

I concur with this review memo. I. Wu. 6/16/23  
I concur with the review memo. A. Shearin. 6/16/23

**FOOD AND DRUG ADMINISTRATION**  
**Center for Biologics Evaluation and Research**  
**Office of Therapeutic Products**  
**Office of Pharmacology/Toxicology**  
**Division of Pharmacology/Toxicology 1**  
**Pharmacology/Toxicology Branch 1**

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BLA NUMBER: STN #125781.000

DATE RECEIVED BY CBER: 9/28/2022

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PRODUCT: ELEVIDYS (Delandistrogene moxeparvovec  
rAAVrh74.MHCK7.Micro-Dystrophin, Non-Replicating,  
Recombinant Adeno-Associated Virus, Serotype rh74  
Containing a Human Micro-Dystrophin Gene Controlled by  
the Muscle-Specific MHCK7 Promoter)

APPLICANT: Sarepta Therapeutics, Inc.

PROPOSED INDICATION:	Treatment of ambulatory patients with Duchenne muscular dystrophy (DMD) with a confirmed mutation in the DMD gene
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## EXECUTIVE SUMMARY:

ELEVIDYS (delandistrogene moxeparvovec or SRP-9001) is a gene therapy consisting of a replication-defective, recombinant adeno-associated virus (AAV), serotype rh74 containing a human micro-dystrophin gene controlled by a muscle-specific MHCK7 promoter. The nonclinical *in vivo* pharmacology, biodistribution, and toxicology data to support this biologics licensing application (BLA) are summarized below.

The pharmacology studies of ELEVIDYS were conducted in rodent models of DMD including *mdx* mice and *Dmd*<sup>*mdx*</sup> rats. In pharmacology studies in 4 to 8 week old *mdx* mice (Report Nos. SR-20-001, SR-19-061, SR-20-014, and SR-21-025), single intravenous (IV) administration of  $\geq 1.33 \times 10^{14}$  vg/kg ELEVIDYS (manufactured by Process A at Nationwide Children Hospital and Process B at ThermoFisher and Catalent) resulted in: i) increased specific force output in the tibialis anterior and diaphragm muscles, ii) reduced dystrophic muscle pathology in skeletal muscles, iii) increased ELEVIDYS micro-dystrophin protein expression in skeletal muscles and heart, with occasional low levels in the liver, and iv) increased  $\beta$ -sarcoglycan staining in the skeletal muscles. Endpoints were evaluated at 12 weeks post-administration in all studies. In a 6-month pharmacology study (Report No. SR-20-012) in 3 to 4 week old *Dmd*<sup>*mdx*</sup> rats, IV administration of  $1.33 \times 10^{14}$  vg/kg ELEVIDYS (manufactured by Process B at Catalent) resulted in i) increased spontaneous activity in an open field assessment, ii) increased heart rate and decreased left ventricle internal diameter during diastole, iii) increased micro-dystrophin protein expression in skeletal muscle and heart, and iv) reduced dystrophic muscle pathology in skeletal muscle and heart. However, administration of ELEVIDYS in older (3-5 month old) *mdx* rats (Report No. SR-20-013) did not result in any statistically significant improvements in the open field assessment or dystrophic muscle pathology, despite ELEVIDYS micro-dystrophin protein expression in skeletal muscles and heart.

A 6-month good laboratory practice (GLP) toxicology and biodistribution study (Report No. SR-19-050) evaluated single IV administration of  $1.33 \times 10^{14}$  and  $4.01 \times 10^{14}$  vg/kg ELEVIDYS (manufactured by Process B at ThermoFisher) in 6 to 7 week old C57BL/6J wild type (WT) and *mdx* mice with interim and terminal sacrifices at 85 and 169 days. No consistent patterns of ELEVIDYS-related adverse findings were observed for body weights, clinical observations, hematology, serum chemistry, or gross and histopathology on a comprehensive list of tissues, and no unscheduled deaths occurred. This study identified a no-adverse-effect-dose-level (NOAEL) of  $4.1 \times 10^{14}$  vg/kg in ELEVIDYS administered to WT and *mdx* mice.

A 12-week GLP toxicology and biodistribution study (Report No. SR-20-066) evaluated single IV administration of  $1.33 \times 10^{14}$  and  $4.01 \times 10^{14}$  vg/kg ELEVIDYS (manufactured by Process B at Catalent) in 7 to 8 weeks old C57BL/6J WT and *mdx* mice. No consistent patterns of ELEVIDYS-related adverse findings for body weights, clinical observations, hematology, serum chemistry, or gross and histopathology on a comprehensive list of tissues in WT mice, or unscheduled deaths were reported. Similar results were also observed in *mdx* mice with the exception of a 20% increased incidence of possible ELEVIDYS-related exacerbation of hydrocephalus and dilation of ventricles at  $4.1 \times 10^{14}$  vg/kg compared to control mice. This study identified NOAELs of  $4.1 \times 10^{14}$  vg/kg in WT mice and  $1.33 \times 10^{14}$  vg/kg in *mdx* mice.

A 12-week non-GLP study (Report No. SR-21-028) was conducted to further evaluate hydrocephalus and hydrocephalus-related unscheduled deaths in 4 to 8 week old *mdx* mice following single IV administration of  $4.01 \times 10^{14}$  vg/kg ELEVIDYS (manufactured by Process B at Catalent). A similar incidence of hydrocephalus and hydrocephalus-related unscheduled deaths was observed in control and  $4.01 \times 10^{14}$  vg/kg ELEVIDYS-injected *mdx* mice; therefore, the occurrence of hydrocephalus was not considered ELEVIDYS-related. Spontaneous hydrocephalus and dilation of ventricles have also been reported in the literature as a background finding in *mdx* mice.

A 3-month GLP toxicology study (Report No. SR-20-015) evaluated single IV administration of ELEVIDYS (manufactured by Process B at Catalent) in neonatal C57BL/6J WT mice administered  $1.33 \times 10^{14}$  and  $4.01 \times 10^{14}$  vg/kg at post-natal day 1. No consistent patterns of ELEVIDYS-related adverse findings for unscheduled deaths, body weights, clinical observations, neurobehavioral tests, femur length, organ weights, or gross and histopathology on a comprehensive list of tissues were observed. This study identified a NOAEL of  $4.1 \times 10^{14}$  vg/kg in ELEVIDYS-administered neonatal WT mice.

Biodistribution of ELEVIDYS was evaluated in two GLP toxicology studies (Report No. SR-19-050 and SR-20-066). Systemic ELEVIDYS distribution was observed, with the highest vector levels in the liver, followed by the adrenal gland, aorta, heart, muscle, and esophagus. The vector was also detected at low levels in other tissues evaluated (e.g., skin, thyroid, trachea, bone, kidney, lung, spleen duodenum, salivary gland, sciatic nerve, jejunum, testes, ileum, stomach, brain, cecum, thymus, pancreas colon, eye, spinal cord, and harderian gland). (b) (4) [REDACTED] for detection of AAV-MHCK and the micro-dystrophin transgene was performed with testes samples in two studies (Report No. SR-20-014 and SR-20-015). Of the limited samples evaluated, positive cells were detected at a low frequency.

Animal reproductive and developmental toxicity and carcinogenicity studies were not conducted with ELEVIDYS, which is acceptable based on the patient population and lack of ELEVIDYS-related adverse findings in the toxicology studies.

**PHARMACOLOGY/TOXICOLOGY RECOMMENDATION:**

There are no nonclinical deficiencies in the pharmacology and toxicology studies or outstanding information requests for ELEVIDYS. The nonclinical data provided in the BLA submission support the approval of this biologics license application.

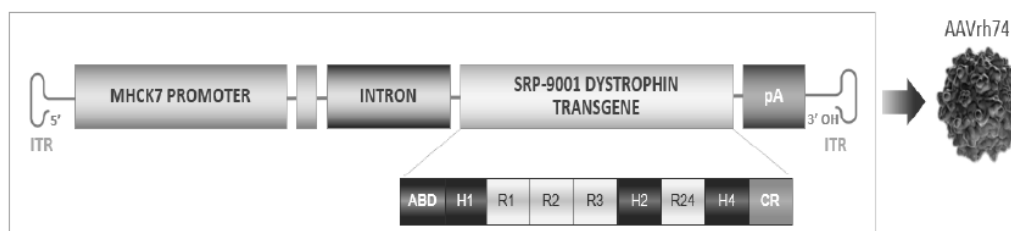
### Formulation and Chemistry:

ELEVIDYS (also named SRP-9001, or rAAVrh74.MHCK7.Micro-Dystrophin) drug substance (DS) is a non-replicating, recombinant adeno-associated virus (AAV), serotype rh74 expressing a human micro-dystrophin gene controlled by a muscle-specific MHCK7 (myosin heavy chain/muscle creatine kinase) promoter, which is derived from the MCK promoter with an additional 5'enhancer from the myosin heavy chain to derive cardiac expression.<sup>1</sup> The micro-

1 (b) (4)

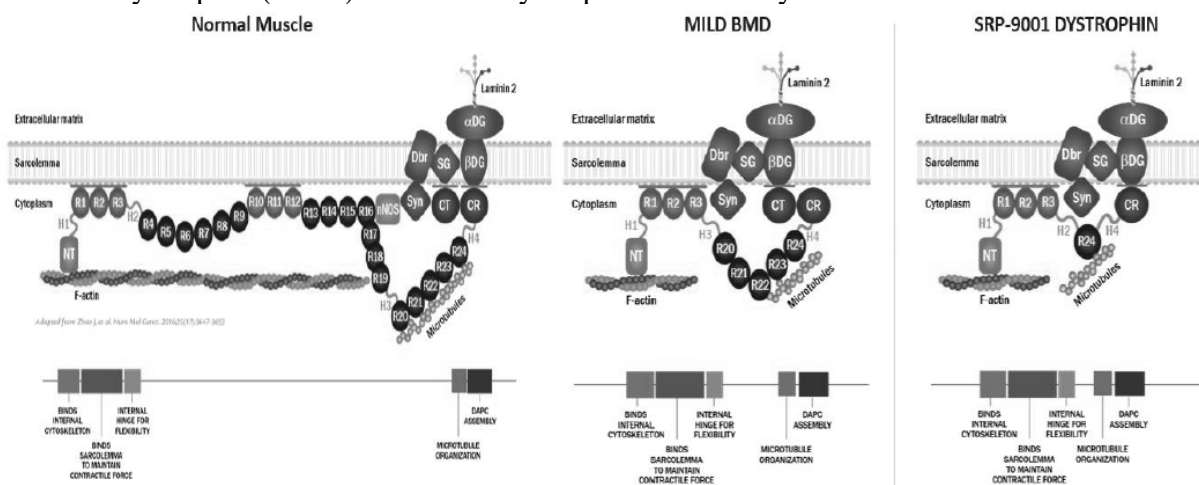
dystrophin protein is a 138.8 kDa protein which includes spectrin repeats R1-R3 and R24, hinge 2, and a cysteine-rich binding domain (page 7 of Module 2.3.S ‘Drug Substance [delandistrogene moxeparvovec]’).<sup>2</sup> The drug product (DP) consists of DS formulated in a buffered solution of 20 mM Tromethamine/ Tromethamine-HCl, 1 mM Magnesium chloride, 200 mM Sodium chloride, and 0.001% Poloxamer 188.

**Figure 1:** ELEVIDYS™ Vector Design



Source- Page 14 of Module 2.4 ‘Nonclinical Overview’

**Figure 2:** Schematic diagram of wild type (WT) normal dystrophin, dystrophin of mild Becker’s Muscular Dystrophy (BMD) and micro-dystrophin encoded by ELEVIDYS



BMD=Becker’s muscular dystrophy; CK=creatine kinase; CR=cysteine-rich region; CT=C-terminus; DAPC=dystrophin-associated protein complex; DMD=Duchenne muscular dystrophy; H=hinge; nNOS=neuronal nitric oxide synthase; NT=N-terminal domain; R=spectrin-like repeat (also named rod).

Source - Page 17 of Module 2.4 ‘Nonclinical Overview’

<sup>2</sup> Harper SQ, et al. (2002) Spectrin-like repeats from dystrophin and a-actinin-2 are not functionally interchangeable 11(16):1807-18115.

## Abbreviations

AAV	Adeno-associated virus
ABD	Actin binding domain
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
ANOVA	Analysis of Variance
AUC	Area under curve
B/C	BUN/creatinine ratio
BD	Biodistribution
BMD	Becker's Muscular Dystrophy
BUN	Blood urea nitrogen
BW	Body weight
CBC	Complete blood count
CK	Creatine kinase
CR	Cysteine-rich region
CREAT	Creatinine
CRO	Contract research organization
CT	C-terminus
DAPC	Dystrophin-associated protein complex
DBil	Direct bilirubin
DGC	Dystrophin-associated glycoprotein complex
DIA	Diaphragm
DR	Drug product
DRG	Dorsal Root Ganglia
DRP	DNase Resistant Particles
DS	Drug substance
ECC	Eccentric Contraction
(b) (4)	
(b) (4)	
F	Female
(b) (4)	(b) (4)
g	Grams
(b) (4)	
GAS	Gastrocnemius
GGT	Gamma-glutamyl transferase
GGTP	Gamma-glutamyl transpeptidase
GLP	Good Laboratory Practice
GLU	Glucose
GLUT	Gluteus maximus
H	Hinge
HAM	Hamstring

HEMOG	Hemoglobin
HEMAT	Hematocrit
H&E	Hematoxylin & Eosin
IF	Immunofluorescence staining
IFN- $\gamma$	Interferon gamma
(b) (4)	
(b) (4)	
IV	Intravenous
Kd	Equilibrium dissociation constant
L	Left
LR	Lactated Ringers
LTA	Left tibialis anterior
LYMPH	Lymphocytes
M	Male
MCH	Mean corpuscular hemoglobin
MCV	Mean corpuscular volume
Mdx	Dystrophin null mutant mouse strain
MHCK7	Myosin heavy chain/muscle creatine kinase promoter
MG	Medial Gastrocnemius
MONO	Monocytes
MPV	Mean platelet volume
NCH-RI	Nationwide Children's Hospital Research Institute
NOAEL	No-adverse-effect-dose-level
nNOS	Neuronal nitric oxide synthase
NT	N-terminal domain
PMDPF	Percent micro-dystrophin positive fiber
PND	Postnatal Day
PSO	Psoas major
qPCR	Quantitative Polymerase Chain Reaction
QUAD (or QD)	Quadriceps femoris
R	Spectrin-like repeat
rAAV	Recombinant adeno-associated virus
RBC	Red blood cell counts
RDW	Red cell distribution width
ROA	Route of administration
SD	Standard deviation
(b) (4)	
SEM	Standard error of mean
TA	Tibialis anterior
TP	Total protein
TPE	Therapeutic plasma exchange
TBIL	Total-value bilirubin
TRI	Triceps brachii

Vg	Vector genome
WB	Western blot
WBC	White blood cell counts
WT	Wild type

## Related File

IND #17763; rAAVrh74.MHCK7.Micro-Dystrophin, Non-Replicating, Recombinant Adeno-Associated Virus, Serotype rh74 Containing a Human Micro-Dystrophin Gene Controlled by the Muscle-Specific MHCK7 Promoter; Duchenne muscular dystrophy; Sarepta Therapeutics Inc.

