Supplementary Online Content

Mendell JR, Sahenk Z, Lehman K, et al. Assessment of systemic delivery of rAAVrh74.MHCK7.microdystrophin in children with Duchenne muscular dystrophy: a nonrandomized controlled trial. *JAMA Neurol.* Published online June 15, 2020. doi:10.1001/jamaneurol.2020.1484

This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods

Vector Production and Titering

For gene transfer, the human micro-dystrophin cassette contained the (R4–R23/Δ71–78) domains as previously described.[1] The complementary DNA was codon-optimized for human usage and synthesized by GenScript (Piscataway, NJ). It includes a consensus Kozak sequence, an SV40 intron, and synthetic polyadenylation site (53 base pairs). The recombinant MHCK7 promoter used to drive transgene expression is a muscle-cardiac specific promoter and is based on the MCK promoter and the promoter described by Dr. Stephen Hauschka (University of Washington, Seattle, WA).[2] This MCK-based promoter utilizes an enhancer derived from the 5' of the transcription start site within the endogenous muscle CK gene with a proximal promoter.[2] This enhancer, along with a modified CK7 cassette from the MCK family of genes, is ligated to an α-MyHC (α-myosin heavy chain) enhancer 5' of the CK portion to enhance cardiac expression.[2] The MHCK7 micro-dystrophin expression cassette was cloned between AAV2 inverted terminal repeats (ITRs) using flanking *Xba*I restriction enzyme sites. *Msc*I/*Sma*I restriction enzyme digestions, as well as Sanger sequencing through the ITRs, were used to confirm ITR integrity.

A quantitative polymerase chain reaction (qPCR)-based titration method based on a supercoiled plasmid standard was used to determine an encapsulated vg titer utilizing a Prism 7500 Fast Taqman detector system (PE Applied Biosystems).[3]

ELISpot Assay

Peripheral blood mononuclear cells (PBMCs) were separated from whole blood through density gradient centrifugation, as previously described.[4] Briefly, fresh blood samples were spun in a table-top centrifuge (Sorval Legend RT) and the top plasma layer was removed and stored in -80°C freezer. The remaining blood was mixed with $PBS^{-/-}$ (Invitrogen), underlayed with Ficoll-Paque (Fisher) and spun for density gradient separation. The middle layer containing the PBMCs was removed and subjected to various wash/spin cycles before a final resuspension in human AIM V media (Fisher) supplemented with human antibody serum. PBMCs were then plated in IP filter plates (Millipore) at a density of $2x10^5$ cells/well with the exception of the positive control wells that are plated at $2.5x10^4$ cells/well. IP plate wells were pre-coated with monoclonal antibody provided in human interferon-γ ELISpot kit (U-Cytech Biosciences). Three peptide pools were used for the AAVrh74 capsid protein (Genemed Synthesis, San Antonio, TX) containing 34-36 peptides, each 18 amino acids long and overlapping by 11 residues. Three peptide pools encompassing the micro-dystrophin transgene (Genemed Synthesis) containing 56 peptides, each 18 amino acids long and overlapping by 11 residues. Concanavalin A (Sigma, 1 μg/mL) served as a positive control and 0.25% dimethylsulfoxide as a negative control. Peptides were added directly to the wells at a final concentration of 1 μg/mL. After the addition of PBMCs and peptides, the plate was incubated at 37°C in 5-7% CO_2 and 100% humidity for 36-48 hours. Following incubation, the plate was developed according to the manufacturer's protocol. Interferon-γ spot formation was counted using Immunospot software (Series 3B Analyzer, Cellular Technology, Ltd, Cleveland, OH).

ELISA

Serum samples were diluted from 1:25 through 1:26,214,400 in blocking solution (5% non-fat dry milk, 1% normal goat serum (Invitrogen) in PBS-/-) and added to wells for 1 hr at room temperature. Sera from previously screened positive and negative human samples were used for assay controls. Goat anti-human HRP IgG-FC conjugated (Bethyl Labs) was used as the secondary antibody at a 1:10,000 dilution for 30 min at room temperature followed by exposure with Ultra TMB-ELISA solution (Thermo Scientific). Absorbance of wells at OD450 was measured and used to calculate titer of positive reaction. The lower limit of detection is 1:25.

Histology

Picrosirius red stain and collagen quantification

Frozen sections placed onto Fisherbrand Superfrost charged microscope slides were fixed in 10% Neutral Buffered Formalin for 5 minutes, then rinsed in distilled water. Slides were then incubated in Solution B (Direct Red 80/2 4 6-Trinitrophenol) from the Picrosirius Red Stain Kit (Polysciences Inc. Mount Arlington, NJ Catalog # 24901) for 15 minutes. After a thorough rinse in distilled water, the slides were placed in Solution C (0.1N hydrochloride acid) for 2 minutes. Slides were counterstained for 5 minutes with 1% Fast Green in 1% Glacial Acetic Acid from Poly Scientific (Bay Shore, NY Catalog #S2114) using a 1:10 dilution in deionized water. Finally, the slides were rinsed again in distilled water, dehydrated in graded ethanol, cleared in xylene, and mounted with coverslips using Cytoseal 60 media from Thermo-Scientific (Catalog #8310). Images were taken using the AxioVision 4.9.1 software. For analysis of Sirius red staining and percent collagen quantification, the contrast between the red and the green colors were

