Assessment of Systemic Delivery of rAAVrh74.MHCK7.micro-dystrophin in Children With Duchenne Muscular Dystrophy
A Nonrandomized Controlled Trial

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IMPORTANCE Micro-dystrophin gene transfer shows promise for treating patients with Duchenne muscular dystrophy (DMD) using recombinant adeno-associated virus serotype rh74 (rAAVrh74) and codon-optimized human micro-dystrophin driven by a skeletal and cardiac muscle-specific promoter with enhanced cardiac expression (MHCK7).

OBJECTIVE To identify the 1-year safety and tolerability of intravenous rAAVrh74.MHCK7.micro-dystrophin in patients with DMD.

DESIGN, SETTING, AND PARTICIPANTS This open-label, phase 1/2a nonrandomized controlled trial was conducted at the Nationwide Children's Hospital in Columbus, Ohio. It began on November 2, 2017, with a planned duration of follow-up of 3 years, ending in March 2021. The first 4 patients who met eligibility criteria were enrolled, consisting of ambulatory male children with DMD without preexisting AAVrh74 antibodies and a stable corticosteroid dose (≥ 12 weeks).

INTERVENTIONS A single dose of 2.0 × 10^{14} vg/kg rAAVrh74.MHCK7.micro-dystrophin was infused through a peripheral limb vein. Daily prednisolone, 1 mg/kg, started 1 day before gene delivery (30-day taper after infusion).

MAIN OUTCOMES AND MEASURES Safety was the primary outcome. Secondary outcomes included micro-dystrophin expression by Western blot and immunohistochemistry. Functional outcomes measured by North Star Ambulatory Assessment (NSAA) and serum creatine kinase were exploratory outcomes.

RESULTS Four patients were included (mean [SD] age at enrollment, 4.8 [1.0] years). All adverse events (n = 53) were considered mild (33 [62%]) or moderate (20 [38%]), and no serious adverse events occurred. Eighteen adverse events were considered treatment related, the most common of which was vomiting (9 of 18 events [50%]). Three patients had transiently elevated γ-glutamyltransferase, which resolved with corticosteroids. At 12 weeks, immunohistochemistry of gastrocnemius muscle biopsy specimens revealed robust transgene expression in all patients, with a mean of 81.2% of muscle fibers expressing micro-dystrophin with a mean intensity of 96% at the sarcolemma. Western blot showed a mean expression of 74.3% without fat or fibrosis adjustment and 95.8% with adjustment. All patients had confirmed vector transduction and showed functional improvement of NSAA scores and reduced creatine kinase levels (posttreatment vs baseline) that were maintained for 1 year.

CONCLUSIONS AND RELEVANCE This trial showed rAAVrh74.MHCK7.micro-dystrophin to be well tolerated and have minimal adverse events; the safe delivery of micro-dystrophin transgene; the robust expression and correct localization of micro-dystrophin protein; and improvements in creatine kinase levels and NSAA scores. These findings suggest that rAAVrh74.MHCK7.micro-dystrophin can provide functional improvement that is greater than that observed under standard of care.

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W]uchenne muscular dystrophy (DMD) is a rare, X-linked, fatal, degenerative neuromuscular disease caused by dystrophin gene (DMD) mutations. Estimated incidence worldwide is 1 in 5000 live male births.1,2 The DMD gene (OMIM 300377) encodes for dystrophin, a 427-kDa cytoskeletal protein required for sarcolemmal stability. Protein loss leads to susceptibility to repeated cycles of necrosis and regeneration as well as diminished regenerative muscle capacity, resulting in fat and connective tissue replacement (fibrosis).1 DMD is progressive, beginning with loss of ambulation between age 9 and 14 years, followed by respiratory complications and cardiac function decline, and ending in death.3-5

As standard of care options have changed, disease progression has improved.6 Corticosteroids have been reported to reduce inflammation7 and to delay the loss of ambulation (by approximately 3 years) and the decline of respiratory function.8 However, long-term corticosteroid use is associated with serious adverse effects, including bone fracture, infection, and gastrointestinal bleeding.7,8

Disease-modifying therapies, such as exon skipping, have been shown to produce functional dystrophin protein and slow the decline in ambulatory and pulmonary function after long-term use.9-12 However, dystrophin production is limited to patients amenable to select exon skipping. Novel therapies with a broader reach are still needed.