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## INTRODUCTION

Duchenne muscular dystrophy (DMD) is an X-linked genetic neuromuscular disorder that affects approximately one in 5000 live male births.<sup>3</sup> DMD is caused by recessive and monogenic deletions or mutations in the *DMD* gene, located on chromosome Xp21, and encoding the dystrophin protein (427 kD) that is essential for assembly of the dystrophin-associated glycoprotein complex (DGC) to support muscle cell integrity in skeletal, diaphragmatic, and cardiac muscle.<sup>4</sup> Diagnosis typically occurs at ages 3 to 5, with loss of ambulation around age 12.<sup>5</sup> The disease leads to muscle atrophy, inflammation, and fibrosis, with progressive weakness and respiratory failure. Additionally, approximately 25% of patients with DMD will have dilated cardiomyopathy.<sup>6, 7</sup> The estimated life expectancy for DMD patients receiving ventilatory support is approximately 30 years.<sup>8</sup>

ELEVIDYS is intended to be administered intravenously (IV) to transduce muscle cells in skeletal, diaphragmatic, and cardiac muscles, leading to expression of micro-dystrophin protein in the transduced muscle cells, and subsequent assembly of the DGC to support muscle integrity in these target tissues.

## NONCLINICAL STUDIES

### Product evaluated

ELEVIDYS (also named SRP-9001 in the nonclinical studies) has been produced from different manufacturing processes and manufacturing sites including Process A at Nationwide Children's Hospital, Process B at ThermoFisher, and Process B at Catalent. The commercial SRP-9001 product is generated using Process B (Catalent). The physical titer of Process A and Process B products were determined by different methodologies (supercoiled plasmid standard-based qPCR assay and linearized plasmid standard-based qPCR assay, respectively). Per the CMC review, Process A and Process B products are not analytically comparable.

The table below identifies the SRP-9001 products administered in the nonclinical and clinical studies.

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<sup>3</sup> Romitti PA, et al. (2015) Prevalence of Duchenne and Becker Muscular Dystrophies in the United States. *Pediatrics* 135:513-21.

<sup>4</sup> Bushby KR, et al. (2010) Diagnosis and management of Duchenne Muscular Dystrophy, part 2: implementation of multidisciplinary care. *Lancet Neurol* 9:177-89.

<sup>5</sup> Brooke MH, et al. (1983) Clinical investigation in Duchenne dystrophy: 2. Determination of the "power" of therapeutic trials based on the natural history. *Muscle & Nerve* 6:91-103.

<sup>6</sup> Juan-Mateu J, et al. (2012) Isolated cardiomyopathy caused by a DMD nonsense mutation in somatic mosaicism: genetic normalization in skeletal muscle. *Clinical Genetics*. 82:574-8.

<sup>7</sup> Yoshida K, et al. (1998) Insertional mutation by transposable element, L1, in the DMD gene results in X-linked dilated cardiomyopathy. *Human Molecular Genetics* 7(7):1129-32.

<sup>8</sup> Landfeldt E (2020) Life expectancy at birth in Duchenne muscular dystrophy: a systemic review and meta-analysis. *Eur J Epidermiol* 35:643-653.

SRP-9001 Product	Nonclinical Study Number	Clinical Trial
Process A SRP-9001	Studies #1, #2, #7, and #8	Study 101- Open label single arm study; Study 102 – Double-blind, placebo control cross-over study
Process B (ThermoFisher) SRP-9001	Studies #3, #3.1, #9, and #10	None
Process B (Catalent) SRP-9001	Studies #4, #5, #6, #11, #12, #13, and #14	Study 103 – Open-label single arm study

## **PHARMACOLOGY STUDIES**

### **Summary List of Pharmacology Studies**

The following pharmacology studies were conducted to support the rationale for the administration of ELEVIDYS to treat the proposed clinical indication.

#### **In Vitro Studies**

Study Number	Study Title / Publication Citation	Report Number
1	(b) (4)	SR-20-009

#### **In Vivo Studies**

Study Number	Study Title / Publication Citation	Report Number
<b>Primary Studies</b>		
2	Efficacy, Toxicity and Biodistribution of rAAVrh74.MHCK7.Micro-Dystrophin After A Single Intravenous Dose Administration in a Mouse Model of Duchenne Muscular Dystrophy With a 12 Week or 24 Week Observational Period	SR-20-001
3	Pharmacodynamics Study: 12-Week Dose Escalation Study Evaluating Pharmacology and Efficacy of Systemic Delivery of SRP-9001 in mdx Mice	SR-19-061
3.1	A Single Dose Intravenous Administration, 12-Week Dose Escalation Study Evaluating Pharmacology and Efficacy of Systemic Delivery of SRP-9001 in mdx Mice	SR-20-014
4	(b) (4) and Systemic Delivery of Catalent Commercially Representative SRP-9001 in the DMD <sup>MDX</sup> Mouse Model: Evaluation of Expression and Functional Outcome Measures	SR-21-025
5	A Single Dose Intravenous Administration Evaluating Pharmacology and Efficacy of Systemic Delivery of SRP-9001 in Young DMD <sup>MDX</sup> Rat Model	SR-20-012
6	A Single Dose Intravenous Administration Evaluating Pharmacology and Efficacy of Systemic Delivery of SRP-9001 in Aged DMD <sup>MDX</sup> Rat Model	SR-20-013
<b>Supporting Studies</b>		
7	A Systemic Dual Dose Evaluation in (b) (4) : Biodistribution, Immunological Response, and Efficacy (non-GLP)	SR-20-007
8	12-Week Safety Study of IV-Delivered Rh74 Adeno-Associated Viral Vector rAAVrh74.MHCK7.Microdystrophin (b) (4) in a Male (b) (4) Monkey	SR-20-002

9	Biodistribution and Efficacy of $7 \times 10^{13}$ Vg/Kg Systemic Dose of SRP-9001 In DMD <sup>MDX</sup> Mice	SR-20-086
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## Reviewer comments:

The following additional study reports were submitted:

- Report No. SR-22-003 titled: ‘Nonclinical PK-PD Analysis of SRP-9001 for the Treatment of Duchenne Muscular Dystrophy’ is an exploratory analysis of studies (#2, #3, #4, and #10) in mdx mice intended to evaluate correlation between relative specific force with percentage of micro-dystrophin positive fibers (PMDPF) by IF or micro-dystrophin expression by Western blot (WB). A correlation between relative specific force and PMDPF by IF was found, but there was no correlation between relative specific force and micro-dystrophin expression by WB. Of note, WB is considered more reliable for quantitative assessment of protein expression due to methodological limitations of using IF (e.g., background fluorescence, variability in intensity, etc.). Additionally, it is unclear whether it is appropriate to pool these data given the differences in methods used for measurement of specific force and IF staining at the different testing facilities where the studies were performed.
- Report No. SR-22-012 evaluated SRP-9001 vector exposure in plasma and excreta following IV administration in mice from Study Report No. SR-19-050 and was reviewed under Study #10 below.
- The following study reports are not reviewed in this memo since they were used to assess product contamination, evaluated early versions of the product, or used a different route of administration (ROA): i) Study Report Nos. SR-20-003 and SR-20-006 that assessed contamination of (b) (4) and vector (b) (4) in Process A SRP-9001 (Lot # (b) (4)), ii) Study Report No. SR-20-031 that assessed various candidate vector constructs (b) (4), iii) Study Report 2021-007 that assessed micro-dystrophin protein expression in mdx mice following (b) (4) administration of Process A and Process B SRP-9001, and iv) Study Report SR020-087 that assessed specific force,  $\beta$ -sarcoglycan staining, PMDPF in TA muscle in mdx mice following (b) (4) administration of Process A and Process B SRP-9001.

## Overview of Pharmacology Studies

### Overview of In Vitro Studies

#### Study #1 (Process A)

Title: (b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

(b) (4)

### Overview of In Vivo Studies

#### ***Animal models of DMD***

##### DMD mouse model

Male C57BL/10ScSn- DMD<sup>mdx</sup>/J mice (also named Dmd<sup>mdx</sup> or *mdx* mice) are dystrophin null.<sup>9</sup> Male *mdx* mice have a mild phenotype with minimal clinical signs compared to the severe muscle dysfunction in patients with DMD. The *mdx* mice undergo an acute phase of skeletal muscle necrosis that peaks around 3-4 weeks of age, followed by robust regeneration and stabilization of the disease phenotype which is not observed in patients with DMD. With the exception of the diaphragm, which displays more severe and progressive pathology, skeletal muscles of the *mdx* mouse remain at a chronic low level of damage and muscle pathology as they cycle between muscle degeneration and regeneration. Damaged skeletal muscle fibers in the *mdx* mouse show a decrease of approximately 20%-30% in specific force; unlike in patients with DMD, myofibers in the *mdx* mouse hypertrophy without atrophy in later stages. The *mdx* mouse has a mild cardiac phenotype, and more severe dystrophic phenotypes such as fibrosis become

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<sup>9</sup> Nulfield G, et al. (1984) X chromosome-linked muscular dystrophy (mdx) in the mouse. *Proc Natl Acad Sci USA* 81:1189-92.

more pronounced around 15 months of age. Finally, the *mdx* mouse has a lifespan equivalent to 80% that of a healthy mouse, whereas the lifespan of patients with DMD is only about one-third of the normal human lifespan.

#### DMD rat model

Male Dmd<sup>mdx</sup> rats are dystrophin-deficient rats which were generated using transcription activator-like effector nucleases targeting exon23 of the *dmd* gene in the Sprague Dawley (SD/Crl) rat background.<sup>10</sup> Male Dmd<sup>mdx</sup> rats display progressive changes with significant motor deficits such as reduction of muscle strength and spontaneous motor activity at 3 months of age; dystrophic muscle pathology in the limbs and diaphragm include necrosis, degeneration, fibrosis, and adipose tissue infiltrations. Cardiac findings include necrosis and fibrosis. Dmd<sup>mdx</sup> rats represent a more severe DMD model compared to *mdx* mice.

#### Study #2 (Process A)

<b>Report Number</b>		<b>SR-20-001</b>
<b>Date of Report Signed</b>		September 15, 2021
<b>Title</b>		Efficacy, Toxicity and Biodistribution of rAAVrh74.MHCK7.Micro-Dystrophin After A Single Intravenous Dose Administration in A Mouse Model of Duchenne Muscular Dystrophy With A 12 Week or 24 Week Observational Period
<b>GLP Status</b>		No
<b>Testing Facility</b>		<ul style="list-style-type: none"><li>In-life: The Research Institute at Nationwide Children's Hospital</li><li>Histopathology: (b) (4)</li></ul>
<b>Objective(s)</b>		To determine efficacy of systemic delivery of rAAVrh74.MHCK7 micro-dystrophin in treating skeletal and cardiac muscle deficits in <i>mdx</i> mice
<b>Study Animals</b>	<b>Strain/Breed</b>	C57BL/10ScSn- DMD <sup>mdx</sup> /J ( <i>mdx</i> )
	<b>Species</b>	Mice
	<b>Age</b>	4-6 weeks old
	<b>Body Weight</b>	14.1-26.2 g
	<b>#/group</b>	3 to 8 males/group
	<b>Total #</b>	40
<b>Test Article</b>		SRP-9001; Lot # TT467-1.1, formulated in (b) (4) Solution
<b>Control Article</b>		LR solution, pH 6.5
<b>ROA</b>		<ul style="list-style-type: none"><li>(b) (4)</li><li>IV injection (via tail vein) at Day 1 (Main study)</li></ul>
<b>Study Design</b>		(b) (4) (b) (4)

<sup>10</sup> Larcher T, et al. (2014) Characterization of dystrophin deficient rats: A new model for Duchenne Muscular Dystrophy. PLoS One 9(10):e110371.

<sup>11</sup> (b) (4) .

<b>Study Design</b>	<b>Main study by IV injection</b>					
	<b>Study group</b>	<b>Mouse strain</b>	<b>Dose level (vg/mouse)</b>	<b>Dose (vg/kg)</b>	<b>Sacrifice</b>	<b>Number of animals/group</b>
	1	mdx	0	0	12 weeks	8
	2	mdx	2 x 10 <sup>12</sup>	1 x 10 <sup>14</sup>	12 weeks	5
	3	mdx	6 x 10 <sup>12</sup>	3 x 10 <sup>14</sup>	6 weeks	3
	4	mdx	6 x 10 <sup>12</sup>	3 x 10 <sup>14</sup>	12 weeks	8
	5	mdx	1.2 x 10 <sup>13</sup>	6 x 10 <sup>14</sup>	12 weeks	8
	6	mdx	6 x 10 <sup>12</sup>	3 x 10 <sup>14</sup>	24 weeks	5
	7	C57BL/6	0	0	12 weeks	6
	8	C57BL/6	6 x 10 <sup>12</sup>	3 x 10 <sup>14</sup>	12 weeks	5
Untreated age matched male C57BL/6J mice served as positive control mice						
<b>Note:</b> Groups 3 and 6 did not have a concurrent control at 6- and 24-weeks; therefore, test article related changes for Group 6 could not be determined. The results were mostly generated at the 12 week sacrifice time point.						
<b>Dosing Regimen</b>	Single administration					
<b>Randomization</b>	Not specified					
<b>Description of Masking</b>	Not specified					
<b>Scheduled sacrifice time points</b>	<ul style="list-style-type: none"> <li>Groups 1, 2, 4, 5, 7, and 8 (12 weeks)</li> <li>Group 3 (6 weeks)</li> <li>Group 6 (24 weeks)</li> </ul>					

*Key Evaluations and Assessments:*

(b) (4)

**IV injected *mdx* or C57BL/6 mice (Main study)**

*In-life evaluations*

- Clinical observations - weekly
- Body weights (BW) - before injection and before sacrifice

*Terminal evaluations – at scheduled sacrifice*

- Clinical pathology (CBC<sup>13</sup> and serum chemistry<sup>14</sup>)

<sup>12</sup> The percent micro-dystrophin positive muscle fiber (PMDPF) across the examined muscle were determined.

<sup>13</sup> CBC parameters include WBC, RBC, HEMOG, HEMAT, MCV, MCH, MCHC, RDW, Platelet, MPV, LYMPH, and MONO.

<sup>14</sup> Serum chemistry parameters include ALT, ALP, AST, BUN, B/C, CREAT, GLU, TP, TBil, and DBil, CK was evaluated in a subset of animals.

- Functional assessment/specific force output of the isolated diaphragm and TA muscle following eccentric contractions (ECCs)
- Dystrophic muscle pathology including: 1) central nucleation, 2) collagen deposition, and 3) muscle fiber diameter. Evaluated in the TA, gastrocnemius (GAS), psoas major (PSO), triceps (TRI), gluteus maximus (GLUT), and quadriceps femoris (QUAD), and/or diaphragm.
- Micro-dystrophin protein expression in the membrane of muscle fibers in TA, GAS, PSO, TRI, GLUT, QUAD, diaphragm, and heart by IF staining
- $\beta$ -sarcoglycan in the membrane of muscle fibers, which is a member of the dystrophin-associated glycoprotein complex (DGC), by IF staining
- Biodistribution (BD)<sup>15</sup>
- Micro-dystrophin protein expression in tissue homogenates by Western blot<sup>16</sup>
- Histopathology
  - Muscles: Skeletal (GAS, GLUT, PSO, QUAD, TA, and TRI) muscle, and diaphragm
  - Heart (ventricular myocardium)
  - Kidney, liver, lung, spleen, and testis

*Key Results:*

(b) (4)

**IV injected mdx or C57BL/6 mice (Main study)**

- There were no consistent patterns of test article-related findings for BWs, CBC and serum chemistry.
- Unscheduled deaths:
  - One Group 1 (vehicle control) animal and one Group 4 (SRP-9001 at  $3 \times 10^{14}$  vg/kg) animal were found dead at 10- and 40-days post-administration, respectively. Necropsies were not performed due to autolysis.