enhanced using Adobe Photoshop. The color deconvolution plugin in the ImageJ software program was selected and the RGB color deconvolution option was used. The red image includes all connective tissue from the Sirius red stain. The green image includes all muscle from the Fast Green counterstain. Only the red image and the original image were used. A threshold was applied to the images to obtain black and white images with areas positive for collagen in black and negative areas in white. Using the measure function, the area of collagen was calculated. The total tissue area was determined by converting the original image to “8-bit” and adjusting the threshold to 254 (one unit below completely saturating the image). The total tissue area was measured as done previously and total area was recorded. The percentage of collagen was calculated by dividing the area of collagen by total tissue area and used to determine the mean percentage for each individual.

Immunohistochemistry/Immunofluorescence

Frozen sections were mounted onto slides (Fisherbrand Superfrost Plus) and incubated in a blocking buffer (1 × TBS, 10% Goat Serum) for 1 hr prior to incubation with primary antibody (monoclonal human Dystrophin 3 [Leica Biosystems, New Castle, UK; Cat. No. NCL-DYS3] at 1:3 or monoclonal human ββ-sarcoglycan [Leica Biosystems, New Castle, UK; Cat. No. NCL-L-b-SARC] at 1:50) for 1 hr at room temperature in a humidity chamber. Sections were washed with a wash buffer (1 X TBS,1% Goat Serum) three times, each for 5 minutes and then incubated with Alexa Fluor 594 conjugated goat anti-mouse secondary IgG antibody (Life Technologies, Grand Island, NY, USA; Cat. No. A11032) at a 1:300 dilution for 1 hr. Sections were

washed with the wash buffer three times for 5 min and mounted with glass coverslips using Vectashield mounting medium (Vector Laboratories, Burlingame, CA, USA).

Image Capture

Non-overlapping 20x or 40x-magnification images of tissue sections from were obtained using the Zeiss Axioskop microscope and Axiovision Rel software. Images are captured at a fixed exposure with no pixel saturation. Positive and negative controls are captured in an identical manner. All images for analysis were saved in .tif format with 8 bits/channel. All annotated images were saved as .jpg. Systematic random sampling method was employed to select microscope fields for image capture. The slide was placed on the microscope with the appropriate objective in place, a random point of entry outside of sample was selected. Repeatedly paced the microscope stage using a series of systematic steps following a raster type pattern of 4 quadrants. Image was captured when the first position at which a full field of tissue was encountered. Using the same step process, moved to the next quadrant point of the section that was encountered and capture image. Repeated until all images are captured. If the initial selected microscope imaging field contained an unacceptable artifact, then the immediate microscope image field above was selected for image capture. If this alternate image also contained an artifact, then the image field immediately below the initially selected image field was selected for image capture. Continued systematic fields left, then right and repeated as needed; 2 microscope fields above and 2 microscope fields below to avoid artifact(s). Artifacts were restricted to conditions that obstruct image interpretation, and were limited to fluorescent or obscuring debris, tissue folds, tissue tears, section edges, extensive areas of fatty or fibrotic tissue that did not

represent muscle fibers, air bubbles created by coverslip mounting, or areas of inconsistent staining.

BIOQUANT Image Analysis

40x images were measured using BIOQUANT software (version 2015). To determine the upper and lower threshold for analysis, the normal and negative control images were used by selecting the stained membrane, including the various shades of intensity within the run. Threshold settings for the data set were obtained from two trained technical analysts independently. The threshold values obtained for red, green, and blue channels were averaged, with the average value for each channel as the consensus threshold for that data set. A sequence of images was analyzed by selecting the field measurement tool. Values for Fluorescence Quantitation (D6) and Red Fluorescence Intensity (D3) BIOQUANT measurement algorithms were recorded. An average value was calculated for expression percentage of normal controls for the data set which was normalized as 100%.

Percent Positive Fiber Analysis

20x images were assessed for the relative number of dystrophin positive fibers to total fibers based on visual examination of the digital images. Dystrophin positive fiber established criteria was defined as positive if the membrane contained approximately 30% or greater additive staining of the fiber perimeter (membrane) above the low intensity levels of the run including controls. The total percentage of the fiber perimeter did not need to be continuous and will be subjectively determined by consciously combining all segments of perimeter positivity to determine a total perimeter intensity percentage. Fibers counted were defined by the structural appearance of the fibers

cross section. To facilitate scoring, NIH ImageJ with the Cell Counter plugin was used to count total fibers. By convention, a “Type #” and color was selected to score/mark positive fibers. The Cell Counter tracked the counts as you selected the fibers. Positive fibers were scored based on the original image exposure- there was no adjustment to the brightness or contrast of any image during the positive image scoring process. Once positive fiber selections were completed, the brightness and contrast of the image was adjusted to visualize the negative fibers more easily. No additional positive fibers were scored once the brightness/contrast was adjusted. The remaining fibers were scored as negative using a different “Type #” and color for the score/mark. Once scoring was complete, “Export Image” was selected from the Cell Count workspace. This image was saved separately as an annotated .jpeg image. Total fiber counts were determined after all fibers were counted.