Gene transfer therapy has emerged as the most promising molecular approach for neuromuscular diseases. For spinal muscular atrophy (SMA), delivery of the SMN gene (SMN1 AC005031) to infants with SMA type 1 using adenovirus (AAV) serotype 9 (onasemnogene abeparvovec; Zolgensma) has been shown to dramatically prolong life and improve function.13,14 In the original trial, 12 infants with disease onset before age 6 months who were treated with high-dose onasemnogene abeparvovec (2.0 × 1014 vg/kg) survived beyond 2 years, far exceeding the 8% predicted survival.13 The infants continue to gain strength, achieve long-term survival, and develop new motor milestones, demonstrating the medication’s safety beyond the trial.14,15

A translational DMD gene transfer trial is designed to achieve clinical efficacy without compromising safety. However, the large size of the DMD gene (2.4 MB) limits its ability to be packaged into AAV vector systems (with a capacity of approximately 4.7 kB). Seminal studies in the 1990s demonstrated the ability to deliver truncated forms of dystrophin to improve muscular function in murine models.16,17 The most notable evidence of shortened dystrophin protein retaining function was the case of a 61-year-old ambulatory patient with Becker muscular dystrophy who harbored a large (approximately 46%) deletion mutation of exons 17 to 48; this case report highlighted the critical domains essential for muscle function.18

We designed a micro-dystrophin transgene (AAVrh74.MHCK7.micro-dystrophin) that would enhance functional efficacy on delivery. This micro-dystrophin transgene contains the N-terminus for binding to F-actin; spectrin repeats 1 to 3 and 24; hinges 1, 2, and 4; and the cysteine-rich domain. Spectrin repeats 1 to 3 bind directly to the sarcolemma,19 providing enhanced resistance to eccentric contraction-induced injury.20,21 Spectrin repeat 24 is critical for microtubule binding, and the cysteine-rich domain is essential for binding to β-dystroglycan, leading to the restoration of dystrophin-associated protein complex.22

For clinical translation, we placed micro-dystrophin under the control of an MHCK7 promoter. The MHCK7 promoter is associated with high levels of expression in skeletal muscles, including the diaphragm, and includes an enhancer to especially drive expression in the heart, whereas expression in off-target tissues is minimal.23

Careful selection of the AAV capsid is also important to ensure transduction efficiency and tissue targeting while minimizing immune response. Selection of AAV serotypes that demonstrate muscle (skeletal and cardiac) tissue tropism is critical. The AAVrh74 (AAV serotype rh74) used in preclinical mouse and nonhuman primate experiments was isolated from rhesus monkeys on the basis of high tropism for skeletal and cardiac muscle as well as no adverse events.24 Being of nonhuman primate origin, it is hoped that AAVrh74 would decrease the likelihood of patients having preexisting immunity to the vector. To date, AAVrh74 as a delivery vehicle has been found to be safe and tolerable in human studies, with minimal immune response.25

Recently, we completed proof-of-principle, preclinical dosing experiments in the mdx mouse. In preclinical studies, systemic delivery of AAVrh74.MHCK7.micro-dystrophin at doses (2.0 × 1014 vg/kg) comparable to those shown to be safe and effective for SMA type 1 demonstrated widespread dystrophin expression to the diaphragm, skeletal, and cardiac muscles.26 These preclinical study findings were the impetus for bringing AAVrh74.MHCK7.micro-dystrophin forward in this pilot nonrandomized controlled trial of a young cohort of boys with DMD. Our intent was to identify the potential for muscle fiber rescue before fiber loss and high degree of endomysial connective tissue replacement. We hypothesized that robust gene expression and correct localization to the sarcolemma could protect muscle fibers from progressive decline typical of DMD.

### Key Points

**Question** Is rAAVrh74.MHCK7.micro-dystrophin gene transfer safe and well tolerated in patients with Duchenne muscular dystrophy?

**Findings** In this nonrandomized controlled trial of 4 young patients with Duchenne muscular dystrophy, rAAVrh74.MHCK7.micro-dystrophin gene transfer was well tolerated, with minimal adverse events, and was associated with robust micro-dystrophin expression, reduced serum creatine kinase levels, and functional improvement as measured by the North Star Ambulatory Assessment.