<sup>15</sup> BD was determined by (b) (4). Tissues for BD analysis include: heart, lung, liver, kidney, spleen, gonad, diaphragm, skeletal muscles (PSO, TRI, GD, GAS, TA).

<sup>16</sup> Each sample of (b) (4)

. Tissues for WB analysis are the same as for BD.

- Functional assessment/specific force output: Groups 4 and 5 demonstrated dose-dependent increases in specific force output in the diaphragm and TA muscle compared to Group 1 at Week 12.
- Muscle pathology: Groups 4 and 5 demonstrated i) reduced mean central nucleation in skeletal muscles (QUAD, GAS, TRI, GLUT, PSO) and diaphragm muscles ii) reduced mean collagen deposition in diaphragm, and iii) increased mean fiber diameters in skeletal muscles (QUAD, GAS, TRI, GLUT, PSO) and diaphragm compared to Group 1 at Week 12.
- Micro-dystrophin expression in muscle fiber by IF: Groups 4 and 5 showed detectable micro-dystrophin protein expression in the membrane of muscle fibers of skeletal muscles (TA and PSO), diaphragm, and heart compared to none in Group 1 at Week 12.
- $\beta$ -sarcoglycan staining by IF: Groups 2, 4, and 5 showed dose-dependent positive signals of  $\beta$ -sarcoglycan staining in the membrane of muscle fiber in skeletal muscles (TA, GAS, QUAD, GLUT, PSO, and TRI) and diaphragm at week 12, while Group 1 did not.
- BD: Vector distribution was evident in all examined tissues, with the highest presence in the liver, followed by the heart, diaphragm, lung, kidney, limbs/skeletal muscle, spleen, and gonads (Table #1).

**Table #1:** BD Average Vector Genome Copy Numbers in Vector Administered Mice at Week 12

Tissue	2.00E+12		6.00E+12		1.20E+13	
	(average vg copies/ $\mu$ g DNA)	(average vg copies/nucleus)	(average vg copies/ $\mu$ g DNA)	(average vg copies/nucleus)	(average vg copies/ $\mu$ g DNA)	(average vg copies/nucleus)
Heart	2.84E+04	0.17	7.65E+05	4.57	5.35E+06	31.98
Lung	3.14E+04	0.19	2.52E+05	1.51	1.49E+06	8.90
Liver	4.36E+04	0.26	1.11E+07	66.56	1.80E+07	107.62
Kidneys	1.96E+04	0.12	3.27E+05	1.95	1.06E+06	6.31
Spleen	5.69E+04	0.34	5.27E+05	3.15	5.78E+05	3.46
Gonads	5.74E+04	0.34	3.68E+04	0.22	3.50E+05	2.09
Diaphragm	2.22E+04	0.13	3.55E+05	2.12	2.32E+06	13.87
Psoas	1.28E+05	0.77	1.60E+05	0.96	1.57E+06	9.36
Tricep	1.60E+05	0.96	5.45E+05	3.26	2.50E+06	14.93
Quadriceps	2.66E+06	15.88	6.57E+05	3.93	2.29E+06	13.69
Gastrocnemius	1.69E+05	1.01	5.80E+05	2.31	2.93E+06	17.54
Tibialis Anterior	5.86E+05	3.50	1.25E+05	0.74	1.32E+06	7.89

- Micro-dystrophin protein expression by WB – Groups 4 and 5 showed micro-dystrophin protein expression in the homogenates of the target tissues (heart, diaphragm, and limb skeletal muscles), but not in the non-target tissues (lung, spleen, liver, kidney, or gonads) at 12-weeks post-administration.
- Histopathology -
  - Muscle pathology in skeletal muscles – Groups 4 and 5 showed a trend towards reduced muscle pathology compared to Group 1 including reduced myofiber size, mononuclear cell inflammation (lymphocytes and few macrophages), increased interstitial space, dense myofibers with dark basophilic, and cytoplasmic mineral deposits at 12-weeks post-administration.
  - Muscle pathology in diaphragm – Groups 4 and 5 showed dose-dependent reduction of dystrophic muscle pathology compared to Group 1 at 12-weeks post-administration.
  - There were no consistent patterns of test article related findings in other examined tissues (heart, kidney, liver, lung, spleen and testis) at 12-weeks post-administration.
  - The 24-week histopathology data did not include a concurrent control group of *mdx* mice injected with vehicle so no comparisons could be made.

### Reviewer's Conclusion

- *mdx* mice administered  $\geq 3 \times 10^{14}$  vg/kg SRP-9001 showed: a) higher specific force in the skeletal muscle and diaphragm, b) lower dystrophic muscle pathology, c) micro-dystrophin expression in membrane of muscle fiber of skeletal muscles, diaphragm, and heart, d)  $\beta$ -sarcoglycan staining in the membrane of muscle fibers of skeletal muscle and the diaphragm, and e) lower dystrophic muscle pathology in skeletal muscle and diaphragm compared to vehicle-injected control mice at 12-weeks post-administration.
- Assessment of CK values was inconclusive due to the frequency of missing data and high variability in the individual animal data.
- IV administration of SRP-9001 resulted in systemic vector distribution with the highest level of detection in liver.
- Due to the use of a different manufacturing process and assay for physical titer determination compared to the commercial manufacturing process for SRP-9001 (Process B product manufactured at Catalent), the dose levels evaluated in this study cannot be compared directly to the commercial lots of SRP-9001.

### Study #3 (Process B ThermoFisher)

Report Number	SR-19-061
Date Report Signed	June 24, 2020
Title	Pharmacodynamics Study: 12-Week Dose Escalation Study Evaluating Pharmacology and Efficacy of Systemic Delivery of SRP-9001 in <i>mdx</i> Mice
GLP Status	No
Testing Facility	Sarepta Therapeutics

<b>Objective(s)</b>		To demonstrate efficacy of systemic delivery of commercial process B material using SRP-9001 in treating skeletal and cardiac muscle deficits in the <i>mdx</i> mouse model at 12 weeks post systemic delivery				
<b>Study Animals</b>	<b>Strain/Breed</b>	C57BL/10ScSn- DMD <sup>mdx</sup> /J				
	<b>Species</b>	Mice				
	<b>Age</b>	6-8 weeks old				
	<b>Body Weight</b>	13-30 g				
	<b>#/group</b>	8 males/group				
<b>Total #</b>		32				
<b>Test Article(s)</b>		SRP-9001 Lot # REC-000945-132756				
<b>Control Article(s)</b>		0.9% Sodium Chloride Lot (b) (4)				
<b>Route of Administration</b>		IV at Day 0				
<b>Study Groups and Dose Levels</b>	<b>Strain</b>	<b>Group<sup>a</sup></b>	<b>Subgroup</b>	<b>No. of Animals</b>	<b>Dose Level (vg/kg)</b>	<b>Dose Concentration (DRP/mL)</b>
				<b>Male</b>		
	C57BL/6J	1 (Control)	1	8	0	0
	DMD <sup>MDX</sup>	2 (Control)	2	8	0	0
	DMD <sup>MDX</sup>	3 (Low)	3	8	4.43x10 <sup>13</sup>	7.92x10 <sup>12</sup>
	DMD <sup>MDX</sup>	4 (Mid)	4	8	1.33x10 <sup>14</sup>	7.92x10 <sup>12</sup>
	DMD <sup>MDX</sup>	5 (High)	5	8	4.01x10 <sup>14</sup>	7.92x10 <sup>12</sup>
<sup>a</sup> Groups 1 and 2 were administered vehicle control article only. Source – page 15 of sr-19-061.pdf under Module 4.2.1.1 ‘Primary Pharmacodynamics’  Untreated age matched male C57BL/6J mice served as positive control mice						
<b>Dosing Regimen</b>		Single administration				
<b>Randomization</b>		Not specified				
<b>Description of Masking</b>		Not specified				
<b>Scheduled Sacrifice Time Points</b>		12 weeks (±15 days)				

*Key Evaluations and Assessments:*

- Mortality/morbidity (once daily)
- Clinical observations (pre-dose and weekly post-dose)
- Serum chemistry<sup>17</sup> (scheduled sacrifice)

Terminal evaluations

- Functional assessment/specific force output of diaphragm and TA muscle
- Micro-dystrophin protein expression in the membrane of muscle fibers<sup>18</sup> and PMDPF<sup>19</sup> by IF
- DGC formation by IF staining for β-sarcoglycan<sup>20</sup>

<sup>17</sup> Serum chemistry parameters include ALT, AST, ALP, total bilirubin, direct bilirubin, and indirect bilirubin.

<sup>18</sup> Micro-dystrophin protein expression was determined in skeletal muscle, diaphragm and heart.

<sup>19</sup> IF of micro-dystrophin staining was performed on right (R) and left (L) skeletal muscles [GAS, TA, TRI, GLUT, QUAD, PSO] and diaphragm. The percent of micro-dystrophin positive fiber (PMDPF) across the examined muscles and mean ±SEM of each group were determined.

<sup>20</sup> A list of tissues for DGC include TA muscle, diaphragm and heart.

- Histopathology of muscles to determine dystrophic pathology including muscle fiber diameter, myofibers with centrally located nuclei, and collagen deposition
- BD<sup>21</sup>
- Micro-dystrophin protein expression in tissue homogenates of target tissues by WB (skeletal muscles [TA, GAS, GLUT, TRI], and diaphragm, heart) and non-target tissues (spinal cord, brain, lymph node, gonads, pancreas stomach, kidney, spleen, liver, and lung)

*Key Results:*

All study animals survived until scheduled sacrifice. There were no consistent patterns of test article-related abnormal findings for clinical observations and serum chemistry.

**Reviewer's Comment:**

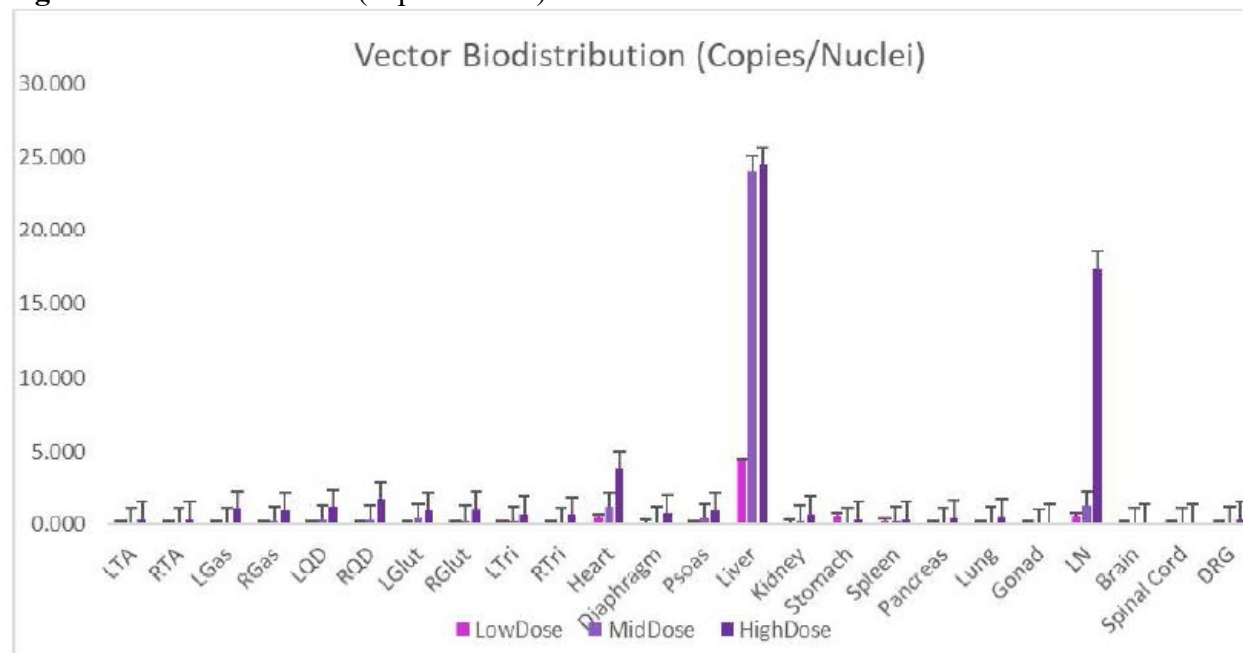
- Data for ALP, AST, and ALP are difficult to interpret because these parameters are also elevated following muscle damage in *mdx* mice. Groups 3, 4, and 5 showed a trend toward lower ALT, AST, and ALP compared to Group 2.
- Functional assessment of diaphragm and TA muscle:
  - Groups 4 and 5 (but not Group 3) showed increased mean specific force in diaphragm compared to Group 2 at Week 12.
  - Groups 3, 4, and 5 showed increased mean specific force in TA muscle compared to Group 2 at Week 12.
- Micro-dystrophin protein expression and PMDPF: In representative micrographs Groups 3, 4, and 5 showed, in comparison to Group 2: i) detectable micro-dystrophin protein in the membrane of muscle fibers of skeletal muscle, diaphragm, and heart, and ii) a dose-dependent increase in mean PMDPF in skeletal muscle and diaphragm.
- DGC formation: In representative micrographs Group 3 showed DGC formation in muscle fibers of skeletal muscle, diaphragm, and heart, while Group 2 did not. Data for Groups 4 and 5 were not provided.
- Histopathology of muscles: In comparison to Group 2, Groups 3-5 showed: i) an increase in mean fiber diameter in TA and TRI muscles and diaphragm, ii) a decrease in mean central nucleation in TA, GAS, and PSO muscles and diaphragm, and iii) a decrease in mean collagen deposition in diaphragm.

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<sup>21</sup> A list of tissues for BD evaluation include: brain, diaphragm, DRG, heart, kidney, liver, lung, lymph node, pancreas, spinal cord, spleen, stomach, gonads, and left and/or right skeletal muscles (GAS, TA, TRI, GLUT, QD, and PSO)

- BD: Groups 3, 4, and 5 showed a dose-dependent increase in vector distribution in all examined tissues compared to Group 2. The levels of detection were highest in the liver and lymph node, followed by the rest of the examined tissues, including gonads (Figure #3).

**Figure #3:** Biodistribution (copies/nuclei) in SRP-9001-administered *mdx* mice



Results depict mean  $\pm$  SEM (n=3 mice/group) at  $4.43 \times 10^{13}$  vg/kg (low dose),  $1.33 \times 10^{14}$  vg/kg (mid dose) and  $4.01 \times 10^{14}$  vg/kg (high dose) 12 weeks post-administration. Source: page 39 of sr-198-061 under Module 4.2.1.1 'Primary Pharmacodynamics'

- Micro-dystrophin protein expression in tissue homogenates by WB:
  - In target tissues: Groups 3, 4, and 5 showed detectable micro-dystrophin protein in the TA, GAS, GLUT, and TRI muscles, diaphragm, and heart, with the strongest signal detected in the heart. Group 2 showed little to no micro-dystrophin protein expression in the skeletal muscles and diaphragm.
  - In non-target tissues: Groups 2-5 showed no detectable micro-dystrophin protein in most of the examined non-target tissues (spinal cord, brain, lymph node, gonad, pancreas stomach, kidney, spleen, and lung). However, Groups 4 and 5 showed detectable micro-dystrophin protein in the liver.

### Reviewer's Conclusion

- *mdx* mice administered  $\geq 1.33 \times 10^{14}$  vg/kg SRP-9001 showed: i) increased specific force in the skeletal muscle and diaphragm, ii) micro-dystrophin protein expression in the membrane of muscle fibers of skeletal muscles, diaphragm, and heart, iii) increased PMDPF, and iv) reduced dystrophic muscle pathology (increased muscle diameters, reduced central

nucleation, and reduced collagen deposition) in skeletal muscle and diaphragm compared to vehicle-injected control mice at Week 12.