eReferences

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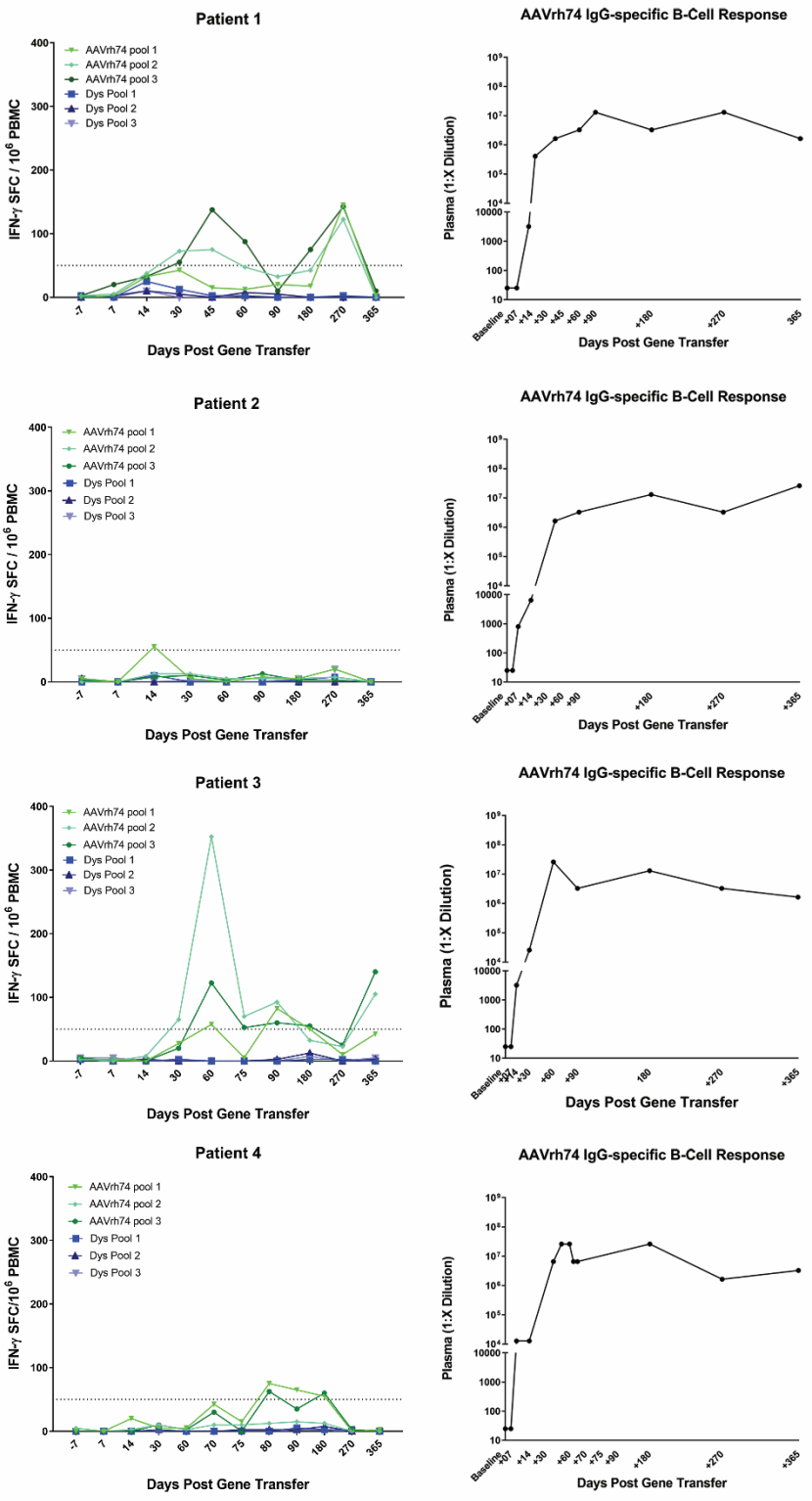
4. Labikova J, Vcelakova J, Ulmannova T, Petruzelkova L, Kolouskova S, Stechova K. The cytokine production of peripheral blood mononuclear cells reflects the autoantibody profile of patients suffering from type 1 diabetes. *Cytokine.* 2014;69(2):189-195.

