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

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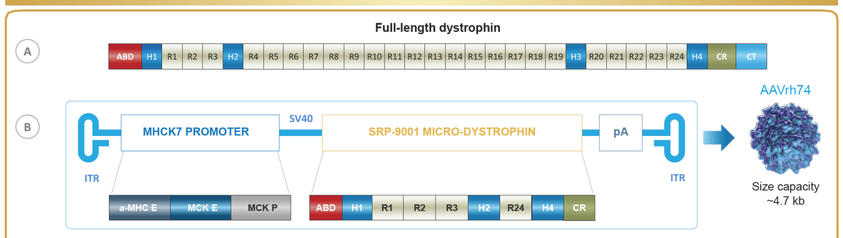
BACKGROUND AND OBJECTIVE

- Duchenne muscular dystrophy (DMD) is a rare, X-linked, fatal, neuromuscular disease caused by mutations in the *DMD* gene that disrupt the production of functional 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 package limitation of AAV.[3,4]
- AAV-mediated gene transfer 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.[5]
- Here we report, for the first time, the long-term (2-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.

Figure 1. rAAVrh74.MHCK7.micro-dystrophin construct



ITR: AAV2 inverted tandem repeat; SV40: intron; pA: synthetic polyadenylation signal

Components	Expectations Based on Preclinical and Clinical Studies
AAVrh74 Vector	AAVrh74 efficient transduction to muscles and favorable safety profile[6-8]
MHCK7 Promoter	MHCK7 regulates and drives transgene expression selectively in skeletal and cardiac muscle[8-9]
Micro-dystrophin Transgene	Shortened sequence that produces a functional protein; includes spectrin-like repeats 1, 2 and 3 that are required for enhancing protection against eccentric force loss, and repeat 24 that contributes to microtubule organization[8]

Figure 1. (A) Full-length dystrophin protein structure. (B) The 3 essential components of the SRP-9001 construct

METHODS

Trial Design

- Single-dose, open-label, Phase I/IIa study (NCT03375164, SRP-9001-101)
- Inclusion criteria
  - Male patients with DMD (aged 4-7 years)
  - Confirmed molecular diagnosis of DMD (frameshift [deletion or duplication] or premature stop codon mutation between exons 18 to 58 in the *DMD* gene)
  - Negative for AAVrh74 and AAV8 antibodies (≤1:400 titer assessed by ELISA)[8]
  - Symptomatic muscular dystrophy
    - Creatine kinase (CK) elevation >1000 U/L
    - Below average on the 100-Meter Timed Test (100 m), defined as ≤80% predicted[10]
  - 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)[8]
- rAAVrh74.MHCK7.micro-dystrophin was infused over 1-2 hours via the peripheral limb vein at a dose of $2x10^{14}$ vg/kg (10 mL/kg) 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 rAAVrh74 by ELISA (B-cell response); and T-cell response was assessed against AAVrh74 capsid and micro-dystrophin proteins

Secondary Outcome Measures

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

Efficacy Outcome Measures

- Decrease in CK
- North Star Ambulatory Assessment (NSAA; 10-Meter Timed Test [10 m] and Time to Rise included)†
- Timed function tests: 100 m, 4-Stair Climb

†Planned analysis for NSAA 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

	Patient 1	Patient 2	Patient 3	Patient 4
Age (yrs)	5	4	6	4
Height (cm)	109.9	104.3	110.0	95.7
Weight (kg)	18.4	18.9	21.4	13.7
BMI	15.2	17.4	17.7	15.0
Creatine kinase (U/L)	20,691	23,414	34,942	29,210
NSAA	18	19	26	19

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 chemistry panels, which included liver function tests
- 3 patients had elevated γ-glutamyl transpeptidase in the first 3 months post-treatment, which resolved with steroid treatment
  - No other clinically significant laboratory findings were reported
- Platelets remained within normal range (mean range, 232.2–398.5)
- The most common treatment-related adverse event (TRAE) was vomiting (9 of 18 TRAEs)
  - Patients had transient vomiting generally within the first week post-infusion
  - It did not correlate with liver enzyme elevations or any other abnormality
- All TRAEs occurred within 90 days post-infusion
- None of the adverse events were associated with complement activation

Dystrophin Expression and Quantification

- Systemic gene transfer resulted in robust levels of micro-dystrophin protein expression localized to the sarcolemma as shown 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)[8]
- 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 2. rAAVrh74.MHCK7.micro-dystrophin uptake into skeletal muscle increases mean levels of micro-dystrophin protein