**Meaning** These results indicated the safe systemic delivery of micro-dystrophin transgene and targeted expression of functional micro-dystrophin protein product, suggesting the potential for rAAVrh74.MHCK7.micro-dystrophin to provide clinically meaningful functional improvement that is greater than the standard of care.
ing contributions from muscle vs liver can be difficult. In this trial, we also monitored bilirubin (direct and total), alkaline phosphatase, and albumin levels (eTable 2 in Supplement 2). Additional biomarkers might also be included in future trials, including glutamate dehydrogenase and ammonia, and depending on severity, ultrasonography and magnetic resonance imaging could be used to more clearly identify pathological changes in the liver.

Vector Production and Dosing Procedures

The cassette containing the MHCK7 promoter and micro-dystrophin transgene were packaged into the AAVrh74 capsid using the standard triple transfection protocol, as described elsewhere.24,31,32 Additional information on vector production and titering can be found in the eMethods in Supplement 2. Patients received a single dose of $2.0 \times 10^{14}$ vg/kg rAAVrh74.MHCK7.micro-dystrophin in approximately 10 mL/kg. This dose was infused through peripheral limb vein over 1.25 to 1.5 hours in the pediatric intensive care unit at Nationwide Children’s Hospital.

Methods

Study Design and Participants

This phase 1/2a nonrandomized controlled trial tested the safety and biological efficacy of a single systemic infusion of rAAVrh74.MHCK7.micro-dystrophin in patients with DMD. The trial was approved by the institutional review board of Nationwide Children’s Hospital. All parents of minor participants signed informed consent in compliance with Code of Federal Regulations Title 21 Part 50 and the International Conference on Harmonization guidelines.25 Detailed inclusion and exclusion criteria are reported in the trial protocol (Supplement 1). We followed the Transparent Reporting of Evaluations With Nonrandomized Designs (TREND) reporting guideline.

This single-site trial was conducted at the Nationwide Children’s Hospital in Columbus, Ohio, and began on November 2, 2017. The duration of follow-up is 3 years, and the final date for follow-up is planned for March 2021.

Patients were excluded if rAAVrh74 (recombinant adeno-associated virus serotype rh74) binding antibody was detected at a dilution greater than 1:400 at enrollment (lower limit of detection 1:25). Eligible patients were ambulatory boys (n = 4) aged 4 to 7 years with confirmed DMD gene mutations with frameshift (deletion or duplication) or premature stop codon mutation between exons 18 and 58 (exons not encoded by the micro-dystrophin transgene to limit immune responses to the transgene)26; creatine kinase (CK) level elevation of more than 1000 U/L (to convert unit per liter to microkat per liter, multiply by 0.0167); and below-average 100-m timed test29 (Figure 1). Eligible patients were required to be on a stable dose of corticosteroids for at least 12 weeks before entry. All patients were on a weekend dose30 of prednisone sodium (n = 1) or prednisolone sodium phosphate (n = 3). After gene transfer, they received daily prednisolone, 1 mg/kg, for 5 days in between the weekend dose for 30 days tapered over 2 to 4 weeks, depending on serum $\gamma$-glutamyltransferase (GGT) level after gene delivery (eTable 1 in Supplement 2).

Vector Production and Dosing Procedures

Enzyme-linked immunosorbent assays (ELISAs) were performed in 96-well Immulon 4HBX ELISA plates coated with $2 \times 10^{10}$ vg AAVrh74 per well in carbonate buffer. Additional information can be found in the eMethods in Supplement 2.

Biological Assessments

Pretreatment and posttreatment (90-day) needle muscle biopsy specimens were obtained, with general anesthesia, from gastrocnemius muscles. An interventional radiologist (M.H.) guided by ultrasonography performed the procedures with a biopsy needle (Vacora; Bard Peripheral Vascular).33

Western Blot and Histological Study

Western blot was validated and performed under Good Clinical Laboratory Practice standards.34 Western blot was executed according to methods adapted from Charleston et al11 and Schnell et al.35 Dystrophin levels of treatment-blinded
samples were calculated from a 5-point standard curve ranging from 5% to 80%. Information on analysis of picrosirius red staining, collagen quantification (percentage), and immunohistochemistry or immunofluorescence staining and quantification is provided in the eMethods in Supplement 2.