- BD analysis of SRP-9001-administered *mdx* mice showed: i) systemic vector distribution in all examined tissues with the highest level of detection in liver, and ii) micro-dystrophin protein expression in target tissues (skeletal muscles, diaphragm, and heart) and at low levels in the liver despite the muscle-specific promoter.

### **Study #3.1 (Process B ThermoFisher)**

**Study Report No. SR-20-014:** ‘A Single Dose Intravenous Administration, 12-Week Dose Escalation Study Evaluating Pharmacology and Efficacy of Systemic Delivery of SRP-9001 in *mdx* Mice.’ Per Module 2.4 ‘Nonclinical Overview’ (page 26), Study Report No. SR-20-014 is a report of H&E and (b) (4) of testes of animals from Study Report No. SR-19-061 (Study #3 reviewed above)

#### *Methods:*

- A (b) (4) assay of tissues from murine testes was performed on samples from 12 weeks post-administration of  $4.43 \times 10^{13}$ ,  $1.33 \times 10^{14}$ , or  $4.01 \times 10^{14}$  vg/kg of SRP-9001 (Groups 3-5); vehicle injected *mdx* control mice (Group 2), and vehicle injected C57BL/6J mice (Group 1). Detection of AAV-MHCK by (b) (4) was performed with a (b) (4) and detection of the micro-dystrophin transgene by (b) (4) with performed using a (b) (4)
- Histopathology (H&E) of murine testicular tissues was performed for samples from Week 12 post-administration in Groups 1-5.

#### *Key Results:*

- (b) (4) assay of testes
  - AAV-MHCK genome in testes - Groups 3-5 showed low (b) (4) scores (average between 1 to 2 and low MHCK positive cells (average between 1 to 2%) in testes with no dose-dependent response; while Groups 1-2 showed no MHCK7 positive cells in the testes.
  - Micro-Dystrophin expression in testes - Groups 3-5 showed low (b) (4) scores (average between 1 to 3) and low percentage micro-dystrophin positive cells (average between 1 to 6%) in the testes, both of which were not dose dependent; while Groups 1-2 showed no micro-dystrophin positive cells in the testes.
- Histopathology (H&E) of testes – There were no consistent patterns of test article-related findings.

#### **Reviewer’s Comment:**

- Although the pathology report concluded that there was a low likelihood of germline transmission by SRP-9001 based on the low (b) (4) scores of both AAV-MHCK and micro-dystrophin staining in Sertoli cells, spermatogonia, and primary spermatocytes, without dose dependent effects, and that the resulting scores were within the expected staining for background staining, the possible transduction of germline cells by SRP-9001 cannot be excluded based on the data and lack of MHCK7 and micro-dystrophin positive staining in control groups.

**Study #4 (Process B Catalent)**

<b>Report Number</b>		<b>SR-21-025</b>
<b>Date Report Signed</b>		September 30, 2021
<b>Title</b>		(b) (4) and Systemic Delivery of Catalent Commercially Representative SRP-9001 in the DMD <sup>mdx</sup> Mouse Model: Evaluation of Expression and Functional Outcome Measures
<b>GLP Status</b>		No
<b>Testing Facility</b>		Sarepta Therapeutics
<b>Objective(s)</b>		To assess bioactivity of SRP-9001 manufactured by Catalent in <i>mdx</i> mice following IV (b) (4) administration
<b>Study Animals</b>	<b>Strain/Breed</b>	C57BL/10ScSn- DMD <sup>mdx</sup> /J (mdx)
	<b>Species</b>	Mice
	<b>Age</b>	4 weeks old [±5 days]
	<b>Body Weight</b>	11-24 g
	<b>#/sex/group</b>	8 males/group
	<b>Total #</b>	32

Test Article(s)	SRP-9001 (commercially representative Lot #s A-634-SRP9001-20-0004, A-634-SRP9001-20-0006, A-634-SRP9001-20-0008) (Supplier: Catalent) Results of (b) (4) and % full capsid are listed below:															
	<ul style="list-style-type: none"> <li>Lot #A-634-SRP9001-20-0004</li> </ul> <table border="1"> <thead> <tr> <th>Test</th><th>Method</th><th>Vendor</th><th>Acceptance Criteria</th><th>Results</th></tr> </thead> <tbody> <tr> <td colspan="4">(b) (4)</td><td>(b) (4)</td></tr> <tr> <td colspan="4"></td><td>49%</td></tr> </tbody> </table>		Test	Method	Vendor	Acceptance Criteria	Results	(b) (4)				(b) (4)				
Test	Method	Vendor	Acceptance Criteria	Results												
(b) (4)				(b) (4)												
				49%												
Source – pages 90-91 of sr-21-025.pdf under Module 4.2.1.1 ‘Primary Pharmacodynamics’																
<ul style="list-style-type: none"> <li>Lot #A-634-SRP9001-20-0006</li> </ul> <table border="1"> <thead> <tr> <th>Test</th><th>Method</th><th>Vendor</th><th>Acceptance Criteria</th><th>Results</th></tr> </thead> <tbody> <tr> <td colspan="4">(b) (4)</td><td>(b) (4)</td></tr> <tr> <td colspan="4"></td><td>53%</td></tr> </tbody> </table>		Test	Method	Vendor	Acceptance Criteria	Results	(b) (4)				(b) (4)					53%
Test	Method	Vendor	Acceptance Criteria	Results												
(b) (4)				(b) (4)												
				53%												
	Source – pages 98-99 of sr-21-025.pdf under Module 4.2.1.1 ‘Primary Pharmacodynamics’															
	<ul style="list-style-type: none"> <li>Lot #A-634-SRP9001-20-0008</li> </ul> <table border="1"> <thead> <tr> <th>Test</th><th>Method</th><th>Vendor</th><th>Acceptance Criteria</th><th>Results</th></tr> </thead> <tbody> <tr> <td colspan="4">(b) (4)</td><td>(b) (4)</td></tr> <tr> <td colspan="4"></td><td>58%</td></tr> </tbody> </table>		Test	Method	Vendor	Acceptance Criteria	Results	(b) (4)				(b) (4)				
Test	Method	Vendor	Acceptance Criteria	Results												
(b) (4)				(b) (4)												
				58%												
Source – pages 102-103 of sr-21-025.pdf under Module 4.2.1.1 ‘Primary Pharmacodynamics’																
		Control Article(s)														
		Saline (Lot (b) (4))														
		Routes of Administration														
		<ul style="list-style-type: none"> <li>IV (Day 0)</li> <li>(b) (4)</li> </ul>														

<b>Study Groups and Dose Levels</b>	IV dosed groups				
	<b>Group</b>	<b>Strain</b>	<b>Test Article</b>	<b>Dose Level (vg/kg)</b>	<b>Dose Volume (ul)</b>
	1	C57BL/6J	Saline	N/A	200
	2	DMD <sup>MDX</sup>	Saline	N/A	200
	3	DMD <sup>MDX</sup>	A-634-SRP9001-20-004	1.33x10 <sup>14</sup>	184-204
	4	DMD <sup>MDX</sup>	A-634-SRP9001-20-006	1.33x10 <sup>14</sup>	202-220
	5	DMD <sup>MDX</sup>	A-634-SRP9001-20-006	2.66x10 <sup>14</sup>	255-338
	6	DMD <sup>MDX</sup>	A-634-SRP9001-20-008	1.33x10 <sup>14</sup>	171-223
Source- page 22 of sr-21-025.pdf under Module 4.2.1.1 'Primary Pharmacodynamics'					
(b) (4)					
Source- pages 22-23 of sr-21-025.pdf under Module 4.2.1.1 'Primary Pharmacodynamics'					
Untreated age matched male C57BL/6J mice served as positive control mice					
<b>Dosing Regimen</b>			Single administration		
<b>Randomization</b>			Not specified		
<b>Description of Masking</b>			Not specified		
<b>Scheduled Sacrifice Time Points</b>			IV dosed groups (12 weeks)		
			(b) (4)		

*Key Evaluations and Assessments:*

- Mortality/morbidity and clinical observations (once daily)

Terminal evaluations – at scheduled sacrifice

- Functional assessment
  - Specific force and eccentric contraction (ECC) of TA muscle (IV (b) (4) dosed groups)
  - Specific force of diaphragm (IV dosed groups)
- BD (IV dosed groups) - left skeletal muscle (TA, GAS, QD, GLUT, TRI, PSO), heart, diaphragm, and liver

- Micro-dystrophin protein expression in homogenates of target tissues (skeletal muscles [TRI, GLUT, CAS, TA], diaphragm, and heart) and non-target tissue (liver only) determined by WB analysis and quantitative determination<sup>22</sup> (IV dosed groups)
- Micro-dystrophin expression and  $\beta$ -sarcoglycan staining in membrane of muscle fiber by IF<sup>23</sup> (b) (4) IV dosed groups)
- PMDPF<sup>24</sup> by IF (IV dosed groups)

*Key Results:*

- Mortality/morbidity and clinical observations:
  - There were unscheduled deaths in one Group 1 and one Group 6 *mdx* mouse, which were sacrificed at 40- and 26- days post-administration, respectively, due to hydrocephaly.
- Functional assessment:
  - Specific force and ECC of TA muscle: SRP-9001-administered *mdx* mice showed: i) (b) (4) at  $\geq 1.33 \times 10^{14}$  vg/kg via the IV route compared to control mice, ii) dose-dependent increase in ECC at  $\geq 1.33 \times 10^{14}$  vg/kg by IV route, and iii) similar results in mice administered Lot #A-634-SRP9001-20-0004, Lot #A-634-SRP9001-20-0006, and Lot #A-634-SRP9001-20-0008
  - Specific force of diaphragm: *mdx* mice administered  $\geq 1.33 \times 10^{14}$  vg/kg by the IV route showed i) increased mean specific force (without dose-response) in diaphragm compared to control mice, and ii) similar results in mice administered Lot #A-634-SRP9001-20-0004, Lot #A-634-SRP9001-20-0006, and Lot #A-634-SRP9001-20-0008.
- BD: *mdx* mice administered  $\geq 1.33 \times 10^{14}$  vg/kg SRP-9001 via the IV route showed: i) dose-dependent vector distribution in skeletal muscle, diaphragm, heart, and liver with the highest levels of detection in liver, followed by heart, skeletal muscle and diaphragm, and ii) similar results in mice administered Lot #A-634-SRP9001-20-0004, Lot #A-634-SRP9001-20-0006, and Lot #A-634-SRP9001-20-0008.
- Micro-dystrophin protein expression and quantitative determination: *mdx* mice administered  $\geq 1.33 \times 10^{14}$  vg/kg SRP-9001 via the IV route showed: i) micro-dystrophin protein expression in tissue homogenates of heart, skeletal muscles, diaphragm, and liver, with the highest level detected in heart and lowest in liver; and ii) similar results in mice administered

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<sup>22</sup> Each sample of (b) (4)

The detected dystrophin bands were quantitatively determined using (b) (4) system and software.

<sup>23</sup> IF staining of micro-dystrophin and  $\beta$ -sarcoglycan was performed in left skeletal muscle (TA, GAS, QD, GLUT, TRI, PSO) and diaphragm.

<sup>24</sup> IF staining of micro-dystrophin was performed in left skeletal muscles [GAS, TA, TRI, GLUT, QD, PSO] and diaphragm. The percent of micro-dystrophin positive fiber (PMDPF) across the examined muscles and mean  $\pm$ SEM of individual group were determined.

Lot #A-634-SRP9001-20-0004, Lot #A-634-SRP9001-20-0006, and Lot #A-634-SRP9001-20-0008.

- $\beta$ -sarcoglycan staining: *mdx* mice administered  $\geq 1.33 \times 10^{14}$  vg/kg SRP-9001 via the IV route showed  $\beta$ -sarcoglycan staining in the membrane of muscle fibers of skeletal muscle, diaphragm, and heart.
- PMDPF: *mdx* mice administered  $\geq 1.33 \times 10^{14}$  vg/kg SRP-9001 via the IV route showed: i) an average PMDPF above 60% in skeletal muscles (TA, GAS, QUAD, GLUT, TRI and PSO) and diaphragm, but without dose response, and ii) similar results in mice administered Lot #A-634-SRP9001-20-0004, Lot #A-634-SRP9001-20-0006, and Lot #A-634-SRP9001-20-0008.

#### Reviewer's Conclusion:

- *mdx* mice administered  $\geq 1.33 \times 10^{14}$  vg/kg SRP-9001 via the IV route showed an increase in specific force in TA muscles and diaphragm compared to control mice at Week 12 and BD to the liver and target tissues (skeletal muscles, diaphragm, and heart). The results were similar in mice administered three different commercial lots of Process B (Catalent) SRP-9001.

#### Study #5 (Process B Catalent)

<b>Report Number</b>		<b>SR-20-012</b>
<b>Date Report Signed</b>		June 29, 2022
<b>Title</b>		A Single Dose Intravenous Administration Evaluating Pharmacology and Efficacy of Systemic Delivery of SRP-9001 in Young DMD <sup>MDX</sup> Rat Model
<b>GLP Status</b>		No
<b>Testing Facility</b>		Sarepta Therapeutics
<b>Objective(s)</b>		To evaluate the overall activity and myocardial activity of commercially representative Process B material in young DMD <sup>MDX</sup> rats
<b>Study Animals</b>	<b>Strain/Breed</b>	(b) (4) DMD mutated (DMD <sup>MDX</sup> ), internal breeding colony from the (b) (4)
	<b>Species</b>	Rat
	<b>Age</b>	3 to 4 weeks old
	<b>Body Weight</b>	50-85 g
	<b>#/sex/group</b>	6 to 10 male animals/group
	<b>Total #</b>	45
<b>Test Article(s)</b>		SRP-9001 Lot #A-634-SRP9001-20-0006
<b>Control Article(s)</b>		Saline Lot (b) (4)
<b>Route of Administration</b>		IV at Day 0

Study Groups and Dose Levels	Group <sup>a</sup>	Strain	Test Article	Time point	Dose Level (vg/kg)	Dose Volume (ml/kg)	Total No. of Animals <sup>b</sup>	No. Animals in Interim Report
	1	DMD <sup>MDX</sup>	SRP-9001	12 Week	1.33 x 10 <sup>14</sup>	9.5	10	10
	2	DMD <sup>MDX</sup>	SRP-9001	24 Week	1.33 x 10 <sup>14</sup>	9.5	8	6
	3	DMD <sup>MDX</sup>	SRP-9001	Survival	1.33 x 10 <sup>14</sup>	9.5	6	0
	7	DMD <sup>MDX</sup>	Saline	12 Week	N/A	9.5	8	8
	8	DMD <sup>MDX</sup>	Saline	24 Week	N/A	9.5	7	5
	9	DMD <sup>MDX</sup>	Saline	Survival	N/A	9.5	6	0
Source – page 10 of sr-20-012.pdf under Module 4.2.3.7.3 ‘Mechanistic Studies’								
Dosing Regimen			Single administration					
Randomization			Not specified					
Description of Masking			Not specified					
Scheduled Sacrifice Time Points			12 and 24 weeks					

