ADENO-ASSOCIATED VIRUS VECTOR DELIVERY OF B-SARCOGLYCAN AND THE TREATMENT OF MUSCULAR DYSTROPHY

[0001] This application claims priority to U.S Provisional Applciation No. U. S. Provisional Application No. 62/810,917, filed February 26, 2019, U. S. Provisional Application No. 62/834,012, filed April 15, 2019, U. S. Provisional Application No. 62/858,644, filed June 7, 2019, U. S. Provisional Application No. 62/881,901, filed August 1, 2019, U. S. Provisional Application No. 62/909,564, filed October 2, 2019 and U. S. Provisional Application No. 62/910,779, filed October 4, 2019, all of which are incorporated by reference herein in their entirey.

INCORPORATION BY REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[0002] This application contains, as a separate part of the disclosure, a Sequence Listing in computer-readable form which is incorporated by reference in its entirety and identified as follows: Filename: 54016_Seqlisting.txt; Size: 33,466 bytes; Created: February 12, 2020.

FIELD OF THE INVENTION

[0003] Described herein are therapy vectors such as AAV vectors expressing β-sarcoglycan and method of using these vectors to reduce and prevent fibrosis in subjects suffering from a muscular dystrophy.

BACKGROUND

[0004] Limb-girdle muscular dystrophy (LGMD) type 2E (LGMD2E) is an autosomal recessive disorder resulting from mutations in the gene encoding β-sarcoglycan (SGCB), causing loss of functional protein. LGMD2E represents a relatively common and severe form of LGMD in the United States with worldwide reports of incidence of 1/200,000–1/350,000.(2) The absence of β-sarcoglycan leads to a progressive dystrophy with chronic muscle fiber loss, inflammation, fat replacement and fibrosis, all resulting in deteriorating muscle strength and function. (3,4) As a complex, the sarcoglycans (α-, β, γ-, δ-), ranging in size between 35 and 50 kD,(5) are all transmembrane proteins that provide stability to the sarcolemma offering protection from mechanical stress during muscle activity.(3) Loss of 3-sarcoglycan in LGMD2E usually results in varying degrees of concomitant loss of

other sarcoglycan proteins contributing to the fragility of the muscle membrane leading to loss of myofibers.1 Although the range of clinical phenotype of LGMD2E varies, diagnosis typically occurs before age 10 and with loss of ambulation occurring by mid to late teens. Patients present with elevated serum creatine kinase (CK), proximal muscle weakness, difficulty arising from the floor and progressive loss of ambulation. Cardiac involvement occurs in as many as fifty percent of cases

[0005] Adeno-associated virus (AAV) is a replication-deficient parvovirus, the single-stranded DNA genome of which is about 4.7 kb in length including two 145 nucleotide inverted terminal repeat (ITRs). There are multiple serotypes of AAV. The nucleotide sequences of the genomes of the AAV serotypes are known. For example, the complete genome of AAV-1 is provided in GenBank Accession No. NC_002077; the complete genome of AAV-2 is provided in GenBank Accession No. NC_001401 and Srivastava et al., J. Virol., 45: 555-564 {1983); the complete genome of AAV-3 is.provided in GenBank Accession No. NC_1829; the complete genome of AAV-4 is provided in GenBank Accession No. NC_001829; the AAV-5 genome is provided in GenBank Accession No. AF085716; the complete genome of AAV-6 is provided in GenBank Accession No. NC_00 1862; at least portions of AAV-7 and AAV-8 genomes are provided in GenBank Accession Nos. AX753246 and AX753249, respectively; the AAV -9 genome is provided in Gao et al., J. Virol., 78: 6381-6388 (2004); the AAV-10 genome is provided in Mol. Ther., 13(1): 67-76 (2006); and the AAV-11 genome is provided in Virology, 330(2): 375-383 (2004). The sequence of the AAV rh.74 genome is provided in see U.S. Patent 9,434,928, incorporated herein by reference. Cis-acting sequences directing viral DNA replication (rep), encapsidation/packaging and host cell chromosome integration are contained within the AAV ITRs. Three AAV promoters (named p5, p19, and p40 for their relative map locations) drive the expression of the two AAV internal open reading frames encoding rep and cap genes. The two rep promoters (p5 and p19), coupled with the differential splicing of the single AAV intron (at nucleotides 2107 and 2227), result in the production of four rep proteins (rep 78, rep 68, rep 52, and rep 40) from the rep gene. Rep proteins possess multiple enzymatic properties that are ultimately responsible for replicating the viral genome. The cap gene is expressed from the p40 promoter and it encodes the three capsid proteins VP1, VP2, and VP3. Alternative splicing and non-consensus translational start sites are responsible for the production of the three related capsid proteins. A single consensus polyadenylation site is located at map position 95 of

the AAV genome. The life cycle and genetics of AAV are reviewed in Muzyczka, Current Topics in Microbiology and Immunology, 158: 97-129 (1992).