**Functional Assessments**

Muscle function was assessed using the North Star Ambulatory Assessment (NSAA), a 17-item measure of ambulatory functions with a score range of 0 to 34 (the highest score indicating perfect). Other functional outcomes included time to rise, 4-stair climb, 100-m timed test, and hand-held dynamometry for knee extensors and flexors as well as elbow flexors and extensors (eTable 5 in Supplement 2).

**Statistical Analysis**

Baseline and demographic characteristics and safety and biopsy data were summarized using descriptive statistics. SAS, version 9.4 (SAS Institute Inc), was used for demographics and safety data, and Prism, version 5 (GraphPad Software), was used for biopsy data.

**Results**

**Patient Demographics**

Four male patients were screened for entry, with no screening failures. Patients with confirmed DMD, demonstrated by genotype and CK level elevations (Table 1), received a single intravenous infusion of 2.0 × 10¹⁴ vg/kg rAAVrh74. MHCK7.micro-dystrophin. Mean (SD) age at enrollment was 4.8 (1.0) years, mean (SD) weight was 18.1 (3.2) kg, and mean (SD) body mass index (BMI) (calculated as weight in kilograms divided by height in meters squared) was 16.3 (1.4). Demographics and baseline disease states, as indicated by CK levels and NSAA score, are shown in Table 1. Mean (SD) CK level at enrollment was 27 064.3 (6340.5) U/L, and the mean (SD) NSAA score was 20.5 (3.7) points.

**Safety and Immunogenicity**

All adverse events (n = 53) were considered mild (33 [62%]) or moderate (20 [38%]) (eTable 6 in Supplement 2); 35 adverse events were considered unrelated to treatment, and 18 were treatment related. The most common treatment-related adverse event was vomiting (9 of 18 events [50%]) after rAAVrh74 administration. No serious adverse events were detected from hematological and chemistry panels, which included liver function tests (eTable 2 in Supplement 2). Aminotransaminases were more difficult to assess in DMD because of skeletal muscle origin; nevertheless, aminotransaminase levels never reached more than 3 times the baseline levels for any patient. In 3 patients, moderate elevations of liver enzyme levels (GGT) increased to 4-fold greater than the upper normal limit (normal range: 8-78 U/L; baseline mean, 10 U/L), peaking at day 60 without clinical manifestations (peak value, 257-280 U/L) and then returning to normal levels with corticosteroids.

No adverse immune responses occurred, and no remarkable T-cell responses were observed toward 3 distinct peptide pools of micro-dystrophin corresponding to the N-terminus, middle, and C-terminus (eFigure 1 in Supplement 2). T-cell response toward AAV peptide pools 1 to 3 demonstrated transient increases as early as 14 days after gene delivery (eFigure 1 in Supplement 2), ranging from 50 to 352 spot-forming colonies per million peripheral blood mononuclear cells as measured through ELISpot. Elevations were not associated with liver enzymes or transgene expression. Expected increases in AAVrh74 antibodies were observed in all 4 patients, increasing by 14 days (range, 1:800-1:12 000) with postgene therapy titers peaking around day 30 (range, 1:13-1:26 million) and remaining stable through 1 year (eFigure 1 in Supplement 2). None of the adverse events was associated with complement activation. Platelets remained within normal range (mean range, 232.2-398.5) (eTable 2 in Supplement 2).