*Key Evaluations and Assessments:*

- Open field activity measured by (b) (4) shortly before scheduled sacrifices at Weeks 12 and 24
- Echocardiography at Weeks 12 and 24
- Blood troponin I and creatine kinase (CK) at Weeks 1 and 12
- BD by (b) (4) at Weeks 12 and 24
- Micro-dystrophin protein expression by WB analysis<sup>26</sup> at Weeks 12 and 24
- Dystrophic muscle pathology determined by histopathology including central nucleation of muscles and muscle fiber diameter by H&E staining; fibrosis of muscle tissues by Masson’s Trichrome staining; and PMDPF by IF of micro-dystrophin staining at Weeks 12 and 24

*Key Results:*

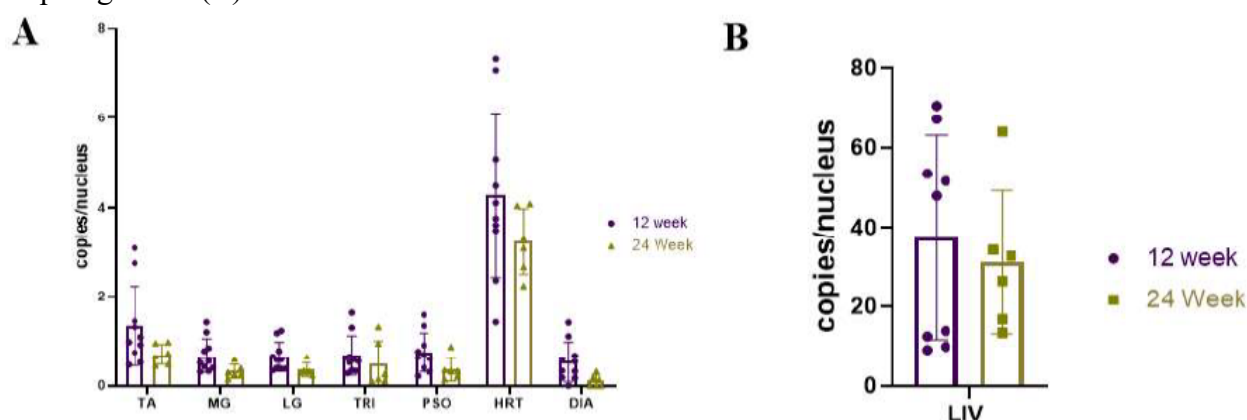
- Open field activity: SRP-9001-administered Dmd<sup>mdx</sup> rats showed statistically significant increase in mean ambulation and vertical activity compared to vehicle-injected control *mdx* rats at Weeks 12 and 24.
- Echocardiography:
  - SRP-9001-administered Dmd<sup>mdx</sup> rats showed statistically significant increased heart rates and decreased left ventricular internal diameter during diastole compared to vehicle-injected control Dmd<sup>mdx</sup> rats at Week 12.

<sup>25</sup> A list of tissues for BD include: (TA, medial GAS, lateral GAS, TRI, and PSO) skeletal muscle, diaphragm, heart and liver.

<sup>26</sup> A list of tissues for micro-dystrophin protein expression determination include: left TA, left medial GAS, left lateral GAS, left TRI, heart, diaphragm, liver, kidney, lung, spleen, stomach, gonad, lymph node, and brain.

- Blood troponin I and CK: There were no consistent patterns of test article-related changes in troponin I at Weeks 1 and 12 or CK at Weeks 12. However, the provided data are from part but not the whole groups, e.g., troponin I from 5/21 control animals (Groups 7-9), 5/24 1.33 x 10<sup>14</sup> vg/kg SRP-9001 dosed animals (Groups 1-3) at Weeks 1 and 12; and CK from 6/21 control Groups 7-9 and 6/24 SRP-9001 dosed Groups 1-3.
- BD: SRP-9001-administered Dmd<sup>mdx</sup> rats showed vector distribution in skeletal, diaphragm, heart muscles and liver, with the highest detection levels in liver followed by heart, and skeletal muscles and diaphragm at Weeks 12 and 24 (Figure #4).

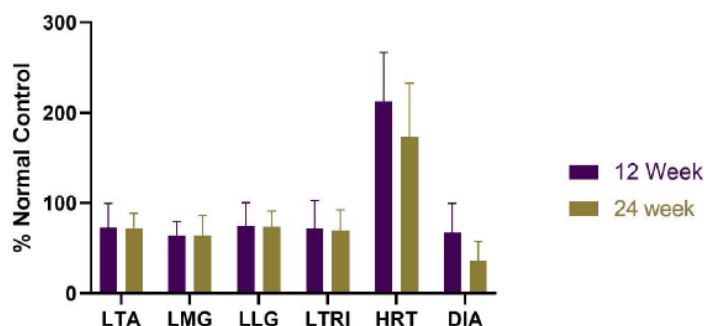
**Figure #4:** Biodistribution of SRP-9001 administered mdx rats in (A) skeletal muscles, heart, diaphragm and (B) liver



Results depict vector copies/nucleus (mean ± SD and dot of individual animals). Source: page 46 of sr-20-012.pdf under Module 4.2.3.7.3 'Mechanistic Studies'

- Micro-dystrophin protein expression: SRP-9001-administered Dmd<sup>mdx</sup> rats showed micro-dystrophin protein expression in tissue homogenates of skeletal muscle, diaphragm, and heart but not in liver, kidney, lung, spleen, stomach, gonads, lymph node, and brain at Weeks 12 and 24. The highest level of micro-dystrophin protein expression was in the heart (Figure #5).

**Figure #5:** Quantification of micro-dystrophin protein expression by WB analysis



Results depict mean ± SD from representative animals (numbers were not specified). Source: page 48 of sr-20-012.pdf under Module 4.2.3.7.3 'Mechanistic Studies'

- Muscle histology: SRP-9001-administered Dmd<sup>mdx</sup> rats showed: i) decreased central nucleation and increased muscle fiber diameter in skeletal muscle, ii) reduced fibrosis in skeletal muscle, diaphragm, and heart, and iii) increased PMDPF in skeletal muscles (average 60 – 90%) and diaphragm (average 50-62.5%) compared to vehicle-injected control Dmd<sup>mdx</sup> rats at Weeks 12 and 24.

#### Reviewer's Conclusion:

- SRP-9001-administered young (3-4 weeks old) Dmd<sup>mdx</sup> rats dosed at  $1.33 \times 10^{14}$  vg/kg IV showed: i) improvement of motor activity, ii) increased heart rates and decreased left ventricular internal diameter during diastole, iii) vector distribution and micro-dystrophin protein expression, iv) reduced dystrophic muscle pathology in skeletal muscle, diaphragm, and heart, and v) increased PMDPF in skeletal muscles and diaphragm at Weeks 12 and 24.

#### Study #6 (Process B Catalent)

<b>Report Number</b>		<b>SR-20-013</b>						
<b>Date Report Signed</b>		June 29, 2022						
<b>Title</b>		A Single Dose Intravenous Administration Evaluating Pharmacology and Efficacy of Systemic Delivery of SRP-9001 in Aged DMD <sup>MDX</sup> Rat Model						
<b>GLP Status</b>		No						
<b>Testing Facility</b>		Sarepta Therapeutics						
<b>Objective(s)</b>		To evaluate the overall activity, myocardial activity of commercially representative Process B material in aged DMD <sup>MDX</sup> rats						
<b>Study Animals</b>	<b>Strain/Breed</b>	(b) (4) DMD mutated (Dmd <sup>mdx</sup> ), internal breeding colony at (b) (4)						
	<b>Species</b>	Rat						
	<b>Age</b>	3 to 5 months old						
	<b>Body Weight</b>	300-650 g						
	<b>#/sex/group</b>	7 to 8 male animals/group						
	<b>Total #</b>	15						
<b>Test Article(s)</b>		SRP-9001 Lot #A-634-SRP9001-20-0006						
<b>Control Article(s)</b>		Saline Lot (b) (4)						
<b>Route of Administration</b>		IV (Day 0)						
<b>Study Groups and Dose Levels</b>	<b>Group<sup>a</sup></b>	<b>Strain</b>	<b>Test Article</b>	<b>Time point</b>	<b>Dose Level (vg/kg)</b>	<b>Dose Volume (ml/kg)</b>	<b>Total No. of Animals<sup>b</sup></b>	<b>No. Animals in Interim Report</b>
	1	DMD <sup>MDX</sup>	SRP-9001	12 Week	$1.33 \times 10^{14}$	9.5	7	7
	7	DMD <sup>MDX</sup>	Saline	12 Week	N/A	9.5	8	8
	8	DMD <sup>MDX</sup>	Saline	24 Week	N/A	9.5	4	0
	9	DMD <sup>MDX</sup>	Saline	Survival	N/A	9.5	4	0
	Source – page 28 of sr-20-013.pdf under Module 4.2.3.7.3 ‘Mechanistic Studies’							
<b>Dosing Regimen</b>		Single administration						
<b>Randomization</b>		Not specified						
<b>Description of Masking</b>		Not specified						
<b>Scheduled Sacrifice Time Points</b>		12 weeks						

*Key Evaluations and Assessments:*

- Open field activity by (b) (4) measured shortly before scheduled sacrifice at Week 12
- Echocardiography at Week 12
- Blood troponin I and CK at Week 12
- BD by (b) (4) at Week 12
- Micro-dystrophin protein expression by WB analysis<sup>28</sup> at Week 12
- Dystrophic muscle pathology determined by histopathology including central nucleation of muscles and muscle fiber diameter by H&E staining at Week 12

*Key Results:*

- There were five unscheduled deaths: three SRP-9001-administered and two vehicle-administered *mdx* rats that occurred within 90 minutes following IV administration. Per the study report, the unscheduled deaths were not considered test article-related and were attributed to the fragility of the older Dmd<sup>mdx</sup> rats.
- There were no significant test article-related changes in open field activity, blood troponin I and CK, or dystrophic muscle pathology at Week 12.
- Echocardiography data were collected but not analyzed.
- SRP-9001-administered Dmd<sup>mdx</sup> rats showed: i) vector distribution in skeletal muscle, diaphragm, heart, and liver, with the highest level detected in the liver, ii) micro-dystrophin protein expression in skeletal muscle, diaphragm, and heart, with the highest level of detection in the heart, but no detectable expression in the liver, kidney, lung, spleen, stomach, gonads, lymph node, or brain at Week 12, and iii) no change in activity endpoints (open field activity, troponin I and CK, muscle pathology, etc.).

**Reviewer's Conclusion:**

- SRP-9001-administered older (3- to 5-months old) Dmd<sup>mdx</sup> rats did not show test article related changes in motor activity, troponin I and CK, or dystrophic muscle pathology despite robust vector distribution and micro-dystrophin protein expression in the target tissues compared to vehicle-injected control Dmd<sup>mdx</sup> rats at Week 12.

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<sup>27</sup> A list of tissues for BD include: (TA, medial GAS, lateral GAS, TRI, and PSO) skeletal muscle, diaphragm, heart and liver.

<sup>28</sup> A list of tissues for micro-dystrophin protein expression determination include: left TA, left medial GAS, left lateral GAS, left TRI, heart, diaphragm, liver, kidney, lung, spleen, stomach, gonad, lymph node, and brain.

*Supporting Studies*

- **Study #7** (Study Report No. SR-20-007): IV administration of  $2 \times 10^{14}$  vg/kg/dose rAAVrh74.MHCK7.micro-dystrophin (b) (4) (manufactured by Process A) was performed in healthy (b) (4). Group 1 received a single IV administration on Day 0. Groups 2-5 received repeat administration on Day 0 and Week 12. Prior to Week 12 administration, 2-3 rounds of therapeutic plasma exchange (TPE) were conducted. Multiple immunosuppression regimens were explored in groups receiving repeat administration, including prednisone (2 mg/kg/day for different duration) with or without rituximab<sup>29</sup> and sirolimus<sup>30</sup>. The immunosuppression resulted in no change of biodistribution in skeletal muscle, diaphragm, heart, and liver at Weeks 12 and 24 following repeat vector administration, and no change of micro-dystrophin protein expression (detected by (b) (4) in skeletal muscle, diaphragm, and heart at Week 24 compared to non-human primates (NHPs) dosed once. Group 5 NHPs (2/2) that underwent TPE and received prednisone with rituximab and sirolimus had petechiae in the groin, torso, and lower limbs 3 weeks post-first vector administration and acute clinical signs of anaphylaxis including elevated respiratory rate, elevated heart rate, lethargy and rash, after the second vector administration. Similar abnormal clinical signs were not observed in the three other groups that received repeat IV administration of vector without rituximab and sirolimus. Thus, rituximab and sirolimus could not be ruled out as the cause of the findings in Group 5 NHPs. However, this study did not include appropriate concurrent control groups, including one with sirolimus and rituximab alone; therefore, the cause of the abnormal clinical signs could not be determined.
- **Study #8** (Study Report No. SR-20-002): Single IV administration of  $2.4 \times 10^{14}$  vg/kg rAAVrh74.MHCK7.micro-dystrophin (b) (4) (manufactured by Process A) in one (b) (4) resulted in transient increase in liver enzymes (ALT and AST) at Weeks 4 and 8, increased antibody titers against AAVrh74 vector capsid from Weeks 5 to 12, increased cellular immune response against AAVrh74 vector capsid and micro-dystrophin at Week 2 compared to baseline, and microscopic lesions in the liver (minimal periportal fibrosis, and mild periportal mononuclear infiltrates), and minimal mononuclear infiltrate in the heart and skeletal muscles at Week 12. The potential for the histopathology findings in the liver, heart, and skeletal muscles to be related to test article could not be determined due to the lack of a control group. In addition, a possible (b) (4)-related immune response and immune response-related toxicity could not be excluded.
- **Study #9** (Study Report No. SR-20-086): A single IV administration of  $7 \times 10^{13}$  vg/kg SRP-9001 (Process B, ThermoFisher) in *mdx* mice resulted in: i) increased specific force in the TA muscle and diaphragm, ii) increased micro-dystrophin expression in skeletal muscle, diaphragm, and heart, iii) increased PMDPF in skeletal muscles including diaphragm, iv) increased vector distribution in all examined tissues with the level of detection highest in the liver and lymph node, and the rest of examined tissues at lower levels, and v) increased

<sup>29</sup> Rituximab was IM administered at 750 mg/m<sup>2</sup>/day from 3-days prior to and until 3-weeks post the 1<sup>st</sup> vector administration; and 10- to 14-days prior to and at the day of repeat SRP-9001 administration.

<sup>30</sup> Sirolimus was orally administered at 3 mg/m<sup>2</sup>/day 14- and 4-days prior to the 1<sup>st</sup> vector administration and 1-day prior to the repeat vector administration, and daily thereafter until 30-days post-repeat vector administration.

micro-dystrophin protein expression in the target tissues (skeletal muscles [TA, GAS, GLUT, and TRI, diaphragm], and heart), with the highest level of detection in heart at Week 10. Micro-dystrophin expression was also detected in the liver, but not in other non-target tissues (spinal cord, brain, lymph node, gonad, pancreas, stomach, kidney, or spleen).

## **SAFETY PHARMACOLOGY STUDIES**

### **Summary List of Safety Pharmacology Studies**

There were no safety pharmacology studies performed with SRP-9001.

## **PHARMACOKINETIC STUDIES (BD)**

### **Summary List of Pharmacokinetics Studies**

There were no standalone biodistribution studies performed with SRP-9001. Studies #2, #3, #4, #10, #11, #12, and #13 in *mdx* mice and Studies #5, and #6 in *mdx* rats contain biodistribution data. A more comprehensive list of tissues was assessed in Studies #10 and #11.

## **TOXICOLOGY STUDIES**

### **Summary List of Toxicology Studies**

The following toxicology studies were conducted to evaluate the safety of ELEVIDYS following administration in WT and *mdx* mice.