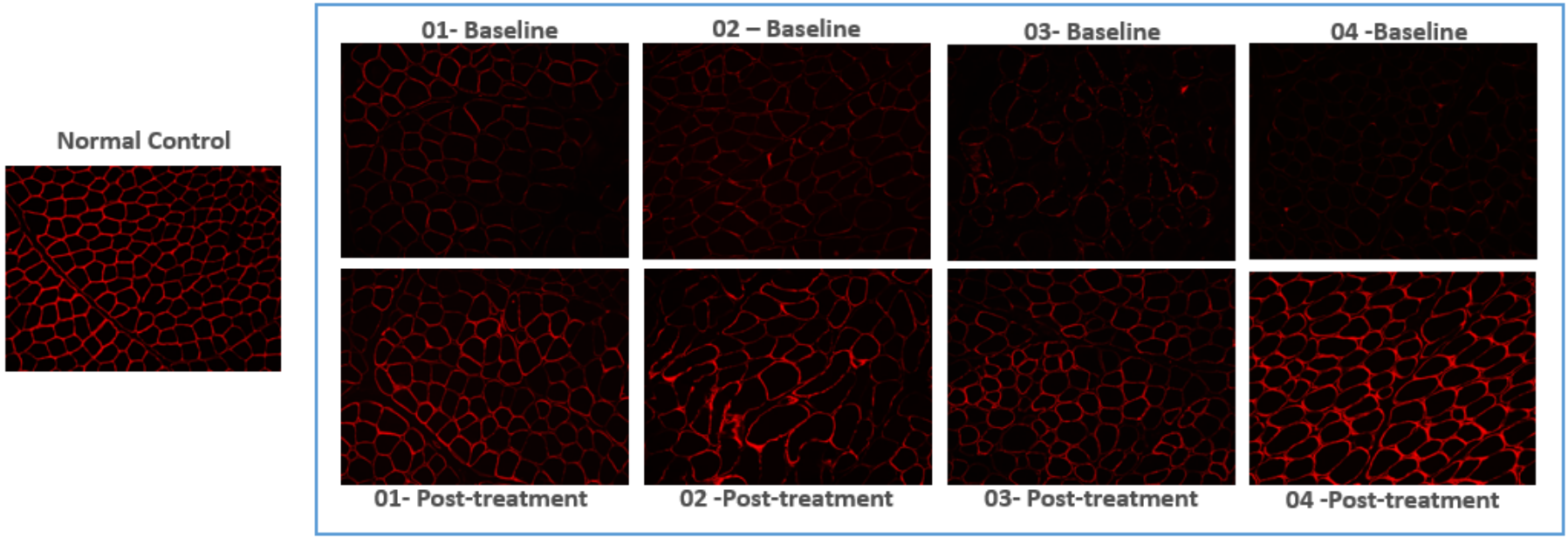
eFigure 1. Immune Responses to the AAVrh74 Capsid Protein and Micro-dystrophin

T-cell responses to 3 different peptide pools of AAVrh74 and micro-dystrophin was assessed from PBMCs throughout the study using an ELISpot assay (left panel). Patients 1 and 3 showed increased IFN-γ responses to different pools of AAV at various timepoints that resolved at subsequent timepoints. The threshold for positive response is 50 spot-forming colonies/10^6 peripheral blood mononuclear cells (PBMCs). B-cell responses to AAV capsid were assessed in serum samples throughout the study using ELISA (right panel). B-cell responses appear to peak for all patients after 30 days and remain stable hereafter.



eFigure 2. Micro-dystrophin Gene Transfer Increases β-Sarcoglycan Expression

Robust increase in expression of β-sarcoglycan in gastrocnemius muscle is shown after gene transfer compared to baseline pre-treatment biopsies. Representative immunofluorescence images of gastrocnemius muscle sections stained for β-sarcoglycan from each patient (01, 02, 03, 04), pre- and post-treatment (top and bottom panels, respectively) compared to normal muscle (far left panel).



eTable 1. Weekend Prednisolone Dose Maintained During Clinical Trial

	Weight (kg)	Actual Dose Received (mg S/S)	Recommended Dose at 5 mg/kg (mg S/S)	Actual Dose Received as a Percentage of Recommended Dose (%)
Patient 1	18.4	84	91.0	92.3
	21.6	84	108.0	77.8
Patient 2	18.9	75	94.5	79.4
	22.4	75	112.0	67.0
Patient 3	21.4	75	103.5	72.5
	24.5	100	122.5	81.6
Patient 4	13.7	62.5	68.5	91.2
	16.1	62.5	80.5	77.6

S/S indicates Saturday/Sunday

For each patient, the first row denotes study entry and the second row denotes the end of the 1-year study period.