[0006] AAV possesses unique features that make it attractive as a vector for delivering foreign DNA to cells, for example, in gene therapy. AAV infection of cells in culture is noncytopathic, and natural infection of humans and other animals is silent and asymptomatic. Moreover, AAV infects many mammalian cells allowing the possibility of targeting many different tissues in vivo. Moreover, AAV transduces slowly dividing and non-dividing cells, and can persist essentially for the lifetime of those cells as a transcriptionally active nuclear episome (extrachromosomal element). The AAV proviral genome is inserted as cloned DNA in plasmids, which makes construction of recombinant genomes feasible. Furthermore, because the signals directing AAV replication and genome encapsidation are contained within the ITRs of the AAV genome, some or all of the internal approximately 4.3 kb of the genome (encoding replication and structural capsid proteins, rep-cap) may be replaced with foreign DNA. To generate AAV vectors, the rep and cap proteins may be provided in trans. Another significant feature of AAV is that it is an extremely stable and hearty virus. It easily withstands the conditions used to inactivate adenovirus (56° to 65°C for several hours), making cold preservation of AAV less critical. AAV may even be lyophilized. Finally, AAV-infected cells are not resistant to superinfection.

[0007] Multiple studies have demonstrated long-term (> 1.5 years) recombinant AAV-mediated protein expression in muscle. See, Clark et al., Hum Gene Ther, 8: 659-669 (1997); Kessler et al., Proc Nat. Acad Sci USA, 93: 14082-14087 (1996); and Xiao et al., J Virol, 70: 8098-8108 (1996). See also, Chao et al., Mol Ther, 2:619-623 (2000) and Chao et al., Mol Ther, 4:217-222 (2001). Moreover, because muscle is highly vascularized, recombinant AAV transduction has resulted in the appearance of transgene products in the systemic circulation following intramuscular injection as described in Herzog et al., Proc Natl Acad Sci USA, 94: 5804-5809 (1997) and Murphy et al., Proc Natl Acad Sci USA, 94: 13921-13926 (1997). Moreover, Lewis et al., J Virol, 76: 8769-8775 (2002) demonstrated that skeletal myofibers possess the necessary cellular factors for correct antibody glycosylation, folding, and secretion, indicating that muscle is capable of stable expression of secreted protein therapeutics.

[0008] An emerging form of therapy for LGMD2E is viral-mediated gene delivery to restore wild-type protein to affected muscle resulting in restoration of muscle function.

Considering that a subset of patients can develop cardiomyopathy, (8, 9, 10, 13) this would have to be considered in the long-term care of these patients. In previous reports, the Sgcb-null mouse was well characterized. Araishi et al.3 developed the β-sarcoglycan-deficient mouse with accompanying loss of all of the sarcoglycans as well as sarcospan, with at least minor preservation of merosin, the dystroglycans and dystrophin, reproducing the clinical picture seen in LGMD2E. The histological changes in this animal model were also a prototype for the clinical counterpart, including the prominence of skeletal muscle fibrosis.(14) Dressman et al. (25) injected the transverse abdominal muscle using rAAV2.CMV.SGCB. Expression persisted for 21 months and muscle fibers were protected from recurrent necrosis. The use of self-complementary AAV to enhance transgene expression,16 a muscle-specific promoter to better target skeletal muscle (20, 26) and the optimization of a human β-sarcoglycan gene (hSGCB) has also been described.

[0009] Functional improvement in patients suffering from LGMD and other muscular dystrophies require both gene restoration and reduction of fibrosis. There is a need for methods of reducing fibrosis that may be paired with gene restoration methods for more effective treatments of LGMD and other muscular dystrophies.

SUMMARY

[0010] Described herein are gene therapy vectors, e.g. AAV, expressing the β-sarcoglycan gene and methods of delivering β-sarcoglycan to the muscle to reduce and/or prevent fibrosis; and/or to increase muscular force, and/or to treat a mammalian subject suffering from muscular dystrophy.

