, fatal, neuromuscular disease caused by mutations in the DMD gene that lead to the production of dysfunctional dystrophin protein.1,2
- Systemic adeno-associated virus (AAV)-based gene therapy for DMD faces challenges that require careful rational design of the construct, given the need to target cardiac muscle and broadly distributed skeletal muscle tissue, and the packaging limitations of AAV.1,3
- AAV-mediated gene therapy has shown early signs of potential to treat DMD. Key considerations include a systematic and stepwise evaluation of safety, transduction, expression, localization, cellular impact, and clinical function. With these considerations in mind, we developed rAAVrh74.MHCK7.micro-dystrophin to achieve targeted skeletal and cardiac muscle expression of a shortened functional dystrophin protein (Figure 1). Long-term durability has been demonstrated in DMD preclinical models for up to 8 years after treatment.1,2
- Here we report, for the first time, the long-term (3-year) functional data from 4 patients with DMD (aged 4-7 years) that supports long-term durability following a single IV infusion of rAAVrh74.MHCK7.micro-dystrophin.

METHODS

Trial Design
- Single-dose, open-label, Phase 1a trial (NCT03375164, Sarpel-9001-101)
- Inclusion criteria
  - Male patients with DMD (aged 4-7 years)
  - Confirmed molecular diagnosis of DMD (trinucleotide or duplication) or premature stop codon mutation between exons 18 to 58 in the DMD gene
- Exclusion criteria
  - Negative for AAV antibodies (NIL-401 test) tested by ELISA1,3
- Symptomatic muscular dystrophy
  - Creative kinase (CK) elevation >1000 U/L
  - Below average on the 100-Meter Timed Test (100 m), defined as ≤80 predicted1,2
- Stable steroid dosing 12 weeks prior to study entry
- Daily prednisolone, 1 mg/kg, started 1 day before gene delivery (30 day taper after infusion)1
- rAAVrh74.MHCK7.micro-dystrophin was infused over 1-2 hours via the peripheral limb vein at a dose of 2×1012 vgp/mL (20 µg/mL) in the Pediatric Intensive Care Unit at Nationwide Children’s Hospital (Columbus, Ohio)

Primary Outcome Measures
- Safety, based on the number of participants with adverse events (AEs), was monitored and recorded throughout the study and included: a serum chemistry panel; immune response (immunogenicity) assessments of AAVrh74 by ELISA (B cell response); and T-cell response was assessed against AAV rh74 capsid and micro-dystrophin proteins

Secondary Outcome Measures
- Micro-dystrophin expression quantified by immunofluorescence and Western blot in pre- and post-treatment muscle biopsy samples at 90 days

Efficacy Outcome Measures
- Decrease in CK
- North Star Ambulatory Assessment (NSA; 10-Meter Timed Test [10 m] and Time to Reach it)8
- Timed function tests: 100-m, 4-M stair Climb

PAAR: Analysis for NSA required in clinic assessments. As Patient 4 did not have in-clinic assessment at Year 2 due to Covid-19-related restrictions at the site, remote assessment at Year 2 was used in the summaries.

RESULTS

Baseline patient demographics are shown in Table 1.

Table 1. Baseline Demographics

<table>
<thead>
<tr>
<th>Age (yr)</th>
<th>Height (cm)</th>
<th>BMI</th>
<th>Creatinine kinase (IU/L)</th>
</tr>
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<tbody>
<tr>
<td>Patient 1</td>
<td>5</td>
<td>109.9</td>
<td>15.2</td>
</tr>
<tr>
<td>Patient 2</td>
<td>4</td>
<td>104.3</td>
<td>17.4</td>
</tr>
<tr>
<td>Patient 3</td>
<td>6</td>
<td>110.0</td>
<td>17.0</td>
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<tr>
<td>Patient 4</td>
<td>4</td>
<td>117.0</td>
<td>17.5</td>
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<table>
<thead>
<tr>
<th>Age at Baseline (yr)</th>
<th>BMI</th>
<th>Creatinine kinase (IU/L)</th>
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<tbody>
<tr>
<td>Patient 1</td>
<td>5.71</td>
<td>18.4</td>
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<tr>
<td>Patient 2</td>
<td>4.79</td>
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<tr>
<td>Patient 3</td>
<td>5.79</td>
<td>21.4</td>
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<tr>
<td>Patient 4</td>
<td>6.02</td>
<td>19.7</td>
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Safety
- No patients experienced a serious adverse event (SAE) or an AE that led to discontinuation
- There were no serious abnormalities observed in hematologic and chemical panels, which included liver function tests.

Dystrophin Expression and Quantification
- Systemic gene transfer resulted in robust levels of microdystrophin protein expression localized to the sarcoglycans by immunofluorescence and western blot (Figure 2). Expression was associated with an increase in β-sarcoglycan expression, promoting the restoration of dystrophin-associated protein complex (DAPC)5
- Demonstration of micro-dystrophin expression was associated with vector genome copies (Figure 2) and robust reductions in CK (mean change baseline to 90 days (n=4, 49.3%), confirming successful delivery of rAAVrh74.MHCK7.micro-dystrophin

Figure 1. rAAVrh74.MHCK7.micro-dystrophin construct

Figure 2. rAAVrh74.MHCK7.micro-dystrophin uptake into skeletal muscle increases mean levels of micro-dystrophin protein

Table 2. Summary of 2-Year Timed Function Tests

<table>
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<th>Time to Rise (sec)</th>
<th>100 m (sec)</th>
<th>100 m (sec)</th>
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</thead>
<tbody>
<tr>
<td>Patient 1</td>
<td>3.7</td>
<td>49.3</td>
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<tr>
<td>Patient 2</td>
<td>3.4</td>
<td>40.9</td>
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<td>Patient 3</td>
<td>3.9</td>
<td>44.7</td>
</tr>
<tr>
<td>Patient 4</td>
<td>2.9</td>
<td>42.0</td>
</tr>
</tbody>
</table>

Figure 4. NSA total score by age at time of assessment over 2 years after rAAVrh74.MHCK7.micro-dystrophin treatment for all 4 patients

CONCLUSIONS

- Systemic administration of rAAVrh74.MHCK7.micro-dystrophin (Sarpel-9001) is well tolerated and has minimal adverse events; most occurred in the first 30 days post-infusion, and all resolved.
- No serious adverse events or immune consequences of vector administration were observed. The majority of adverse events that occurred (vomiting, elevated liver enzymes) were consistent with what is expected from gene therapy delivery.
- Robust expression of micro-dystrophin and correct localization to the sarcoglycans were associated with vector genome copies, reduction in CK levels, and increase of β-sarcoglycan, a DAPC component.
- All 4 patients in the Sarpel-9001-101 open-label trial demonstrated improved functional measures compared to baseline that were maintained, and showed a durable response with respect to NSA 2 years after micro-dystrophin gene therapy administration.
- The enduring response observed in study Sarpel-9001-101 provides proof-of-concept support for the continuation of clinical trials to assess rAAVrh74.MHCK7.micro-dystrophin using single-dose gene transfer in patients with DMD.

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REFERENCES


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