eTable 2. Serum Chemistry Profiles

Parameter/ Statistics	Baseline	Day 1	Day 7	Day 14	Day 30	Day 60	Day 90	Day 180	Day 270	Day 365
WBC (K/mm3)										
Mean (SD)	10.7 (3.52)	6.6 (1.59)	4.8 (1.68)	8.6 (1.37)	7.7 (3.28)	8.3 (2.67)	8.6 (0.93)	8.3 (1.25)	9.1 (1.77)	9.3 (3.06)
Hemoglobin (g/dL)										
Mean (SD)	12.7 (0.92)	12.4 (1.14)	13.3 (1.02)	14.0 (0.34)	13.6 (0.41)	13.6 (0.42)	13.1 (0.61)	12.7 (0.88)	12.7 (0.66)	13.2 (0.77)
Hematocrit (%)										
Mean (SD)	38.4 (2.13)	37.6 (2.70)	40.5 (2.42)	41.9 (0.90)	41.2 (0.99)	41.3 (0.90)	38.9 (1.40)	38.4 (1.81)	38.2 (1.34)	39.1 (2.03)
Platelets (K/mm3)										
Mean (SD)	420.3 (62.97)	319.8 (77.84)	232.3 (37.40)	244.5 (87.88)	314.5 (53.31)	321.8 (66.67)	258.8 (59.21)	332.3 (91.09)	364.0 (48.55)	398.5 (71.70)
MCV (FL)										
Mean (SD)	82.9 (2.11)	83.3 (1.90)	82.9 (2.11)	82.0 (1.67)	82.1 (2.51)	82.2 (2.39)	85.0 (3.40)	83.6 (1.40)	82.4 (2.06)	82.4 (2.05)
MCH (pg)										
Mean (SD)	27.4 (1.02)	27.3 (0.90)	27.2 (1.08)	27.3 (0.84)	27.1 (0.94)	27.1 (0.88)	28.5 (1.27)	27.7 (1.20)	27.3 (1.04)	27.8 (1.19)
MCHC (%)										
Mean (SD)	33.0 (0.57)	32.8 (0.65)	32.9 (0.57)	33.3 (0.56)	33.0 (0.60)	33.0 (0.56)	33.6 (0.51)	33.1 (1.03)	33.2 (0.61)	33.8 (0.75)
RDW (%)										
Mean (SD)	13.7 (0.78)	13.8 (0.93)	13.7 (0.95)	13.8 (0.63)	13.9 (0.82)	16.4 (2.88)	17.0 (2.34)	13.2 (1.03)	13.8 (0.90)	13.3 (0.56)
RBC (M/mm3)										
Mean (SD)	4.6 (0.18)	4.5 (0.32)	4.9 (0.28)	5.1 (0.21)	5.0 (0.21)	5.0 (0.22)	4.6 (0.19)	4.6 (0.14)	4.6 (0.08)	4.7 (0.15)
Neutrophils (%)										

Parameter/ Statistics	Baseline	Day 1	Day 7	Day 14	Day 30	Day 60	Day 90	Day 180	Day 270	Day 365
Mean (SD)	42.1 (19.83)	36.0 (10.98)	2.8	47.6 (21.47)	52.4 (11.47)	31.7 (20.90)	42.3 (15.86)	42.4 (7.34)	37.0 (11.28)	48.2 (13.54)
Seg Neutrophils (%)										
Mean (SD)			42.2 (14.37)	42.4 (18.01)	4.8	52.0 (15.70)	79.3			41.2
Lymphocytes (%)										
Mean (SD)	50.5 (19.46)	55.1 (12.08)	28.7 (23.32)	50.4 (10.32)	38.0 (8.38)	39.6 (20.41)	49.2(16.84)	49.0 (9.88)	54.0 (13.51)	44.2 (8.69)
Monocytes (%)										
Mean (SD)	6.7 (2.38)	7.1 (3.12)	18.0 (11.08)	13.3 (9.57)	9.5 (5.19)	11.6 (5.32)	7.9 (2.84)	7.8 (2.36)	8.0 (3.76)	6.9 (4.16)
Eosinophils (%)										
Mean (SD)	0.6 (0.48)	1.7 (0.92)	3.3 (2.49)	0.1 (0.06)	0.4 (0.45)	0.4 (0.13)	0.5 (0.33)	0.5 (0.26)	0.8 (0.33)	1.3 (1.83)
Basophils (%)										
Mean (SD)	0.5 (0.42)	0.2 (0.24)	2.1 (0.10)	0.1 (0.12)	0.2 (0.19)	0.4 (0.26)	0.3 (0.13)	0.3 (0.17)	0.3 (0.10)	0.4 (0.26)
Bands (%)										
Mean (SD)			10.8 (12.45)	2.0		2.9 (1.06)				3.1
MPV (fL)										
Mean (SD)	10.1 (1.32)	9.4 (0.45)	9.9 (0.97)	10.7 (1.02)	9.7 (0.48)	10.1 (1.08)	9.9 (0.60)	9.9 (0.92)	9.8 (0.84)	9.7 (0.87)
TotProtein (mmol/L)										
Mean (SD)	6.8 (0.58)	6.4 (0.26)	7.1 (0.29)	7.2 (0.37)	6.9 (0.29)	7.0 (0.33)	6.3 (0.19)	6.8 (0.56)	6.7 (0.37)	7.0 (0.24)