#### **Toxicology Studies:**

Study Number	Study Title / Publication Citation	Report Number
<b>Primary Studies</b>		
10	A Single-Dose Intravenous Injection Toxicity and Biodistribution Study with SRP-9001 (rAAVrh74.MHCK7.Micro-dystrophin) in Mice with a 12-Week or 24-Week Observation Period	<b>SR-19-050</b>
11	A Single-Dose Intravenous Injection Toxicity Study with SRP-9001 (rAAVrh74.MHCK7.Micro-dystrophin) in Mice with a 12-Week Observation Period	<b>SR-20-066</b>
12	A Safety Evaluation of Systemic Delivery of SRP-9001 and Dose Formulations in DMD <sup>MDX</sup> Mice to Evaluate Dilatation of Brain Ventricles	<b>SR-21-028</b>
13	A Single Dose Juvenile Toxicity Temporal Vein Injection Study with SRP-9001 (rAAVrh74.MHCK7.Micro-dystrophin) in C57BL/6 Mice with a 13-Week Postdosing Period	<b>SR-20-015</b>
<b>Supporting Study</b>		
14	A Single Dose Juvenile Toxicity Temporal Vein Injection Study with SRP-9001 (rAAVrh74.MHCK7.Micro-dystrophin) in C57BL/6 Mice with a 4-Week Recovery Period	<b>SR-20-060</b>

## **Toxicology Studies**

### **Study #10 (Process B ThermoFisher)**

<b>Report Number</b>	<b>SR-19-050</b>						
<b>Date Report Signed</b>	September 10, 2021						
<b>Title</b>	A Single-Dose Intravenous Injection Toxicity and Biodistribution Study with SRP-9001 (rAAVrh74.MHCK7.Micro-dystrophin) in Mice with a 12-Week or 24-Week Observation Period						
<b>GLP Status</b>	Yes						
<b>Testing Facility</b>	(b) (4)						
<b>Objective(s)</b>	To evaluate the toxicity of SRP-9001 when administered as a single dose via IV injection to male mice; animals were observed for either 12 or 24 weeks to assess the biodistribution of SRP-9001 viral genome in tissues, the duration of SRP-9001 viral shedding, the systemic immunological response to SRP-9001, and the reversibility of observed toxicological effects.						
<b>Study Animals</b>	<b>Strain/Breed</b>	C57BL/6J					
	<b>Species</b>	Mice					
	<b>Age</b>	6-7 weeks old					
	<b>Body Weight</b>	17-25.7 g					
	<b>#/sex/group</b>	Toxicity arm: 20 males/group Satellite arm: 6 to 12 males/group					
	<b>Total #</b>	90					
<b>Study Animals</b>	<b>Strain/Breed</b>	C57BL/10ScSn- DMD <sup>mdx</sup> /J ( <i>mdx</i> )					
	<b>Species</b>	Mice					
	<b>Age</b>	6-7 weeks old					
	<b>Body Weight</b>	19.8-29.3					
	<b>#/sex/group</b>	Toxicity arm: 20 males/group Satellite arm: 6 to 12 males/group					
	<b>Total #</b>	90					
<b>Test Article(s)</b>	SRP-9001 (Lot # REC-000945-132756; retest date February 2020) (Process B GMP lot produced at ThermoFisher)						
<b>Control Article(s)</b>	0.9% NaCl (b) (4)						
<b>Route of Administration</b>	IV						
<b>Study Groups and Dose Levels</b>			<b>No. of Animals<sup>b</sup></b>		<b>Dose Volume</b>	<b>Dose Level</b>	<b>Dose Concentration</b>
	<b>Strain</b>	<b>Group<sup>a</sup></b>	<b>Subgroup</b>	<b>Male</b>	<b>(µL/g)</b>	<b>(DRP/kg)</b>	<b>(DRP/mL)</b>
	C57BL/6J	1 (Control)	1 (Toxicity)	20	50.6	0	0
			2 (Satellite)	6			
	C57BL/6J	2 (Low)	1 (Toxicity)	20	16.8	1.33x10 <sup>14</sup>	7.92x10 <sup>12</sup>
			2 (Satellite)	12			
	C57BL/6J	3 (High)	1 (Toxicity)	20	50.6	4.01x10 <sup>14</sup>	7.92x10 <sup>12</sup>
			2 (Satellite)	12			
	DMD <sup>MDX</sup>	4 (Control)	3 (Toxicity)	20	50.6	0	0
			4 (Satellite)	6			
	DMD <sup>MDX</sup>	5 (Low)	3 (Toxicity)	20	16.8	1.33x10 <sup>14</sup>	7.92x10 <sup>12</sup>
			4 (Satellite)	12			
	DMD <sup>MDX</sup>	6 (High)	3 (Toxicity)	20	50.6	4.01x10 <sup>14</sup>	7.92x10 <sup>12</sup>
			4 (Satellite)	12			
	DRP = DNase Resistant Particles a Groups 1 and 4 were administered vehicle control article only. b Toxicity animals designated for interim sacrifice (up to 10 animals/group, dependent on survival) were terminated on Day 85 of the dosing phase.						
	Source – page 14 of sr-19-050.pdf under Module 4.2.3.1 ‘Single-Dose Toxicity’						
<b>Dosing Regimen</b>	Single administration						
<b>Randomization</b>	Yes						
<b>Description of Masking</b>	Not specified						
<b>Scheduled Sacrifice Time Points</b>	Day 85 and Day 169						

*Key Evaluations and Assessments:*

- Mortality/morbidity assessed twice daily
- Clinical observations assessed weekly
- Body Weights measured weekly
- Clinical pathology: evaluated for Toxicity subgroups; Groups 1-6
  - Hematology
    - red blood cell (erythrocyte) count
    - hemoglobin
    - hematocrit
    - mean corpuscular volume
    - mean corpuscular hemoglobin
    - mean corpuscular hemoglobin concentration
    - red cell distribution width
    - absolute reticulocyte count
    - platelet count
    - white blood cell (leukocyte) count
    - absolute neutrophil count
    - absolute lymphocyte count
    - absolute monocyte count
    - absolute eosinophil count
    - absolute basophil count
    - absolute large unstained cell count
    - blood smear

Source – page 26 of sr-19-050.pdf under Module 4.2.3.1 ‘Single-Dose Toxicity’

- Serum chemistry
  - glucose
  - urea nitrogen
  - total protein
  - albumin
  - globulin
  - albumin:globulin ratio
  - total bilirubin
  - alanine aminotransferase
  - glutamate dehydrogenase
  - cholesterol
  - gamma glutamyltransferase
  - aspartate aminotransferase
  - alkaline phosphatase

Source – page 26 of sr-19-050.pdf under Module 4.2.3.1 ‘Single-Dose Toxicity’

- Gross pathology conducted for Toxicity subgroups; Groups 1-6
- Histopathology conducted for Toxicity subgroups; Groups 1-6 at Day 85 and Groups 1, 3, 4, and 6 at Day 169

Organ/Tissue		Organ/Tissue	
adrenal (left)	P,E	liver	P,E
animal identification		lung with large bronchi	P,E
aorta	P,E	lymph node (mandibular)	P,E
bone, femur with bone marrow (articular surface of the distal end to include stifle joint)	P,E	lymph node (mesenteric)	P,E
bone, sternum with bone marrow	P,E	muscle, biceps femoris	P,E
brain	P,E	optic nerve (2) <sup>a</sup>	P,E
cecum	P,E	pancreas	P,E
coagulating gland (intact with seminal vesicles)	P,E	pituitary gland	P,E
colon	P,E	prostate	P,E
dorsal root ganglion (lumbar) - (2)	P,E	rectum	P,E
duodenum	P,E	salivary gland (mandibular)	P,E
epididymis (left)	P,E	sciatic nerve	P,E
esophagus	P,E	seminal vesicle	P,E
eye (left) <sup>a</sup>	P,E	skin/subcutis	P,E
gall bladder	P,E	spinal cord (cervical, thoracic, and lumbar)	P,E
gut-associated lymphoid tissue (GALT) - (Peyer's Patch)	P,E	spleen	P,E
Harderian gland (left) <sup>a</sup>	P,E	stomach	P,E
heart	P,E	testis (left) <sup>a</sup>	P,E
ileum	P,E	thymus (entire left aspect)	P,E
injection site	P,E	thyroid (left lobe) with parathyroid	P,E
jejunum	P,E	tongue	P,E
kidney (2)	P,E	trachea	P,E
lesions	P,E	urinary bladder	P,E

E = Examined microscopically; P = Processed.

Source – page 27 of sr-19-050.pdf under Module 4.2.3.1 ‘Single-Dose Toxicity’

- Viral shedding in the plasma, feces and urine (Groups 1-6 at Days 2, 5, 8, 11, 14, 17, 20, 23, 26, 29, 32, 35, 38, 41, and 44)
- BD (Groups 1-6 at Days 85 and 169)
  - Tissues

Organ/Tissue	Organ/Tissue
adrenal (right, entire)	lung- entire right cranial lobe
brain (right frontal cortex)	muscle, biceps femoris (right, entire)
colon	sciatic nerve (right, entire)
epididymis (right, entire)	skin/subcutis-dorsal thoracic
eye (right, entire)	spleen
Harderian gland (right, entire)	testis (right, entire)
heart-apex	thymus (entire right aspect)
jejunum	thyroid (right lobe, entire) with parathyroid
kidney (right caudal pole)	trachea
Liver - entire remaining following collection for microscopic evaluation	

Source – page 28 of sr-19-050.pdf under Module 4.2.3.1 ‘Single-Dose Toxicity’

- Immunogenicity: anti-AAV antibody titer evaluated for satellite subgroups (Groups 1-6 at Day 7, Weeks 3, 4, 8, 12, 16, 20, and 25)

*Key Results:*

- There were no consistent patterns of test article-related findings for morbidity/mortality, clinical observations, and BWs.
  - Unscheduled deaths occurred in both control and SRP-9001-administered *mdx* mice as follows:

Group	Unscheduled deaths	Animal IDs [Cause of deaths]
4	2/26 mice (7.7%)	IDs: <u>M0311</u> [found dead at day 39; cause of death undetermined] and <u>M0327</u> [moribund sacrifice at day 9; hydrocephalus; histopathologic finding of moderate brain <b>ventricle dilatation</b> (cause of death) ]
5	6/32 mice (18.8%)	IDs: <u>M0408</u> [accidental death after blood sampling at day 35], <u>M0410</u> [moribund sacrifice at day 29; hydrocephalus; histopathologic finding of marked brain <b>ventricle dilatation</b> (cause of death)], <u>M0412</u> [moribund sacrifice at day 8; hydrocephalus; histopathologic finding of moderate brain <b>ventricle dilatation</b> (cause of death)], and <u>M0419</u> [found dead day 8, cause of death undetermined]; <u>M0423</u> [moribund sacrifice at day 29, cause of death undetermined], <u>M0424</u> [found dead at day 8; cause of death undetermined]
6	2/32 mice (6.3%)	IDs: <u>M0516</u> [moribund sacrifice at day 29; hydrocephalus histopathologic finding of marked brain <b>ventricle dilatation</b> (cause of death)] and <u>M0518</u> [found dead at day 33; cause of death undetermined]

**Reviewer's Comment:** As shown above, four unscheduled deaths were related to moderate to marked brain ventricle dilatation, which was observed in vehicle control and SRP-9001-administered *mdx* mice. Due to the lack of a consistent pattern of test article-related microscopic findings, and that spontaneous brain ventricle dilatation has been reported in *mdx* mice,<sup>31, 32</sup> the independent anatomic pathologist did not consider these unscheduled deaths test article-related. This reviewer agrees.

- Clinical pathology: There were no consistent patterns of test article-related adverse findings
  - Hematology-
    - SRP-9001-administered C57BL/6J and *mdx* mice showed slightly lower mean platelet counts at Days 85 and 169 compared to control mice.

<sup>31</sup> Xu S, et al. (2015) Abnormalities in brain structure and biochemistry associated with *mdx* mice measured by in vivo MRI and high resolution localized (1)H MRS. Neuromuscul Disord; 25(10):764-772.

<sup>32</sup> Bagatlioglu et al. (2020) Cognitive impairment appears progressive in the *mdx*. Neuromuscul Disord; 30(5):368-388.

- SRP-9001-administered *mdx* mice showed transiently lower mean WBC counts compared to control mice at Day 85, which resolved by Day 169.

**Reviewer's Comment:** Per the study director, due to the transient nature and/or small magnitude of these changes, the CBC findings were not considered adverse. This reviewer agrees.

- Serum chemistry –
  - There were no consistent patterns of test article-related adverse findings in SRP-9001-administered C57BL/6J mice.
  - SRP-9001-administered *mdx* mice had decreased AST, ALT, GLDH, and BUN compared to control *mdx* mice at Days 85 and 169.

**Reviewer's Comment:** Decreased AST, ALT, GLDH, and BUN in the SRP-9001-administered *mdx* mice compared to control mice were attributed to reduced muscle injury, consistent with histopathology observations of reduced muscle degeneration in heart and skeletal muscles of SRP-9001-administered *mdx* mice compared to control mice.

- Histopathology:
  - Liver - Possible SRP-9001-related findings of increased incidence of macroscopic and microscopic lesions were observed in the liver of C57BL/6J mice but not in *mdx* mice including: macroscopic lesions of rough surface of liver lobes (20% higher in Group 3 compared to Group 1) at Day 65 but not at Day 169; microscopic lesions with minimal severity (hyperplasia, hypertrophy, hepatocyte mitosis, hepatocyte necrosis, and/or pigmented) (up to 40% higher in Groups 2 and 3 without dose-dependent effect compared to Group 1) at Day 85 but not Day 169; and hepatocyte vacuolation with slight to moderate severity (10% and 40% higher in Groups 2 and 3 compared to Group 1, respectively) at Day 169.
  - Muscle – Possible SRP-9001-related findings of reduced incidence and severity in muscle degeneration in skeletal muscles and heart of *mdx* mice at Day 65 and Day 169.

**Reviewer's Comment:** The independent anatomic pathologist did not consider the liver findings adverse based on the low severity and lack of correlated clinical pathologic findings indicating no functional impact on the liver. This reviewer agrees with pathologist's conclusion.

- Viral shedding: SRP-9001 related viral shedding was observed in C57BL/6J and *mdx* mice at  $\geq 1.3 \times 10^{14}$  vg/kg in urine, plasma, and feces, which peaked at Day 2 for feces, at Day 7 for plasma, and Day 2 for urine, which were mostly reduced to below the limit of detection by Day 44.
- BD: SRP-9001 vector distribution was seen in all examined tissues in C57BL/6J and *mdx* mice at  $\geq 1.3 \times 10^{14}$  vg/kg. The highest levels were observed in the liver, followed by the

adrenal gland, heart, and muscle; low levels of vector DNA were also detected in the other tissues evaluated (e.g., thyroid, kidney, skin, lung, trachea, spleen, jejunum, testes, sciatic nerve, thymus, colon, brain, eye, epididymis, and hardierian gland). Vector levels were similar at Days 85 and 169.

- Anti-AAV antibody titer: Increased anti-AAV antibodies were observed in C57BL/6J and *mdx* mice administered SRP-9001 at  $\geq 1.3 \times 10^{14}$  vg/kg compared to baseline when assessed at Weeks 3 or 4, and plateaued by Weeks 20 or 25.