Evaluation by histopathological study showed that vector treatment was not associated with harmful alterations in the morphological features of muscle, demonstrated by the lack of remarkable alterations in central nucleation and the absence of prominent alterations in the distribution or morphology of other cell components. Performance of histological and immunohistochemical staining methods was performed on randomly selected samples from each patient. Six months after gene delivery, histological analysis showed that vector delivery in all patients resulted in the presence of dystrophin (Table 1).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
<th>Patient 4</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>4</td>
<td>6</td>
<td>4</td>
<td>4.8 (1.0)</td>
</tr>
<tr>
<td>Height, cm</td>
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<td>104.3</td>
<td>110</td>
<td>95.7</td>
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<tr>
<td>Weight, kg</td>
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<td>18.9</td>
<td>21.4</td>
<td>13.7</td>
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<td>BMI</td>
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<td>17.4</td>
<td>17.7</td>
<td>15.0</td>
<td>16.3 (1.4)</td>
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<td>DMD mutation</td>
<td>Deletion of exons 46-50</td>
<td>Deletion of exons 46-49</td>
<td>Premature stop codon exon 27</td>
<td>Partial deletion of exon 44</td>
<td>NA</td>
</tr>
<tr>
<td>Duration of steroids before treatment, mo</td>
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<td>15</td>
<td>23</td>
<td>11</td>
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<td>Creatine kinase, U/L</td>
<td>20 691</td>
<td>23 414</td>
<td>34 942</td>
<td>29 210</td>
<td>27 064.3 (6340.5)</td>
</tr>
<tr>
<td>NSAA score</td>
<td>18</td>
<td>19</td>
<td>26</td>
<td>19</td>
<td>20.5 (3.7)</td>
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<td>Time to rise, s</td>
<td>3.7</td>
<td>3</td>
<td>3.9</td>
<td>4.1</td>
<td>3.68 (0.5)</td>
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<td>4-Stair climb, s</td>
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<td>3.8</td>
<td>1.9</td>
<td>4.8</td>
<td>3.48 (1.2)</td>
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<td>100 m, s</td>
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<td>49.9</td>
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<td>67.2</td>
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<td>4.3</td>
<td>4.7</td>
<td>5.4</td>
<td>4.88 (0.5)</td>
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</table>

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); DMD, Duchenne muscular dystrophy; NA, not applicable; NSAA, North Star Ambulatory Assessment.
sence of ringed myofibers (Figure 2A). Vector treatment was not associated with increased muscle fibrosis but was associated with improvement in all 4 patients, demonstrated by a reduction in the percentage of collagen content in the muscle tissue after treatment compared with at baseline (mean [SD], 26.7% [8.4%]) (Figure 2B and C). Moreover, results from cardiac magnetic resonance imaging and echocardiography demonstrated no adverse findings in all 4 boys at the time points assessed and were within normal ranges for children with or without DMD.\(^\text{36,37}\) (eTable 3 in Supplement 2). These results suggest a lack of toxic effects from vector exposure.

**Micro-dystrophin Expression, Correct Localization, and Restoration of Dystrophin-Associated Protein Complex**

Successful gene transfer of micro-dystrophin was assessed by quantitative polymerase chain reaction for vector-specific genome molecular analysis, immunofluorescence (Figure 3A), and Western blot (Figure 3B). Transduction was confirmed in all 4 patients, showing a mean of 3.3 vector genome copies per nucleus detected by quantitative polymerase chain reaction (eTable 4 in Supplement 2), indicating the successful delivery of AAVrh74.MHCK7.micro-dystrophin in skeletal muscle. At 12 weeks, immunohistochemistry of muscle biopsy speci-
Mens showed a mean of 81.2% micro-dystrophin-positive fibers in the gastrocnemius muscle, with a mean intensity of 96.0% (Figure 3A; eTable 4 in Supplement 2). Findings at 12 weeks were confirmed by Western blot (Figure 3B), in which mean micro-dystrophin expression in the sample was 74.3% of normal dystrophin expression level without adjustment for fator fibrosis. When adjusted for fator fibrosis, mean micro-dystrophin expression was 95.8%.

In addition to expression of micro-dystrophin at the sarcolemmal membrane, immunofluorescence for β-sarcoglycan, a critical component of dystrophin-associated protein complex, showed a robust increase in sarcolemma expression compared with pretreatment in all 4 patients (eFigure 2 in Supplement 2). This increase suggests that the micro-dystrophin transgene can promote restoration and reconstitution of the dystrophin-associated protein complex.

**Functional Outcomes**

All 4 patients showed improvements in NSAA scores and reductions in CK levels from baseline throughout the study and up to 1 year after treatment (Table 2). The 1-year NSAA score improvement was 7 points in patient 1, 8 points in patient 2, 2 points in patient 3, and 5 points in patient 4 (mean, 5.5 points). Patient 3 (aged 6 years) would be expected to decline, and thus the true Δ of improvement was likely greater than 2 points. The mean (SD) CK level at baseline was 27064 (6340.5) U/L and after treatment was 8035 (3312.5) U/L. Patient 2 had CK levels that rose to greater than 40000 U/L, and neither this participant nor any other participant had myoglobinuria.