Parameter/ Statistics	Baseline	Day 1	Day 7	Day 14	Day 30	Day 60	Day 90	Day 180	Day 270	Day 365
GGT (U/L)										
Mean (SD)	10.0 (0.00)	10.0 (0.00)	10.0 (0.00)	11.5 (3.00)	10.8 (0.96)	149.3 (111.91)	62.3 (28.39)	19.3 (11.98)	13.0 (4.76)	11.8 (2.87)
ALT (U/L)										
Mean (SD)	556.0 (197.31)	421.3 (128.72)	493.8 (171.86)	503.3 (156.42)	341.5 (104.34)	1216.5 (879.15)	311.0 (35.66)	302.5 (97.63)	357.8 (132.61)	334.3 (65.38)
AST (U/L)										
Mean (SD)	393.0 (147.80)	213.3 (34.44)	347.3 (155.97)	628.8 (209.45)	235.3 (13.20)	709.0 (565.77)	248.8 (184.45)	169.0 (81.95)	216.8 (99.80)	166.5 (41.01)
TotBil (mg/dL)										
Mean (SD)	0.3 (0.10)	0.4 (0.13)	0.1 (0.12)	0.3 (0.06)	0.3 (0.00)	0.7 (0.31)	0.3 (0.13)	0.2 (0.10)	0.2 (0.06)	0.3 (0.13)
Gluc (mg/dL)										
Mean (SD)	87.3 (8.02)	94.3 (5.68)	94.8 (16.19)	95.0 (16.21)	90.5 (18.59)	85.3 (23.91)	90.5 (13.18)	78.3 (15.52)	87.5 (4.93)	93.3 (10.72)
CaDiox (mmol/L)										
Mean (SD)	24.0 (1.41)	21.5 (0.58)	26.3 (1.71)	23.8 (1.89)	24.3 (0.96)	23.8 (1.50)	23.8 (2.22)	25.5 (1.73)	26.8 (2.06)	26.0 (1.83)
Cl (mmol/L)										
Mean (SD)	106.3 (1.71)	107.8 (2.87)	102.8 (2.06)	104.5 (0.58)	104.8 (1.71)	105.0 (0.82)	106.5 (0.58)	106.3 (0.96)	106.5 (1.29)	106.0 (2.31)
Pot (mmol/L)										
Mean (SD)	3.7 (0.59)	3.9 (0.10)	3.9 (0.38)	4.3 (0.36)	3.8 (0.26)	3.8 (0.50)	3.7 (0.34)	3.6 (0.19)	3.6 (0.38)	3.7 (0.26)
Sodium (mmol/L)										
Mean (SD)	142.8 (1.50)	140.3 (0.96)	140.0 (1.63)	140.0 (2.16)	140.8 (2.06)	141.3 (2.22)	141.0 (1.41)	141.8 (1.50)	141.0 (0.82)	139.5 (1.00)
Creat (mg/dL)										

Parameter/ Statistics	Baseline	Day 1	Day 7	Day 14	Day 30	Day 60	Day 90	Day 180	Day 270	Day 365
Mean (SD)	0.2 (0.03)	0.2 (0.03)	0.2 (0.04)	0.2 (0.02)	0.2 (0.06)	0.2 (0.03)	0.3 (0.09)	0.3 (0.08)	0.2 (0.02)	0.2 (0.09)
BUN (mg/dL)										
Mean (SD)	10.8 (1.50)	11.3 (2.22)	11.0 (2.00)	6.8 (2.36)	9.3 (2.50)	10.3 (3.86)	8.8 (3.30)	9.5 (1.00)	10.3 (2.63)	11.0 (2.58)
Cysc (mg/dL)										
Mean (SD)	0.7 (0.05)	0.6 (0.00)	0.7 (0.00)	0.6 (0.05)	0.7 (0.05)	0.7 (0.10)	0.7 (0.05)	0.8 (0.10)	0.8 (0.13)	0.8 (0.12)
AlkPhos (U/L)										
Mean (SD)	109.8 (22.10)	97.8 (21.65)	114.3 (27.62)	90.5 (21.06)	75.5 (21.06)	179.8 (93.09)	133.8 (23.26)	144.8 (27.58)	142.8 (18.10)	147.8 (28.36)
Amylase (U/L)										
Mean (SD)	66.8 (15.20)	62.0 (13.88)	69.0 (24.01)	82.8 (23.80)	56.3 (4.16)	64.8 (15.63)	71.5 (17.79)	56.5 (10.25)	55.3 (12.20)	58.0 (21.21)
PT (sec)										
Mean (SD)	13.2 (0.63)	13.5 (0.45)	12.8 (0.88)	12.2 (0.76)	12.7 (1.17)	13.4 (1.77)	13.4 (1.34)	14.0 (1.11)	13.6 (1.04)	13.8 (0.64)
PTT (sec)										
Mean (SD)	26.8 (1.71)	26.8 (2.06)	27.8 (1.26)	23.8 (1.26)	24.3 (0.50)	25.5 (1.91)	26.8 (1.26)	27.3 (0.96)	28.0 (1.83)	28.3 (1.26)
INR										
Mean (SD)	1.0 (0.05)	1.1 (0.03)	1.0 (0.10)	0.9 (0.05)	1.0 (0.08)	1.0 (0.17)	1.0 (0.12)	1.1 (0.11)	1.0 (0.10)	1.0 (0.06)