[0011] In one aspect, described herein is a method of treating muscular dystrophy in a subject in need thereof comprising the step of administering a recombinant adeno-associated virus (rAAV) scAAVrh74.MHCK7.hSGCB to a subject in need thereof, wherein the rAAV is administered using a systemic route of administration and at a dose of about 1.0×10^{12} vg/kg to about 5.0×10^{14} vg/kg based on a supercoiled plasmid as the quantitation standard or 1.0×10^{13} vg/kg to about 1.0×10^{14} vg/kg based on a linearized plasmid as the quantitation standard; wherein the serum creatine kinase (CK) level in the subject is decreased after administration of the rAAV as compared to serum CK level before administration of the rAAV.

[0012] In another aspect, provided is a method of treating muscular dystrophy in a subject in need thereof comprising the step of administering a recombinant adeno-associated virus (rAAV) scAAVrh74.MHCK7.hSGCB, wherein the level of beta-sarcoglycan gene expression in a cell of the subject is increased after administration of the rAAV as compared to the level of beta-sarcoglycan gene expression before administration of the rAAV; wherein the number of beta-sarcoglycan positive fibers in the muscle tissue of the subject is increased after administration of the rAAV as compared to the number of beta-sarcoglycan positive fibers before administration of the rAAV; or wherein motor function is improved in said subject as compared to the motor function of said subject before administration of the rAAV, and wherein the motor function is determined by a 100 meter timed walk test.

[0013] In another aspect, this disclosure provides a method of treating a limb-girdle muscular dystrophy in a subject in need, comprising administering to the subject an rAAV intravenous infusion over approximately 1 to 2 hours at a dose of about 5.0×10^{13} vg/kg or about 2.0×10^{14} vg/kg based on a supercoiled plasmid as the quantitation standard, or about 1.85×10^{13} vg/kg or 7.41×10^{13} vg/kg based on a linearized plasmid as the quantitation standard, and wherein the rAAV comprises a nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19. In antoher aspect, the disclosure describes a method of expressing beta-sarcoglycan gene in a subject's cell comprising administering to the subject the scAAVrh74.MHCK7.hSGCB construct that comprises a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 19. In one aspect, the disclosure provides a method of increasing beta-sarcoglycan positive fibers and/or decreasing CK level in a subject's muscle tissue comprising administering to the subject the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 19.

[0014] In one aspect, described herein is a method of increasing the expression of alpha-sarcoglycan in a subject in need thereof comprising administering to the subject an rAAV comprising a scAAVrh74.MHCK7.hSGCB construct with a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 3 or SEQ ID NO: 19. In another aspect, provided herein is a method of increasing localization of alpha-sarcoglycan to a cell membrane in a subject in need thereof comprising administering to the subject the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 3 or SEQ ID NO: 19. In another aspect, provided is a

method of increasing sarcoglycan expression in muscle tissue or improving muscle function of a subject comprising administering to the subject an rAAV comprising a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 19. In another aspect, the disclosure provides a method of increasing sarcoglycan expression in muscle tissue of a subject comprising administering to the subject a construct comprising a nucleotide sequence encoding a first sarcoglycan, and detecting increased expression of at least a second sarcoglycan in the cell membrane of the cell expressing said first sarcoglycan.

[0015] In another aspect, described is a composition, comprising an rAAV scAAVrh74.MHCK7.hSGCB vector, a buffer agent, an ionic strength agent, and a surfactant. In another aspect, described herein is a pharmaceutical composition comprising a recombinant AAV (rAAV) scAAVrh74.MHCK7.hSGCB, wherein the scAAVrh74.MHCK7.hSGCB comprising a nucleotide sequence that is at least 90%, 95% or 99% identical to SEQ ID NO: 19.

[0016] In another aspect, provided is a method of generating a recombinant AAV scAAVrh74.MHCK7.hSGCB, comprising transferring a plasmid to a cell, wherein the plasmid comprises a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 24. In particular, the plasmid comprises a nucleotide sequence of SEQ ID NO: 24. In another embodiment, the plasmid comprises a nucleotide sequence of SEQ ID NO: 19.

[0017] In another aspect, described here in a recombinant AAV vector comprising a polynucleotide sequence encoding β-sarcoglycan. In some embodiments, the polynucleotide sequence encoding β-sarcoglycan comprises a sequence e.g. at least 65%, at least 70%, at least 75%, at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, or 89%, more typically 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the nucleotide sequence set forth in SEQ ID NO: 1 and encodes protein that retains β-sarcoglycan activity. In some embodiments, the polynucleotide sequence encoding β-sarcoglycan comprises the nucleotide sequence set forth in SEQ ID NO: 1. In some embodiments, the polynucleotide sequence encoding β-sarcoglycan consists of the nucleotide sequence set forth in SEQ ID NO: 1.