**Reviewer's Conclusion:** C57BL/6J and *mdx* (6-7 weeks old) mice that received single IV administration of SRP-9001 up to  $4.02 \times 10^{14}$  vg/kg showed no test article-related adverse findings for the 169-day study duration. The study concluded that the NOAEL was  $4.01 \times 10^{14}$  vg/kg in C57BL/6J and *mdx* mice. This reviewer agrees with the identified NOAEL.

#### Study #11 (Process B Catalent)

<b>Report Number</b>		SR-20-066
<b>Date Report Signed</b>		September 30, 2021
<b>Title</b>		A Single-Dose Intravenous Injection Toxicity Study with SRP-9001 (rAAVrh74.MHCK7.Micro-dystrophin) in Mice with a 12-Week Observation Period
<b>GLP Status</b>		Yes
<b>Testing Facility</b>		(b) (4)
<b>Objective(s)</b>		To evaluate the toxicity of SRP-9001 when administered as a single dose via IV injection to male mice and observed for 12 weeks following administration.
<b>Study Animals</b>	<b>Strain/Breed</b>	C57BL/6J
	<b>Species</b>	Mice
	<b>Age</b>	7-8 weeks old
	<b>Body Weight</b>	18.2-30.8 g
	<b>#/sex/group</b>	15 males/group (Toxicity); 5 males/group (biodistribution)
	<b>Total #</b>	60
<b>Study Animals</b>	<b>Strain/Breed</b>	C57BL/10ScSn- DMD <sup>mdx</sup> /J
	<b>Species</b>	Mice
	<b>Age</b>	7-8 weeks old
	<b>Body Weight</b>	18.2-30.8 g
	<b>#/sex/group</b>	15 males/group (Toxicity); 5 males/group (biodistribution)
	<b>Total #</b>	60
<b>Test Article(s)</b>		SRP-9001 (Lot #A-634-20-0003)
<b>Control Article(s)</b>		0.9% Sodium Chloride (Lot (b) (4))
<b>Route of Administration</b>		IV at Day 1

Study Groups and Dose Levels			No. of Animals <sup>b</sup>	Dose Volume (µL/g)	Dose Level (vg/kg)	Dose Concentration (vg/mL)
	Strain	Group <sup>a</sup>	Subgroup	Male		
	C57BL/6J	1 (Control)	1 (Toxicity)	15	25.1	0
			3 (Biodistribution)	5		
	C57BL/6J	2 (Low)	1 (Toxicity)	15	8.3	1.33x10 <sup>14</sup>
			3 (Biodistribution)	5		
	C57BL/6J	3 (High)	1 (Toxicity)	15	25.1	4.02x10 <sup>14</sup>
			3 (Biodistribution)	5		
	DMD <sup>MDX</sup>	4 (Control)	2 (Toxicity)	15	25.1	0
			4 (Biodistribution)	5		
	DMD <sup>MDX</sup>	5 (Low)	2 (Toxicity)	15	8.3	1.33x10 <sup>14</sup>
			4 (Biodistribution)	5		
	DMD <sup>MDX</sup>	6 (High)	2 (Toxicity)	15	25.1	4.02x10 <sup>14</sup>
			4 (Biodistribution)	5		
vg = vector genome (equivalent to DNase Resistant Particles)						
a Groups 1 and 4 were administered vehicle control article only.						
Source- page 18 of sr-20-066.pdf under Module 4.2.3 'Single-Dose Toxicity'						
Dosing Regimen			Single administration			
Randomization			Yes			
Description of Masking			Not specified			
Scheduled Sacrifice Time Points			12 weeks (Day 85)			

*Key Evaluations and Assessments:*

- Mortality/morbidity evaluated twice daily
- Clinical observations evaluated weekly
- BWs measured twice weekly
- Food consumption measured quantitatively Days 1 to 8 of dosing phase, weekly thereafter until Week 12
- Clinical pathology for Groups 1 to 6 toxicity subgroups at Week 12
  - Hematology

red blood cell (erythrocyte) count	platelet count
hemoglobin	white blood cell (leukocyte) count
hematocrit	absolute neutrophil count
mean corpuscular volume	absolute lymphocyte count
mean corpuscular hemoglobin	absolute monocyte count
mean corpuscular hemoglobin concentration	absolute eosinophil count
red cell distribution width	absolute basophil count
absolute reticulocyte count	absolute large unstained cell count
	blood smear

Source- page 21 of sr-20-066.pdf under Module 4.2.3 'Single-Dose Toxicity'

- Clinical Chemistry

Glucose	albumin:globulin ratio
urea nitrogen	total bilirubin
total protein	alanine aminotransferase
albumin	alkaline phosphatase
globulin	aspartate aminotransferase
creatinine	

Cholesterol  
Source- page 21 of sr-20-066.pdf under Module 4.2.3 'Single-Dose Toxicity'

gamma glutamyltransferase

- Necropsy and organ weights for Groups 1-6 toxicity subgroups
- Histopathology (Groups 1 to 6 toxicity subgroups)

Organ/Tissue		Organ/Tissue	
adrenal (2)	P,E	liver	P,E
animal identification		lung with large bronchi	P,E
aorta	P,E	lymph node (mandibular)	P,E
bone, femur with bone marrow (articular surface of the distal end to include stifle joint)	P,E	lymph node (mesenteric)	P,E
bone, sternum with bone marrow	P,E	muscle, biceps femoris	P,E
Brain	P,E	optic nerve (2) <sup>a</sup>	P,E
cecum	P,E	pancreas	P,E
coagulating gland (2) - (intact with seminal vesicles)	P,E	pituitary gland	P,E
colon	P,E	prostate	P,E
dorsal root ganglion (lumbar) - (2)	P,E	rectum	P,E
duodenum	P,E	salivary gland (mandibular [2])	P,E
epididymis (2)	P,E	sciatic nerve	P,E
esophagus	P,E	seminal vesicle	P,E
eye (2) <sup>a</sup>	P,E	skin/subcutis	P,E
gall bladder	P,E	spinal cord (cervical, thoracic, and lumbar)	P,E
gut associated lymphoid tissue (GALT)/Peyer's patch	P,E	spleen	P,E
Harderian gland <sup>a</sup>	P,E	stomach	P,E
heart	P,E	testis (2) <sup>a</sup>	P,E
ileum	P,E	thymus	P,E
injection site <sup>b</sup>	P,E	thyroid (2 lobes) with parathyroid	P,E
Jejunum	P,E	tongue	P,E
kidney (2)	P,E	trachea	P,E
Lesions	P,E	urinary bladder	P,E

E = Examined microscopically; P = Processed.

<sup>a</sup> Collected in modified Davidson's fixative and stored in 10% neutral-buffered formalin.

<sup>b</sup> The entire tail was collected; only the site of injection was processed for microscopic evaluation.

Source- page 22 of sr-20-066.pdf under Module 4.2.3 'Single-Dose Toxicity'

- BD (Groups 1 to 6 at Week 12)

adrenal (right, entire)	liver (left lateral lobe)
aorta	lung, entire right cranial lobe
bone, sternum with bone marrow (one sternebrae)	muscle, biceps femoris (right, entire)
brain (right frontal cortex)	pancreas
cecum	salivary gland (mandibular [right, entire])
colon	sciatic nerve (right, entire)
duodenum	skin/subcutis-dorsal thoracic
esophagus	spinal cord (cervical, thoracic, and lumbar)
eye (right, entire)	spleen
Harderian gland (right, entire)	stomach
heart-apex	testes (right)
ileum	thymus (entire right aspect)
jejunum	thyroid (right lobe, entire) with parathyroid
kidney (right caudal pole)	trachea

Source- page 23 of sr-20-066.pdf under Module 4.2.3 'Single-Dose Toxicity'

### Key Results:

- Mortality/morbidity: Unscheduled deaths were observed in Groups 5 and 6

Group	Unscheduled deaths	Animal IDs [Cause of deaths]
5	1/20 (5%)	IDs: M0417 [found dead at day 79; gross pathology – bilateral large iliac lymph nodes; histopathology – not performed; cause of death not determined]
6	2/20 mice (10%)	IDs: M0501 [moribund sacrificed at day 26; abnormal brain shape; hydrocephalus; histopathological finding of marked ventricle dilatation (cause of death)] M0504 [moribund sacrificed at day 43; abnormal brain shape; hydrocephalus; histopathological findings of moderate ventricle dilatation (cause of death)]

**Reviewer's Comment:** The independent anatomic pathologist did not consider these unscheduled deaths test article-related because the death of animal M0417 was considered incidental and brain ventricle dilatation has been reported as a background finding in *mdx* mice.<sup>33, 34</sup> This reviewer agrees with the assessment.

- BWs: Group 6 showed possible test article-related decreased mean BWs of 12% less compared to Group 4.

**Reviewer's Comment:** Per the study director, the changes in BW occurred mostly during the first week of the study and did not continue for the study duration; therefore, these decreases in BW were not considered adverse. This reviewer agrees.

- Clinical observations and food consumption: There were no consistent patterns of test article-related findings.
- Clinical pathology: There were no consistent patterns of test article-related adverse findings.

<sup>33</sup> Xu S, et al. (2015) Abnormalities in brain structure and biochemistry associated with *mdx* mice measured by in vivo MRI and high resolution localized (1)H MRS. *Neuromuscul Disord*; 25(10):764-772.

<sup>34</sup> Bagatlioglu et al. (2020) Cognitive impairment appears progressive in the *mdx*. *Neuromuscul Disord*; 30(5):368-388.

- CBC – Group 6 showed slightly decreased mean reticulocyte counts, platelet counts, WBC counts, and neutrophils compared to Group 4.
- Clinical chemistry – Groups 5 and 6 showed decreased mean AST and ALT compared to Group 4.

**Reviewer's Comment:** Per the study director, due to the small magnitude of these changes, the observed CBC findings were not considered adverse. The observed AST and ALT changes in SRP-9001-administered *mdx* mice were attributed to decreased muscle injury, consistent with the histopathology observations of reduced muscle degeneration in heart and skeletal muscles of SRP-9001-administered *mdx* mice compared to control mice.

- Organ weights: There were no consistent patterns of test article-related adverse findings.
- Gross and histopathology:
  - Brain - Possible SRP-9001-related higher incidence and severity of dilatation of the brain ventricles was observed in 2/9 surviving *mdx* mice at the high dose level ( $4.02 \times 10^{14}$  vg/kg) compared to control mice at Day 85.
  - Liver – Possible SRP-9001-related higher incidence of mononuclear cell infiltrates at minimal severity was observed in *mdx* mice at  $\geq 1.3 \times 10^{14}$  vg/kg compared to Group 4.
  - Muscle – Possible SRP-9001 related lower incidence and severity in muscle degeneration in skeletal muscles and heart of *mdx* mice at  $\geq 1.3 \times 10^{14}$  vg/kg.
- BD – SRP-9001-related vector distribution to all examined tissues was observed in C57BL/6J and *mdx* mice that received SRP-9001. The highest levels were observed in the liver, followed by adrenal gland, heart, aorta, and skeletal muscle; low levels of vector DNA were also detected in the other tissues evaluated (e.g., skin, thyroid, trachea, bone, kidney, lung, spleen, duodenum, salivary gland, sciatic nerve, jejunum, testes, ileum, stomach, brain, cecum, thymus, pancreas, colon, eye, spinal cord and harderian gland.)

**Reviewer's Conclusion:** Potential SRP-9001 related exacerbation of dilatation of ventricles was observed in 7-8 week old *mdx* mice at the high dose level ( $4.02 \times 10^{14}$  vg/kg) but not in C57BL/6J mice. The clinical significance of this finding is unclear. The study concluded that the NOAEL is  $4.02 \times 10^{14}$  vg/kg in C57BL/6J mice and  $1.33 \times 10^{14}$  vg/kg in *mdx* mice.

#### **Study #12 (Process B Catalent)**

<b>Report Number</b>	<b>SR-21-028</b>
<b>Date Report Signed</b>	June 28, 2022
<b>Title</b>	A Safety Evaluation of Systemic Delivery of SRP-9001 and Dose Formulations in DMD <sup>MDX</sup> Mice to Evaluate Dilatation of Brain Ventricles
<b>GLP Status</b>	No
<b>Testing Facility</b>	Sarepta Therapeutics

<b>Objective(s)</b>		To evaluate the safety of SRP-9001 at mid and high dose ( $2.0 \times 10^{14}$ and $4.01 \times 10^{14}$ vg/kg, respectively) concurrently with control treatment of saline or formulation buffer in the DMD <sup>MDX</sup> mouse model				
<b>Study Animals</b>	<b>Strain/Breed</b>	C57BL/10ScSn- DMD <sup>mdx</sup> /J ( <i>mdx</i> )				
	<b>Species</b>	Mice				
	<b>Age</b>	4-8 weeks old				
	<b>Body Weight</b>	12-35 g				
	<b>#/sex/group</b>	8 to 15 males/group				
<b>Total #</b>		48				
<b>Test Article(s)</b>		SRP-9001 Lot #P-634-SRP9001-20-0014FF				
<b>Control Article(s)</b>		Saline Lot (b) (4) Formulation buffer Lot #DM-001378-74				
<b>Route of Administration</b>		IV (tail vein)				
<b>Study Groups and Dose Levels</b>	<b>Group</b>	<b>Strain</b>	<b>Test Article</b>	<b>Dose Level (vg/kg)</b>	<b>Dose Volume (µl)</b>	<b>No. of Animals</b>
	1 <sup>a</sup>	DMD <sup>MDX</sup>	Saline	N/A	700	15
	2 <sup>a</sup>	DMD <sup>MDX</sup>	Formulation Buffer	N/A	(b) (4)	15
	3 <sup>a</sup>	DMD <sup>MDX</sup>	SRP-9001	$4.01 \times 10^{14}$	702 - 889	15
	4 <sup>b</sup>	DMD <sup>MDX</sup>	SRP-9001	$2.0 \times 10^{14}$	433 - 538	8
Source – page 25 of sr-21-028 under Module 4.2.3.7.7 ‘Other’ <b>Note:</b> ➤ Per the study report (page 7), animals in Group 4 were under evaluation for functional improvement, vector genome biodistribution, and protein expression by WB and IF only. Tissues for histopathology were only collected from Groups 1-3.						
<b>Dosing Regimen</b>		Single administration				
<b>Randomization</b>		Not described				
<b>Description of Masking</b>		Not described				
<b>Scheduled Sacrifice Time Points</b>		12 weeks (±15 days) post-dose				

*Key Evaluations and Assessments:*

- Mortality/morbidity assessed daily
- Specific force of the TA muscle (Group 4 only) – 12 weeks
- BD by (b) (4) for Group 4 was assessed in the skeletal muscle (TA, TRI, QUAD, GLUT), heart diaphragm, brain, spinal cord, and liver – 12 weeks
- Micro-dystrophin expression by WB for Group 4 was assessed in the skeletal muscle (TA, TRI, QUAD, GLUT), heart, diaphragm, and liver – 12 weeks
- PMDPF by IF for Group 4 was assessed in the skeletal, diaphragm, and heart muscles – 12 weeks
- Histopathology of ventricular dilatation of the brain only (Groups 1-3) – 12 weeks

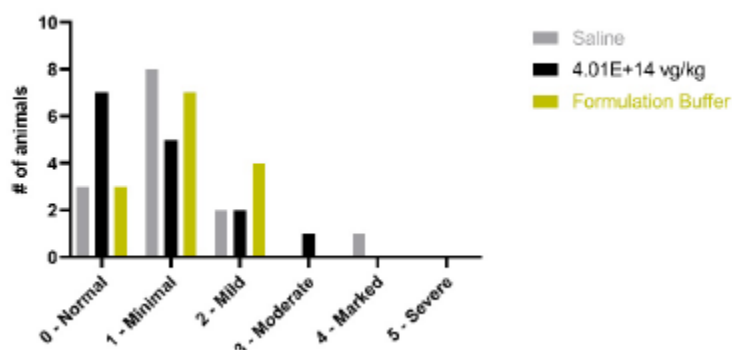
**Reviewer’s Comment:**

- This study was primarily conducted to evaluate the brain of Study Groups 1-3. The rest of the assessments (specific force, BD, micro-dystrophin and PMDPF) for Group 4 were to confirm bioactivity of SPR-9001.