AlkPhos indicates alkaline phosphate; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; CaDiox, calcium dioxide; Cl, chlorine; Creat, serum creatinine; Cysc, cystatin C; GGT, gamma-glutamyl-transferase; Gluc, glucose; INR, international normalized ratio; MCH, mean corpuscular hemoglobin; MCHC, mean corpuscular hemoglobin concentration; MCV, mean corpuscular volume; MPV, mean platelet volume; Pot, potassium; PT, prothrombin time; PTT, partial thromboplastin time; RBC, red blood cell (count); RDW, red (cell) distribution width; Seg, segmented; TotBil, total bilirubin; TotProtein, total protein; WBC, white blood cell (count).

eTable 3. Cardiac Magnetic Resonance Imaging at Baseline and Post Treatment

Patient 1	**Left Vent EF**	**Echo EF**	**Echo Interpretation**	**MRI Evidence of fibrosis**	**Right and left atrial size**	**Right and left ventricle chamber dimensions**
Baseline	64%	65%	Normal	none	Normal	Normal
30 days	N/A	58%	Normal			
6 months	58%	N/A		none	Normal	Normal
1 Year	55%	67%	Normal	none	Normal	Normal
Patient 2						
Baseline	56%	Not recorded	Normal	none	Normal	Normal
30 days	N/A	70%	Normal			
6 months	57%	N/A		none	Normal	Normal
1year	59%	62%	Normal	none	Normal	Normal

Patient 3						
Baseline	62%	60%	Normal	none	Normal	Normal
30 days	N/A	68%	Normal			
6 months	63%	N/A		none	Normal	Normal
1 year	58%	61%	Normal	none	Normal	Normal
Patient 4						
Baseline	56%	57%	Normal	none	Normal	Normal
30 days	N/A	61%	Normal			
6 months	59%	N/A		Mid lateral left ventricular wall	Normal	Normal
1 Year	57%	63%	Normal	none	Normal	Normal

Evidence of fibrosis: based on late gadolinium enhancement imaging; mid lateral wall change on Patient 4 was reviewed by consulting cardiologist and not considered clinically significant.

Left Vent EF indicates left ventricle ejection fraction; MRI, magnetic resonance imaging

eTable 4. Quantitative Summary of Protein Expression by Intensity and Percentage of Dystrophin-Positive Fibers and Transduction by qPCR of Vector Genome

Measurement	Patient 1	Patient 2	Patient 3	Patient 4	Mean (SD)
Immunohistochemistry					
Intensity	82%	59%	83%	160%	96% (44)
Percentage of Dystrophin-Positive Fibers	78%	74%	77%	96%	81% (10)
Vector Genome Number					
Vector Copies/ug DNA	$>10^5$	$>10^5$	$>10^5$	$>10^5$	
Copies per Nucleus	1.7	1.3	1.9	8.1	3.3 (3.2)

eTable 5. Additional Function Outcome Measures Assessed Throughout the Study in Each Patient

Patient 1								
Parameter	**Baseline**	**Day 30**	**Day 60**	**Day 90**	**Day 180**	**Day 270**	**Day 365**	**Change from Baseline at Day 365**
Time to Rise (sec)	3.7	3.6	3	2.8	3.0	3	3.4	-0.3*
4 stair climb (sec)	3.4	2.7	2.5	2.7	2.4	2.3	2.4	-1.0*
100 m (sec)	49.3	52.5	53.1	47.4	46.4	43.8	42.9	-6.4*
HHD (kg)								
Knee extensor (right)	6.2	8.3	7.3	7.6	7.5	4.8	8.2	2.0
Knee extensor (left)	5.0	7.9	7.2	7.3	6.4	5.2	7.6	2.6
Knee flexor (right)	4.1	5.5	4.3	5.2	4.9	2.1	5.1	1.0
Knee flexor (left)	4.2	4.6	3.1	5.3	4.7	3.0	5.2	1.0
Elbow extensor (right)	3.5	3.8	5.0	4.4	5.3	2.6	4.8	1.3
Elbow extensor (left)	3.4	4.3	3.9	3.9	4.7	4.3	3.8	0.4
Elbow flexor (right)	4.2	5.5	5.2	4.4	6.0	4.0	5.8	1.6
Elbow flexor (left)	3.4	4.7	4.8	4.3	5.3	3.4	5.7	2.3
Patient 2								
Parameter	**Baseline**	**Day 30**	**Day 60**	**Day 90**	**Day 180**	**Day 270**	**Day 365**	**Change from Baseline at Day 365**
Time to Rise (sec)	3	3.2	2.8	3.6	3.7	3.3	3.4	0.4*
4 stair climb (sec)	3.8	2.9	2.3	2.7	2.6	2.7	2.6	-1.2*
100 m (sec)	49.9	49.3	47.5	46.9	48.6	50.3	47.4	-2.5*
HHD (kg)								
Knee extensor (right)	8.2	6.2	8.0	9.8	8.7	9.6	7.9	-0.3