[0018] In another aspect, a recombinant AAV vector described herein comprises a polynucleotide sequence encoding β-sarcoglycan that is at least 65%, at least 70%, at least

75%, at least 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, or 89%, more typically at least 90%, 91%, 92%, 93%, or 94% and even more typically at least 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequence of SEQ ID NO: 2, and the protein retains β-sarcoglycan activity.

[0019] In another aspect, described herein is a recombinant AAV vector comprising a polynucleotide sequence encoding functional β-sarcoglycan that comprises a nucleotide sequence that hybridizes under stringent conditions to the nucleic acid sequence of SEQ ID NO: 1, or a complement thereof.

[0020] The term "stringent" is used to refer to conditions that are commonly understood in the art as stringent. Hybridization stringency is principally determined by temperature, ionic strength, and the concentration of denaturing agents such as formamide. Examples of stringent conditions for hybridization and washing are 0.015 M sodium chloride, 0.0015 M sodium citrate at 65-68°C or 0.015 M sodium chloride, 0.0015M sodium citrate, and 50% formamide at 42°C. See Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, (Cold Spring Harbor, N.Y. 1989). More stringent conditions (such as higher temperature, lower ionic strength, higher formamide, or other denaturing agent) may also be used, however, the rate of hybridization will be affected. In instances wherein hybridization of deoxyoligonucleotides is concerned, additional exemplary stringent hybridization conditions include washing in 6x SSC 0.05% sodium pyrophosphate at 37°C (for 14-base oligos), 48°C (for 17-base oligos), 55°C (for 20-base oligos), and 60°C (for 23-base oligos).

[0021] When ranges are used herein for physical properties, such as molecular weight, concentration, or dosage, all combinations and subcombinations of ranges and specific embodiments therein are intended to be included. The term "about" when referring to a number or a numerical range means that the number or numerical range referred to is an approximation within experimental variability (or within statistical experimental error), and thus the number or numerical range may vary from, for example, between 1% and 15% of the stated number or numerical range.

[0022] Other agents may be included in the hybridization and washing buffers for the purpose of reducing non-specific and/or background hybridization. Examples are 0.1% bovine serum albumin, 0.1% polyvinyl-pyrrolidone, 0.1% sodium pyrophosphate, 0.1% sodium dodecylsulfate, $NaDodSO_4$, (SDS), ficoll, Denhardt's solution, sonicated salmon

sperm DNA (or other non-complementary DNA), and dextran sulfate, although other suitable agents can also be used. The concentration and types of these additives can be changed without substantially affecting the stringency of the hybridization conditions. Hybridization experiments are usually carried out at pH 6.8-7.4, however, at typical ionic strength conditions, the rate of hybridization is nearly independent of pH. See Anderson et al., Nucleic Acid Hybridisation: A Practical Approach, Ch. 4, IRL Press Limited (Oxford, England). Hybridization conditions can be adjusted by one skilled in the art in order to accommodate these variables and allow DNAs of different sequence relatedness to form hybrids.

[0023] In another aspect, the recombinant AAV vectors described herein may be operably linked to a muscle-specific control element. For example the muscle-specific control element is human skeletal actin gene element, cardiac actin gene element, myocyte-specific enhancer binding factor MEF, muscle creatine kinase (MCK), tMCK (truncated MCK), myosin heavy chain (MHC), MHCK7 (a hybrid version of MHC and MCK), C5-12 (synthetic promoter), murine creatine kinase enhancer element, skeletal fast-twitch troponin C gene element, slow-twitch cardiac troponin C gene element, the slow-twitch troponin I gene element, hypozia-inducible nuclear factors, steroid-inducible element or glucocorticoid response element (GRE).

[0024] In some embodiments, the muscle-specific promoter is MHCK7 (SEQ ID NO: 4). An exemplary rAAV described herein is pAAV.MHCK7.hSCGB which comprises the nucleotide sequence of SEQ ID NO: 3. Within the nucleotide sequence of SEQ ID NO: 3, the MCHK7 promoter spans nucleotides 130-921, a SV40 chimeric intron (SEQ ID NO: 20) spans nucleotides 931-1078, the β-sarcoglycan sequence (SEQ ID NO: 1) spans nucleotides 1091-2047 and the poly A (SEQ ID NO: 21) spans nucleotides 2054-2106. In some embodiments, the rAAV pAAV.MHCK7.hSCGB comprises a nucleotide sequence of SEQ ID NO: 19. Within the nucleotide sequence of SEQ ID NO: 19, the MCHK7 promoter spans nucleotides 128-919, a SV40 chimeric intron spans nucleotides 929-1076, the β-sarcoglycan sequence spans nucleotides 1086-2042 and the poly A spans nucleotides 2049-2101.