**Key Results:**

- Results from Group 4 confirmed the bioactivity of administered SPR-9001 including: i) higher specific force of skeletal muscle compared to external control mice, ii) vector distribution to skeletal muscle, diaphragm, and heart, iii) micro-dystrophin protein expression in skeletal muscle, diaphragm, and heart, and iv) ~60 ( $\pm 10$ )% PMDPF in skeletal (TA) muscle, diaphragm and heart.
- There were no consistent patterns of test article-related findings for unscheduled deaths and microscopic findings of the brain. The overall severity of ventricular dilatation in the brain evaluated by histopathology indicated similar frequency and severity for control and SRP-9001 groups (Figure #6).

**Figure #6:** Number of animals with ventricular dilatation dosed with saline, vehicle control, or SRP-9001 at various severities (0-5)



Source – page 37 of sr-21-028 under Module 4.2.3.7.7 ‘Other’

- One Group 1 (saline control) animal was found dead on Day 34 without clinical abnormalities.
- One Group 2 (formulation buffer control) animal was found dead on Day 82 with hydrocephalus.
- One Group 3 (SRP-9001 dosed) animal was sacrificed early on Day 34 with hydrocephalus.

**Reviewer’s Conclusion:**

Hydrocephalus was observed in both control and SRP-9001-administered 4-8 weeks old *mdx* mice at similar frequencies and severities; therefore, hydrocephalus was not considered test article-related.

**Study #13 (Process B Catalent)**

Report Number	SR-20-015
Date Report Signed	December 3, 2021
Title	A Single Dose Juvenile Toxicity Temporal Vein Injection Study with SRP-9001 (rAAVrh74.MHCK7.Micro-dystrophin) in C57BL/6 Mice with a 13-Week Postdosing Period
GLP Status	Yes

Testing Facility		(b) (4)					
Objective(s)		To evaluate the biodistribution and potential toxicity of SRP-9001 when administered via a single temporal vein injection to neonatal mice on Postnatal Day (PND) 1 and to assess the neurobehavioral functionality and reversibility of any effects after a 13-week post dosing phase					
Study Animals	Strain/Breed	C57BL/6J					
	Species	Mice					
	Age	PND 1					
	Body Weight	Males 1to 1.6 g; females 1 to 1.7 g					
	#/sex/group	11 to 20/sex/group					
Total #		83					
Test Article(s)		SRP-9001 (Lot #A-634-20-0003)					
Control Article(s)		0.9% Sodium Chloride (Lot #s (b) (4))					
Route of Administration		IV					
Study Groups and Dose Levels	Number of Animals <sup>a</sup>						
		Toxicity (males/females)	Anti-AAV <sup>b</sup> (males/females)	Neurobehavioral (males/females)	Dose Level (Vg/kg)	Concentration <sup>c</sup> (Vg/mL)	Dose Volume (µL/g)
	1 (Control) <sup>c</sup>	20/20	31/31	20/20	0	0	25.1
	2 (Low)	11/10	31/30	20/20	1.33x10 <sup>14</sup>	1.6x10 <sup>13</sup>	8.31
	3 (High)	14/14	41/38	24/24	4.01x10 <sup>14</sup>	1.6x10 <sup>13</sup>	25.1
	AAV = Adeno-associated virus; Vg = Viral Genomes; PND = Postnatal Day						
	a Each litter consisted of four males and four females postdose on PND 1, when possible.						
	b Anti-AAV animals were used for anti-AAV antibody analysis sampling.						
	c Group 1 was administered vehicle control article (0.9% Sodium Chloride) only.						
	Source – page 18 of sr-20-015.pdf under Module 4.2.3.5.4 ‘Studies in which the offspring (juvenile animals) are dosed and/or further evaluated’						
Dosing Regimen		Single administration					
Randomization		Yes					
Description of Masking		Not described					
Scheduled Sacrifice Time Points		<ul style="list-style-type: none"><li>• Toxicity subgroups - PND 90</li><li>• Anti-AAV subgroups - PND 7 or older as applicable; no necropsy was performed</li><li>• Neurobehavioral subgroups - PND 69 or 70</li></ul>					

*Key Evaluations and Assessments:*

- Mortality/morbidity assessed twice daily
- Clinical observations assessed twice daily
- BWs measured on PNDs 4, 7, 10, 14, 17, 21, 25, 29, 32, 36, 39, 43, 46, 50, 53, 57, 60, 64, 67, and 70
- Food consumption measured beginning on PND 21, then at same time interval as BWs
- In the neurobehavioral subgroups, neurobehavioral tests (function observation battery [FOB], grip strength, foot splay, elicited behaviors, etc.) were measured on PND 45(±3), locomotor activity at PND 50(±3), learning and memory by Morris water maze at PND 65(±3), and auditory startle at PND 55(±3).

- Hematology on PND 90 for Toxicity subgroups
 

red blood cell (erythrocyte) count	platelet count
hemoglobin	white blood cell (leukocyte) count
hematocrit	differential blood cell count
mean corpuscular volume	blood smear
mean corpuscular hemoglobin	reticulocyte count
mean corpuscular hemoglobin concentration	red blood cell distribution width

Source – page 25 of sr-20-015.pdf under Module 4.2.3.5.4 ‘Studies in which the offspring (juvenile animals) are dosed and/or further evaluated’

- Clinical chemistry on PND 90 for Toxicity subgroups
 

glucose	alkaline phosphatase
urea nitrogen	gamma glutamyltransferase
creatinine	aspartate aminotransferase
total protein	calcium
albumin	inorganic phosphorus
globulin	sodium
albumin:globulin ratio	potassium
cholesterol	chloride
total bilirubin	triglycerides
alanine aminotransferase	creatine kinase

Source – page 25 of sr-20-015.pdf under Module 4.2.3.5.4 ‘Studies in which the offspring (juvenile animals) are dosed and/or further evaluated’

- Anti-AAV antibodies on PNDs 1, 2, 4, 22, 29, 43, 57, 71, and 85 for Anti-AAV subgroups

#### Terminal (PND 90)

- Femur length for Toxicity subgroups
- Femur imaging for bone mineral density [BMD], bone volume [BV], and bone mineral content [BMC]) by micro–Computed Tomography for Toxicity subgroups
- Necropsy for Toxicity subgroups
- Organ weights for Toxicity subgroups
 

adrenal (2)	ovary with oviduct (2)
brain	pituitary gland
cervix (weighed with uterus)	prostate
epididymis (2)	seminal vesicles (with coagulating gland)
heart	- (2)
kidney (2)	spleen
liver	testis (2)
lung (with large bronchi)	thymus
	thyroid with parathyroid (2)
	uterus

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Note: Organ:body weight percentages and organ:brain weight ratios were calculated.

Source – page 28 of sr-20-015.pdf under Module 4.2.3.5.4 ‘Studies in which the offspring (juvenile animals) are dosed and/or further evaluated’

- Histopathology for Groups 1 and 3 of toxicity subgroups, and Group 2 of neurobehavioral subgroups

adrenal (2)	mandibular lymph node
animal identification	mesenteric lymph node
aorta	optic nerve (2) <sup>b</sup>
brain	ovary (2)
cecum	oviduct (2)
cervix (weighed with uterus)	pancreas
coagulating gland (2) - (intact with seminal vesicles)	pituitary
colon	prostate
duodenum	rectum
dorsal root ganglion (lumbar) - (2)	salivary gland (mandibular [2])
epididymis (2) <sup>a</sup>	sciatic nerve
esophagus	seminal vesicle
eye (2) <sup>b</sup>	skeletal muscle (biceps femoris)
femur with bone marrow, with stifle joint (articular surface of the distal end)	skin, subcutis
gall bladder	spinal cord (cervical, thoracic, and lumbar)
Gut-associated lymphoid tissue (GALT)/Peyer's patch	spleen
Harderian gland <sup>b</sup> (2)	sternum with bone marrow
heart	stomach
ileum	testis (2) <sup>a,b</sup>
jejunum	thymus
kidney (2)	thyroid (2) with parathyroid
lesions	tongue
liver	trachea
lung (with large bronchi)	ureter (2)
mammary gland/region (females and males, if possible)	urinary bladder
	uterus
	vagina

Source – page 29 of sr-20-015.pdf under Module 4.2.3.5.4 ‘Studies in which the offspring (juvenile animals) are dosed and/or further evaluated’

- BD by (b) (4) in the liver, heart, and skeletal muscle
- Germline vector genome and transgene mRNA expression by (b) (4) assay<sup>35</sup> on PND 69 or 70 for Group 1 – testes from 2 males; ovaries from 2 females, Group 2 – testes from 8 males, and ovaries from 8 females, Group 3- testes from 8 males.

### Reviewer's Comment:

- A limited number of samples were available for analysis: Group 1- 1/8 males; Group 2- 3/8 males and 3/8 females; Group 3- 3/8 males. It is unclear why the germline vector genome and transgene mRNA expression were assessed in only a few selected animals.

### Key Results:

<sup>35</sup> (b) (4)

- There were no consistent patterns of test article-related findings for unscheduled deaths, clinical observations, BWs, food consumptions, neurobehavioral tests, hematology, clinical chemistry, femur length, organ weights, gross pathology, and histopathology.
- Unscheduled deaths: As the unscheduled deaths occurred in similar numbers across all groups (7/40 in Group 1, 4/21 in Group 2, 5/28 in Group 3), with many attributed to missing mice/presumed cannibalization, these deaths were not considered test article-related.
- Detection of Anti-AAV antibody: Due to insufficient samples in many animals in Groups 1-3, only limited data were available. These data showed test article-related higher anti-AAV antibody titers in Groups 2 and 3 compared to Group 1.
- BD: Groups 2 and 3 showed a dose dependent increase of vector levels in the liver, heart, and skeletal muscle, with the highest level of vector detection in the liver.
- Germline vector genome/transgene mRNA expression: A limited number of samples were available for analysis: Group 1- 1/8 males; Group 2- 3/8 males and 3/8 females ; Group 3- 3/8 males
  - Group 2 - 2 out of 3 analyzed male samples were positive for micro-dystrophin
  - Group 2 - 1 out of 3 analyzed male samples were positive for AAV-MHCK
  - All other analyzed samples were negative

### **Reviewer's Conclusion**

- There was no consistent pattern of SRP-9001-related adverse findings in neonatal C57/BL6 mice administered up to  $4 \times 10^{14}$  vg/kg SRP-9001 throughout the 90-day study duration. This reviewer concluded that the was NOAEL =  $4 \times 10^{14}$  vg/kg.
- The assessment of germline vector genome/transgene mRNA expression was limited due to the few numbers of samples analyzed. However, of the limited samples evaluated, a few were positive for micro-dystrophin and AAV-MHCK.

### *Supporting Study*

- **Study #14** (Study Report No. SR-20-060): Single IV (temporal vein) administration of  $1.32 \times 10^{14}$  and  $2.49 \times 10^{14}$  vg/kg of Process B (Catalent) SRP-9001 in neonatal C57BL/6 mice resulted in no test article-related findings for mortality/morbidity, clinical observations, body weights, food consumption, and gross pathology. A dose-dependent increase of vector distribution in the liver, heart, and skeletal muscle was observed during the 28-day study duration.

### Developmental and Reproductive Toxicology Studies:

Per the applicant, developmental and reproductive toxicology studies have not been conducted with SRP-9001 for the following reasons:

- The intended clinical population is nearly exclusively male.
- There was no evidence of SRP-9001 related lesions in male reproductive tissue (Report No. SR-19-050 and SR-20-066)
- BD showed low levels of vector distribution in ovaries and testes and germline transmission of SRP-9001 is expected to be low.

Genotoxicity Studies:

Per the applicant, genotoxicity studies have not been conducted with SRP-9001 because the literature thus far, supports random integration of AAV vector into the host genome at low frequencies<sup>36, 37</sup>

Carcinogenicity/Tumorigenicity Studies:

Per the applicant, carcinogenicity/tumorigenicity studies have not been conducted with SRP-9001 for the following reasons:

- AAV of various serotypes including AAV2 and AAVrh74, have been studied in 162 clinical trials; thus far, no cancer of any type has been reported.<sup>38, 39, 40</sup>
- The potential risk of transgene related carcinogenicity/tumorigenicity is low because dystrophin may have tumor suppressor potential in human cancers with myogenic programs.<sup>41</sup>

**Reviewer Comment:** Based on the indication of X-linked DMD which occurs primarily in males and lack of concerning findings in the completed toxicology studies, further assessment of developmental and reproductive toxicology, carcinogenicity/tumorigenicity, and genotoxicity are not warranted at this time.

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<sup>36</sup> Nowrouzi A, et al. (2012) Integration frequency and intermolecular recombination of rAAV vectors in non-human primate skeletal muscle and liver. Mol Ther 20(6):1177-86.

<sup>37</sup> Chandler RJ, et al. (2017) Recombinant adeno-associated viral integration and genotoxicity: insights from animal models. Hum Gene Ther 28(4):314-22.

<sup>38</sup> Berns KI, et al. (2015) Adeno-associated virus type 2 and hepatocellular carcinoma. Hum Gene Ther 26(12):779-81.

<sup>39</sup> Colella P, et al. (2017) Emerging issues in AAV-mediated in vivo gene therapy. Mol Ther Methods Clin Dev 1(8):87-104.

<sup>40</sup> Nault JC, et al. (2016) Wild-type AAV Insertions in Hepatocellular Carcinoma Do Not Inform Debate Over Genotoxicity Risk of Vectorized AAV. Mol Ther 24(4):660-1.

<sup>41</sup> Wang Y, et al. (2014) Dystrophin is a tumor suppressor in human cancers with myogenic programs. Nat Genet 46(6):601-6.

### APPLICANT'S PROPOSED LABEL

- Subsections 8.1-8.3 of Section 8 ('Use in Specific Population') should be revised to comply with 21 CFR 201.56(d)(91), 201.57(c)(9), and 20157(c)(14).<sup>42</sup>
- Section 12..3('Vector Distribution and Vector Shedding') should be revised to accurately reflect nonclinical biodistribution results generated from studies in healthy and *mdx* mice from a comprehensive list of tissues (e.g., Report Numbers SR-19-050 and SR-20-066) and to include tissues where micro-dystrophin protein was detected.
- Section 13 ('Nonclinical Toxicology') should be revised to include only the necessary nonclinical information needed for safe use of the product.

### CONCLUSION OF NONCLINICAL STUDIES

Review of the nonclinical studies did not identify any safety concerns that could not be addressed in the product label. The nonclinical data support approval of the license application.

### KEY WORDS/TERMS

ELEVIDYS™, delandistrogene moxeparvovec, rAAVrh74.MHCK7.micro-dystrophin, adeno-associated vector, replication-defective, serotype 74, MHCK7 promoter, human micro-dystrophin, SPR-9001, IV administration, Duchenne muscular dystrophy

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<sup>42</sup> *Pregnancy, Lactation, and Reproductive Potential: Labeling for Human Prescription Drug and Biological Products* - Content and Format, available at:  
<https://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm450636.pdf>.