Knee extensor (left)	5.9	6.0	9.4	9.0	6.9	7.5	5.3	-0.6
Knee flexor (right)	4.1	2.8	4.1	3.5	5.0	4.8	4.8	0.7
Knee flexor (left)	3.2	3.5	3.7	3.2	4.4	4.4	3.9	0.7
Elbow extensor (right)	3.6	4.6	3.7	3.9	4.3	4.2	4.8	1.2
Elbow extensor (left)	3.7	3.5	4.2	4.3	5.0	4.8	5.1	1.4
Elbow flexor (right)	2.5	3.6	3.5	3.5	3.8	3.3	5.1	2.6
Elbow flexor (left)	3.3	3.5	4.0	3.4	5.3	3.3	5.2	1.9
Patient 3								
Parameter	**Baseline**	**Day 30**	**Day 60**	**Day 90**	**Day 180**	**Day 270**	**Day 365**	**Change from Baseline at Day 365**
Time to Rise (sec)	3.9	2.6	3.2	2.6	3.4	2.8	3.9	0*
4 stair climb (sec)	1.9	1.8	1.8	1.9	1.8	1.9	1.8	-0.1*
100 m (sec)	59.3	49.8	57.3	51.0	48.4	50.7	55.5	-3.8*
HHD (kg)								
Knee extensor (right)	6.3	6.3	5.5	6.4	7.6	6.4	6.8	0.5
Knee extensor (left)	6.6	5.3	5.5	4.8	6.8	5.9	6.8	0.2
Knee flexor (right)	4.6	5.0	4.4	3.0	5.1	5.1	5.1	0.5
Knee flexor (left)	3.6	4.6	4.2	4.2	5.0	4.6	4.3	0.7
Elbow extensor (right)	4.1	4.4	4.2	4.7	4.8	4.5	4.7	0.6
Elbow extensor (left)	5.4	4.9	4.0	4.1	4.6	4.3	5.1	-0.3
Elbow flexor (right)	3.1	4.2	4.5	3.9	5.5	4.5	2.9	-0.2
Elbow flexor (left)	3.7	3.7	3.9	3.6	5.0	5.2	4.1	0.4
Patient 4								
Parameter	**Baseline**	**Day 30**	**Day 60**	**Day 90**	**Day 180**	**Day 270**	**Day 365**	**Change from Baseline at Day 365**
Time to Rise (sec)	4.1	2.9	2.6	2.3	2.2	2.4	2.6	-1.5*
4 stair climb (sec)	4.8	3.0	2.6	2.2	2.3	2.2	2.0	-2.8*

100 m (sec)	67.2	55	52.1	50.7	51.9	49.7	43.6	-23.6*
HHD (kg)								
Knee extensor (right)	6.7	5.8	7.7	9.7	6.7	10.5	12.7	6.0
Knee extensor (left)	6.9	5.7	6.7	9.1	8.7	9.4	11.5	4.6
Knee flexor (right)	2.8	3.1	3.7	4.4	4.4	4.7	4.3	1.5
Knee flexor (left)	3.1	3.5	3.5	4.0	3.6	4.5	4.2	1.1
Elbow extensor (right)	1.8	2.9	3.0	4.3	2.9	3.0	3.7	1.9
Elbow extensor (left)	2.3	2.1	2.7	3.6	4.6	3.7	4.7	2.4
Elbow flexor (right)	1.4	2.2	1.8	2.4	2.1	2.9	3.3	1.9
Elbow flexor (left)	2.3	1.9	1.7	2.4	2.9	3.1	3.4	1.1

HHD indicates hand-held dynamometry.

* Negative numbers indicate improvement as it is the reduction in time required to complete the task.

Table 6. List of Adverse Events by System Organ Class

All adverse events by system organ class	No. of events	Treatment related, No. (%)
GI disorder		
Vomiting	14	9 (50)
Fecal incontinence	1	0
GERD	2	0
Loose stool	1	0
Nausea	1	1 (6)
Abnormal distension	1	0
Infection and infestation		
URI	9	0
Viral illness	2	0
Viral gastroenteritis	1	0
General disorder and administration site condition		
Pain at biopsy site	3	0
Fatigue	2	1 (6)
Asthenia	1	1 (6)
Low-grade fever	1	0
Right foot pain	1	0
Metabolism and nutrition disorder		
Decreased appetite	3	2 (11)
Hypokalemia	1	0
Investigation		
Elevated liver enzymes	4	4 (22)
Respiratory, thoracic, and mediastinal disorder		
Asthma exacerbation	1	0
Cough	1	0
Renal and urinary disorder		
Proteinuria	1	0

Eye disorder		
Eye irritation	1	0
Nervous system disorder		
Headache	1	0
Total	53	18 (34)

Abbreviations: GERD, gastroesophageal reflux disease; GI, gastrointestinal; URI, upper respiratory tract infection.