[0025] In some embodiments, the rAAV pAAV.MHCK7.hSCGB comprises a nucleotide sequence that is at least 65%, at least 70%, at least 75%, at least 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, or about 89%, more typically about 90%, about 91%, about 92%, about 93%, about 94%,

about 95%, about 96%, about 97%, about 98%, or about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 3 or SEQ ID NO: 19, or a nucleotide sequence that encodes a polypeptide that is at least 65%, at least 70%, at least 75%, at least 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, or about 89%, more typically about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to SEQ ID NO: 2.

[0026] In one embodiment, the polynucleotide sequence encodes a protein that retains sarcoglycan activity, including beta- and/or alpha-sarcoglycan activity. In another embodiment, the polynucleotide sequence encodes a protein that retains beta-sarcoglycan activity.

[0027] In some embodiments, the muscle-specific promoter is tMCK (SEQ ID NO: 6). An exemplary rAAV described herein is pAAV.tMCK.hSCGB which comprises the nucleotide sequence of SEQ ID NO: 5. Within the nucleotide sequence of SEQ ID NO: 5, the tMCK promoter spans nucleotides 141-854, an SV40 chimeric intron spans nucleotides 886-1018, the β-sarcoglycan sequence spans nucleotides 1058-2014 and the poly A spans nucleotides 2021-2073. In some embodiments, the polynucleotide sequence encoding a pAAV.tMCK.hSCGB comprises a sequence e.g. at least 65%, at least 70%, at least 75%, at least 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, or about 89%, more typically about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% or more identical to the nucleotide sequence set forth in SEQ ID NO: 5, wherein the polynucleotide sequence encodes a protein that retains sarcoglycan activity, including but not limited to, beta- and/or alpha-sarcoglycan activity.

[0028] The AAV can be any serotype, for example AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV-10, AAV-11, AAV-12, AAV-13 and AAVrh.74. Production of pseudotyped rAAV is disclosed in, for example, WO 01/83692. Other types of rAAV variants, for example rAAV with capsid mutations, are also contemplated. See, for example, Marsic et al., Molecular Therapy, 22(11): 1900-1909 (2014).

[0029] Compositions comprising any of the rAAV vectors described herein are also contemplated.

[0030] In some embodiments, the disclosure provides a composition or pharmaceutical composition that comprises an scAAVrh74.MHCK7.hSCGB rAAV vector comprising a nucleotide sequence that is at least 65%, at least 70%, at least 75%, at least 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, or about 89%, more typically about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to the nucleotide sequence set forth in SEQ ID NO: 3 or SEQ ID NO: 19, or comprising a nucleotide sequence that encodes a polypeptide that is at least 65%, at least 70%, at least 75%, at least 80%, about 81%, about 82%, about 83%, about 84%, about 85%, about 86%, about 87%, about 88%, or about 89%, more typically about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, or about 99% identical to SEQ ID NO: 2. In addition, the disclosure provides a provides a composition or pharmaceutical composition that comprises an scAAVrh74.MHCK7.hSCGB rAAV vector comprising a nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19, or comprising a nucleotide sequence that encodes a polypeptide comprising the amino acid sequence of SEQ ID NO: 2.

[0031] Provided are methods of treating muscular dystrophy in a subject in need thereof comprising the step of administering a recombinant adeno-associated virus (rAAV) scAAVrh74.MHCK7.hSGCB, wherein the rAAV is administered using a systemic route of administration and at a dose of about 1.0×10^{12} vg/kg to about 5.0×10^{14} vg/kg.

[0032] Also provided are composition for treating muscular dystrophy, wherein the composition comprises a recombinant adeno-associated virus (rAAV) scAAVrh74.MHCK7.hSGC at a dose of about 1.0×10^{12} vg/kg to about 5.0×10^{14} vg/kg and the composition is formulated for systemic administration.

[0033] In addition, provided are uses of a recombinant adeno-associated virus (rAAV) scAAVrh74.MHCK7.hSGC for the preparation of a medicament for treating muscular dystrophy, wherein the medicament comprises scAAVrh74.MHCK7.hSGC at a dose of about 1.0×10^{12} vg/kg to about 5.0×10^{14} vg/kg and the medicament is formulated for systemic administration.