NSAA score improvement and the reduction in CK level appeared to be the most sensitive measures, but only a larger sample size and a clinical trial will validate improved motor function. There were different magnitudes of improvement across various other functional outcomes measured, including time to rise, 4-stair climb, 100-m timed test, and handheld dynamometry (eTable 5 in Supplement 2). Variability in clinical outcomes was associated with multiple factors, including age and disease severity. Activity can also suddenly increase serum CK level, leading to levels far above the mean. To avoid this, families should modify activity for at least 1 day before blood draw. An ongoing placebo-controlled random-
zyme levels gradually resolved over 4 to 10 weeks. In this trial, gene delivery. No clinical manifestations associated with el-
seas and vomiting were the most common clinical adverse
effects of AAV administration seen shortly (1-4 weeks) after
the administration. Nausea and vomiting were the most common adverse
effects associated with AAVrh74. Preclinical studies have reported that micro-dystrophin delivery
in an mdx mouse model under control of the MHCK7 pro-
motor was associated with robust cardiac and skeletal muscle
expression without toxic effects, providing compelling rea-
sions to assess this association in a clinical trial. In this study,
enrollment criteria included patients with DMD with out-of-
frame deletions between exons 18 and 58 (exons not encoded
by micro-dystrophin) to minimize the theoretical concern for
immune response to the transgene.28 However, no identified
immune responses to micro-dystrophin were seen, support-
ing the conduct of registration trials applicable to all DMD gene
mutations.

We hypothesized that robust gene expression and cor-
rect localization to the sarcolemma of muscle fibers could protect
muscles, and the decrease in CK levels after micro-
dystrophin gene transfer suggests stabilization of the sarco-
lemma. However, this trial also showed a potential caveat for
using CK level as an outcome measure if assessed at 1 time
point, as we did in this study. We attributed the high CK level
as a response to intense motor activity.41 An important con-
cern for gene transfer therapy is the potential for declining out-
come over time. Until the entire portfolio of gene therapies ex-
pressed clinical trials includes a much greater sample size and will
provide further insight for assessment of outcome measures.38

### Discussion

Results of this pilot nonrandomized controlled trial provided
biological and clinical evidence of systemic gene therapy for
potential use in treating patients with DMD. These findings fol-
low the success reported for exon-skipping therapy for pa-
tients with DMD and gene therapy for patients with SMA.12,13
Specifically, findings to date have shown the advantages of
exon-skipping therapy for promoting the expression of even
small amounts of truncated dystrophin in muscle that can posi-
tively alter the DMD course.9,12 Moreover, the natural history
of a patient with Becker muscular dystrophy who harbored a
dramatically shortened dystrophin suggests that expression
of micro-dystrophin protein can lead to relatively mild
dystrophinopathy.18 Together these findings suggest the pos-
sibility that targeted muscular expression of micro-
dystrophin may lessen DMD severity.

Adverse event findings were minimal with rAAVrh74.
MHCK7.micro-dystrophin. In this trial, the only vector-
related adverse event was elevated transaminase and GGT lev-
eels, which was previously reported in other trials.39,40 Nau-
sea and vomiting were the most common clinical adverse
effects of AAV administration seen shortly (1-4 weeks) after
gene delivery. No clinical manifestations associated with el-
levated liver enzymes were observed, and the elevated en-
zyme levels gradually resolved over 4 to 10 weeks. In this trial
and a previous study,13 glucocorticoids were used beginning
1 day before gene delivery and continuing until CK levels
reached less than 150 U/L. It is speculative to suggest that glucocorti-
coids may be associated with suppression or resolution of the
inflammatory infiltrate provoked by degrading capsids, but we
have seen recurrent elevated liver enzymes as glucocorti-
coids were tapered, followed by GGT decrease after reininiti-
ating the drug.