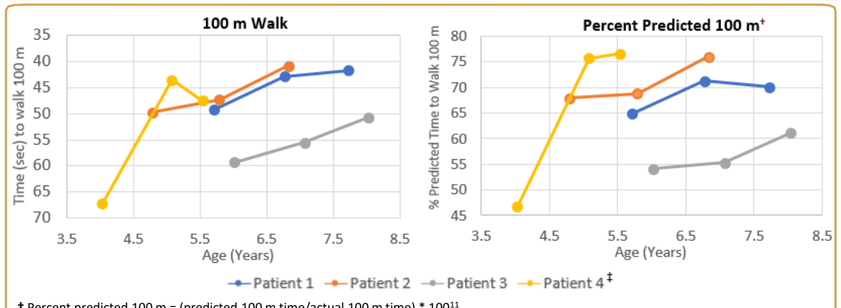
Vector genome number		Micro-dystrophin expression (Western Blot)		Micro-dystrophin expression (Immunohistochemistry)	
Vector copies/ µg DNA	Copies per nucleus	Sarepta†	Nationwide Children's‡	Intensity	% Dystrophin-positive fibers
$>10^5$	3.3	74.3%	95.8%	96.0%	81.2%

† Not adjusted for fat/fibrosis
‡ Adjusted for fat/fibrosis

Efficacy Outcomes

- Motor function was improved in all 4 patients, as measured by increased ambulation (100 m; **Figure 3**, **Table 2**), generally increased muscle strength (Time to Rise and 4-Stair Climb, (**Table 2**), and improvement in overall motor abilities (NSAA, **Figure 4**).
- All 4 patients demonstrated a clinically meaningful improvement on NSAA as early as Day 90, with a mean change from baseline to Year 2 of +7.0.

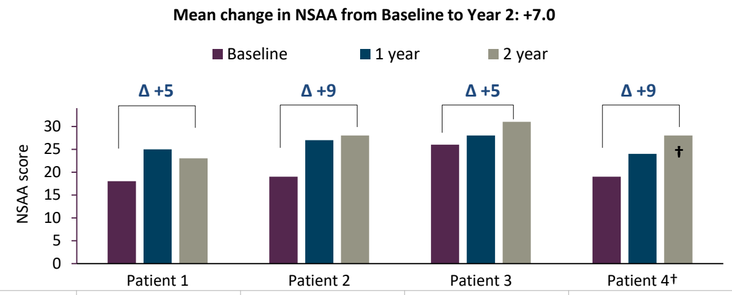
Figure 3. Time to walk 100 m and percent predicted time to walk 100 m



† Percent predicted 100 m = (predicted 100 m time/actual 100 m time) * 100[11]
‡ Patient 4 did not have 2-year timed function tests assessed due to COVID-19-related restrictions at the site. 18-month data are presented

RESULTS (CONT'D)

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



	Patient 1	Patient 2	Patient 3	Patient 4†
Age at Baseline (y)	5.71	4.79	6.02	4.03
Age at Year 1 (y)	6.77	5.79	7.07	5.08
Age at Year 2 (y)	7.73	6.83	8.03	6.31

† Patient 4: 2-year NSAA value was from a remote assessment due to COVID-19-related restrictions at the site

Table 2. Summary of 2-Year Timed Function Tests

	Patient 1			Patient 2			Patient 3			Patient 4†		
	BL	1 year	2 year	BL	1 year	2 year	BL	1 year	2 year	BL	1 year	18 month
Time to Rise (sec)	3.7	3.4	4.1	3.0	3.4	3.2	3.9	3.9	2.8	4.1	2.6	2.4
4-Stair Climb (sec)	3.4	2.4	2.4	3.8	2.6	2.2	1.9	1.8	2.2	4.8	2.0	2.0
100 m (sec)	49.3	42.9	41.8	49.9	47.4	40.9	59.3	55.5	50.7	67.2	43.6	47.5
100 m (%p)	64.8	71.2	70.1	67.9	68.8	76.0	54.1	55.3	61.1	46.7	75.7	76.6

† Patient 4 did not have 2-year timed function tests assessed due to COVID-19-related restrictions at the site. 18-month data are presented

CONCLUSIONS

- Systemic administration of rAAVrh74.MHCK7.micro-dystrophin (SRP-9001) is well tolerated and has minimal adverse events; most occurred in the first 90 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 sarcolemma were associated with vector genome copies, reduction in CK levels, and the rescue of β-sarcoglycan, a DAPC component.
- All 4 patients in the SRP-9001-101 open-label trial demonstrated improvements in functional measures compared to baseline that were maintained, and showed a durable response with respect to NSAA 2 years after micro-dystrophin gene therapy administration.
- The enduring response observed in study SRP-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.
  - The ongoing double-blind placebo-controlled trial, SRP-9001-102, is a confirmatory study that will enable the assessment of functional efficacy of SRP-9001 against placebo.

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Trial registration: NCT03375164.

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