[0034] In any of the provided methods, compositions and uses, the level of beta-sarcoglycan gene expression in a cell of the subject is increased after administration of the rAAV as compared to the level of beta-sarcoglycan gene expression before administration

of the rAAV; wherein the serum creatine kinase (CK) level in the subject is decreased after administration of the rAAV as compared to serum CK level before administration of the rAAV; and/or wherein the number of beta-sarcoglycan positive fibers in the muscle tissue of the subject is increased after administration of the rAAV as compared to the number of beta-sarcoglycan positive fibers before administration of the rAAV.

[0035] In another embodiment, in any of the provided methods, compositions and uses, motor function is improved in said subject as compared to the motor function of said subject before administration of the rAAV, and wherein the motor function is determined by a 100 meter timed walk test. For example, motor function is improved by at least 5% in 1 month or thirty days post-gene transfer, at least 10 % in 2 months or sixty days post-gene transfer, or at least 15% in 3 months or ninety days post gene transfer. In some embodiments, the motor function is improved by at least 5%, 10%, 15%, 20%, 25%, 30%, 40%, 45%, or 50%.

[0036] For example, in any of the provided methods, compositions and uses, the systemic route of administration is an intravenous route. For example, the rAAV is admiministered using an intravenous route and the dose of the rAAV administered is about 1.85x 10^{13} vg/kg or about 7.41 x10^{13} vg/kg based on a linearized plasmid as the quantification standard or the dose of the rAAV administered is about 5 x 10^{13} vg/kg or about 2 x 10^{14} vg/kg based on a supercoiled plasmid as the quantification standard.

[0037] In some embodiments, the dose of rAAV administered using an intravenous route and the dose is about about 1.0x10^{13} vg/kg to about 5x10^{14} based on a supercoiled plasmid as the quantitation standard or about 1.0 x 10^{13} vg/kg to about 1.0 x 10^{14} vg/kg based on a linearized plasmid as the quantitation standard.

[0038] In addition, the dose of the rAAV administered is about 1.5 x 10^{13} vg to about 2 x 10^{16} vg, or 1.5 x 10^{13} vg to 1 x 10^{16} vg, or about 1.5 x 10^{13} vg to about 2 x 10^{15} vg, or about 1.5 x 10^{13} vg to about 1 x10^{15} vg. In addition, in any of the methods, compositions and uses, the dose of rAAV is administered at a concentration of about 10 mL/kg. In any of the methods, compositions or uses provided, the muscular dystrophy is limb-girdle muscular dystrophy.

[0039] In addition, provided are methods of treating muscular dystrophy in a subject in need thereof comprising the step of administering a recombinant adeno- associated virus (rAAV) scAAVrh74.MHCK7.hSGCB, wherein the rAAV is administered using a

systemic route of administration and at a dose of about 1.0 x 10^{12} vg/kg to about 5.0 x 10^{14} vg/kg; wherein the level of beta-sarcoglycan gene expression in a cell of the subject is increased after administration of the rAAV as compared to the level of beta-sarcoglycan gene expression before administration of the rAAV; wherein the serum CK level in the subject is decreased after administration of the rAAV as compared to serum CK level before administration of the rAAV; or wherein the number of beta-sarcoglycan positive fibers in the muscle tissue of the subject is increased after administration of the rAAV as compared to the number of beta-sarcoglycan positive fibers before administration of the rAAV. For example, in any of the provided methods, the systemic route of administration is an intravenous route and the dose of the rAAV administered is about 5.0 x 10^{13} vg/kg based on a supercoiled plasmid as the quantitation standard. In another embodiment, the dose of the rAAV administered is about 2.0 x 10^{14} vg/kg based on a supercoiled plasmid as the quantitation standard. In another embodiment, the dose of the rAAV administered is about 7.41 x10^{13} vg/kg based on a linearized plasmid as the quantitation standard. In another embodiment, the dose of rAAV administered is about 1.85x10^{13} vg/kg based on a linearized plasmid as the quantitated standard. In addition, the dose of the rAAV administered is about 1.5 x 10^{13} vg to about 2 x 10^{16} vg, or 1.5 x 10^{13} vg to 1 x 10^{16} vg, or about 1.5 x 10^{13} vg to about 2 x 10^{15} vg, or about 1.5 x 10^{13} vg to about 1 x10^{15} vg. In addition, in any of the methods, the dose of rAAV is administered at a concentration of about 10 mL/kg. In any of the methods provided, the muscular dystrophy is limb-girdle muscular dystrophy.