In this trial, gene transfer was done with AAVrh74. Pre-
clinical studies have reported that micro-dystrophin delivery
in an mdx mouse model under control of the MHCK7 pro-
motor was associated with robust cardiac and skeletal muscle

<table>
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<tr>
<th>Patient</th>
<th>Parameter</th>
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<td>1</td>
<td>NSAA</td>
<td>18</td>
<td>22</td>
<td>24</td>
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<tr>
<td></td>
<td>CK level, U/L</td>
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<td>2984</td>
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<td>2</td>
<td>NSAA</td>
<td>19</td>
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<td></td>
<td>CK level, U/L</td>
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<td>4283</td>
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<td></td>
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<td>20</td>
<td>20</td>
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<td></td>
<td>CK level, U/L</td>
<td>29210</td>
<td>7215</td>
<td>908</td>
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Abbreviations: CK, creatine kinase; NA, not applicable; NSAA, North Star Ambulatory Assessment.
SI conversion factor: To convert U/L to μkat/L, multiply by 0.0167.
pands through multiple applications, we can only speculate on long-term efficacy on the basis of single-gene administration. Data from a long-term follow-up in a clinical trial of 12 patients with SMA who were treated with a dose range of virus (Zolgensma) similar to the dose delivered in this microdystrophin trial showed sustained efficacy of the therapy for 5 years.

It is important to review the available published natural history data in patients aged 4 to 7 years to help contextualize the findings from this trial. Patients with DMD generally showed improved physical function up to age 7 years, a finding that complicates the interpretation of the treatment effect of a therapeutic agent compared with that of the natural disease course. Recent data from the Collaborative Trajectory Analysis Project demonstrated that approximately 2 points constituted a clinically meaningful change of the NSAA total score over a 48-week period. Early studies reported that approximately 40% of patients up to age 7 years showed some improvement of their NSAA scores over 12 months, but a sizeable proportion did not improve. Furthermore, the literature reported that the expected higher NSAA scores in patients up to age 7 years ranged from approximately 0.7 to 3 points per year. In a 12-month longitudinal cohort study, Mazzone et al found a 0.7-point increase in NSAA score from baseline in patients up to 7 years of age who were receiving continuous corticosteroid treatment. Ricotti et al reported a functional gain of approximately 1.5 points per year on NSAA scores in patients aged 4 to 7 years, with an additional improvement of 1.3 points per year in boys who had started glucocorticoids at an age younger than 5 years compared with boys who started glucocorticoids at an age older than 5 years.

In an extensive systematic natural history study, Muntoni et al reported findings of a cohort of approximately 359 patients aged 2 to 17 years from the UK North Star registry. Mean trajectory of NSAA total score peaked at age 6.3 years, and the population mean NSAA total score initially increased at a rate of approximately 3 units per year, which, following the peak, approached a rate of decline of approximately 3 units per year. The North Star registry cohort included patients with DMD mutations that were generally associated with slower disease progression than typically seen in DMD (eg, 44 skip-amenable patients).

In the present study, the 1-year NSAA score improvement was 7 points in patient 1, 8 points in patient 2, 2 points in patient 3, and 5 points in patient 4 (mean, 5.5 points). Patient 3 (aged 6 years) would be expected to decline, and thus the true Δ of improvement was likely greater than 2 points. Patient 3 also showed the least improvement; however, the NSAA score of this patient constituted a clinically meaningful change according to data from the Collaborative Trajectory Analysis Project. All 4 patients had a clinically meaningful NSAA score improvement and had such improvements as soon as day 90. This finding suggests an overall improvement vs natural history and acquisition of activities that were greater than that expected in patients with DMD who were receiving standard-of-care treatment.

These results, along with biological and clinical markers of efficacy, provide 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. A randomized clinical trial with a much larger sample size of boys with DMD is under way.

**Limitations**

This nonrandomized trial yielded encouraging results for rAAVrh74.MHCK7.micro-dystrophin. However, the safety and efficacy of this therapy will need to be confirmed in a randomized clinical trial.

**Conclusions**

This trial demonstrated that a single systemic infusion of rAAVrh74.MHCK7.micro-dystrophin was well tolerated and had a favorable safety profile. In addition, it described the delivery of transgene to the nuclei and the robust expression and proper localization of micro-dystrophin, which coincided with marked reductions in CK levels and improvement in functional measurements such as NSAA score. These preliminary results, albeit in a small number of patients, suggest the potential for rAAVrh74.MHCK7.micro-dystrophin to provide clinically meaningful functional improvement that is greater than that observed with standard-of-care treatment, including corticosteroids.
Research Original Investigation


Reference


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