[0040] In some embodiments, the disclosure includes a method of treating muscular dystrophy in a subject in need thereof comprising the step of administering a recombinant adeno- associated virus (rAAV) scAAVrh74.MHCK7.hSGCB, wherein motor function is demonstrably improved in said subject as compared to motor function of said subject before administration of the rAAV, and wherein motor function is determined by a 100m timed walk test. In some aspects, motor function is improved by at least 5% in 1 month or thirty days post-gene transfer, at least 10% in 2 months or sixty days post-gene transfer, or at least 15% in 3 months or ninety days post gene transfer. In some aspects, motor function is improved by at least about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 40%, about 45%, or about 50%.

[0041] Provided are methods of increasing the level of alpha-sarcoglycan in a subject in need thereof comprising administering to the subject the scAAVrh74.MHCK7.hSGCB

construct that comprises a nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19. In addition, provided are composition for increasing the level of alpha-sarcoglycan in a subject in need, wherein the composition comprises scAAVrh74.MHCK7.hSGCB construct comprising a nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19. Also provides are uses of scAAVrh74.MHCK7.hSGCB construct that comprises a nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19 for the preparation of a medicament for increasing the level of alpha-sarcoglycan in a subject in need thereof. In some aspects, the alpha-sarcoglycan is colocalized to the membrane of a cell expressing a beta-sarcoglycan encoded by scAAVrh74.MHCK7.hSGCB.

[0042] In some embodiments, the scAAVrh74.MHCK7.hSGCB construct comprises an intron sequence. In one embodiment, the intron sequence comprise a nucleotide sequence of SEQ ID NO: 20. In another embodiment, the scAAVrh74.MHCK7.hSGCB construct comprises a polyA sequence. In one embodiment, the poly A sequence comprises a nucleotide sequence of SEQ ID NO: 21. In another embodiment, the scAAVrh74.MHCK7.hSGCB construct comprises a 5' inverted terminal repeat (ITR) sequence. In one embodiment, the 5'ITR sequence comprises a nucleotide sequence of SEQ ID NO: 22. In another embodiment, the scAAVrh74.MHCK7.hSGCB construct comprises a 3' inverted terminal repeat (ITR) sequence. In one embodiment, the 3'ITR sequence comprises a nucleotide sequence of SEQ ID NO: 23.

[0043] Also provided are methods of increasing sarcoglycan expression in muscle tissue of a subject comprising administering to the subject a construct comprising a nucleotide sequence encoding a first sarcoglycan, and detecting increased expression of at least a second sarcoglycan in the cell membrane of the cell expressing said first sarcoglycan. In some aspects, the first sarcoglycan is β-sarcoglycan (SGCB), and said second sarcoglycan is α-sarcoglycan (SGCA), γ-sarcoglycan (SGCG), or δ-sarcoglycan (SGCD).

[0044] In any of the methods, useses and compositions of treating muscular dystrophy provided, the subject is 4-15 years of age, has confirmed beta-sarcoglycan (SGCB) mutation in both alleles, was negative for AAVrh74 antibodies and/or had >40% or normal 100 meter walk test. In any of the methods, uses and compositions of treating muscular dystrophy provided, the subject is a pediatric subject. In some embodiments, the subject is a pediatric subject, such as a subject ranging in age from 1 to 10 years. In some embodiments, the subject is 4 to 15 years of age. The subject, in on embodiment, is an adolescent subject, such as a subject ranging in age from 10 to 19 years. In addition, the

subject, in on embodiment, is a young adult subject such as a subject ranging in age from late teens or early twenties, such as the subject may range in age from 15 to 29 years of age. In some embodiment, the subject is a middle-aged adult or an elderly subject, such that the middle-aged adult may range in age from 25-55 years of age and the elderly subject may range in age over 50 years of age.

[0045] In some embodiments, the rAAV is administered by injection, infusion or implantation. For example, the rAAV is administered by infusion over approximately 1 to 2 hours. In addition, the rAAV is administered by an intravenous route through a peripheral limb vein.

[0046] In the methods of treating muscular dystrophy in a subject in need thereof comprising the step of administering a recombinant adeno-associated virus (rAAV) scAAVrh74.MHCK7.hSGCB, wherein the rAAV is administered using a systemic route of administration and at a dose of about 1.0×10^{12} vg/kg to about 5.0×10^{14} vg/kg based on a supercoiled plasmid as the quantitation standard and the rAAV comprises the human β-sarcoglycan nucleotide sequence of SEQ ID NO: 1. In addition, the rAAV comprises the MHCK7 promoter sequence of SEQ ID NO: 4. In some embodiments, the rAAV is of the serotype AAVrh.74. In addition, the rAAV comprises the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19.

[0047] In another embodiment, the scAAVrh74.MHCK7.hSGCB construct comprises an intron sequence. In one embodiment, the intron sequence comprise a nucleotide sequence of SEQ ID NO: 20. In another embodiment, the scAAVrh74.MHCK7.hSGCB construct comprises a polyA sequence. In one embodiment, the poly A sequence comprises a nucleotide sequence of SEQ ID NO: 21. In another embodiment, the scAAVrh74.MHCK7.hSGCB construct comprises a 5' inverted terminal repeat (ITR) sequence. In one embodiment, the 5'ITR sequence comprises a nucleotide sequence of SEQ ID NO: 22. In another embodiment, the scAAVrh74.MHCK7.hSGCB construct comprises a 3' inverted terminal repeat (ITR) sequence. In one embodiment, the 3'ITR sequence comprises a nucleotide sequence of SEQ ID NO: 23.

[0048] In an exemplary embodiment, methods of treating muscular dystrophy in a subject in need thereof comprise the step of administering a recombinant adeno- associated virus (rAAV) scAAVrh74.MHCK7.hSGCB, wherein the rAAV is administered using a

systemic route of administration and at a dose of about 1.0 x 10^{12} vg/kg to about 5.0 x 10^{14} vg/kg, wherein the subject is suffering from limb-girdle muscular dystrophy, and the rAAV is administered by intravenous infusion over approximately 1 to 2 hours at a dose of about 5.0 x 10^{13} vg/kg or about 2.0 x 10^{14} vg/kg based on a supercoiled plasmid as the quantitation standard, or about 1.85 x 10^{13} vg/kg or 7.41 x 10^{13} vg/kg based on a linearized plasmid as the quantitation standard, and wherein the rAAV comprises the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19.

[0049] In addition, the disclosure provides a composition for treating limb-girdle muscular dystrophy comprising a dose of recombinant adeno-associated virus (rAAV) scAAVrh74.MHCK7.hSGCB, wherein the rAAV comprises the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19, and the composition is formulated to deliver a dose of about 5.0 x 10^{13} vg/kg or about 2.0 x 10^{14} vg/kg based on a supercoiled plasmid as the quantitation standard, or about 1.85 x 10^{13} vg/kg or about 7.41 x 10^{13} vg/kg based on a linearized plasmid as the quantitation standard by intravenous infusion over approximately 1 to 2 hours.

[0050] The disclosure also provides for use of a dose of recombinant adeno- associated virus (rAAV) scAAVrh74.MHCK7.hSGCB for the preparation of a medicament for the treatment of limb-girdle muscular dystrophy, wherein the dose of rAAV about 5.0 x 10^{13} vg/kg or about 2.0 x 10^{14} vg/kg based on a supercoiled plasmid as the quantitation standard, or about 1.85 x 10^{13} vg/kg or about 7.41 x 10^{13} vg/kg based on a linearized plasmid as the quantitation standard and the medicament is formulated to deliver the dose by intravenous infusion over approximately 1 to 2 hours.

[0051] The disclosure further provides a method of improving muscle function of a subject comprising administering to the subject a construct comprising a nucleotide sequence with at least 90% identity, at least 95% identity, at least 99% identity, or 100% identity to SEQ ID NO: 1, 3, 5, or 19. In addition, provided are composition for improving muscle function of a subject, wherien the composition comprises a construct comprising a nucleotide sequence with at least 90% identity, at least 95% identity, at least 99% identity or 100% identity to SEQ ID NO: 1, 3, 5, or 19. Also provides are uses of a construct comprising a nucleotide sequence with at least 90% identity, at least 95% identity, at least 99% identity or 100% identity to SEQ ID NO: 1, 3, 5, or 19 for the preparation of a medicament for improving muscle function of a subject.

[0052] In any of the provided methodsm uses or compositions, the subject suffers from a genetic mutation in a gene encoding a sarcoglycan or a muscular dystrophy. In some aspects, the sarcoglycan is β-sarcoglycan (SGCB), α-sarcoglycan (SGCA), γ-sarcoglycan (SGCG), or δ-sarcoglycan (SGCD). In some aspects, the sarcoglycan is β-sarcoglycan or α−sarcoglycan.

[0053] In any of the provided methods, uses or compositions, the level of beta-sarcoglycan gene expression in a cell of the subject is increased after administration of the rAAV as compared to the level of beta-sarcoglycan gene expression before administration of the rAAV.

[0054] In addition, in any of the provided methods, uses or compositions, the expression of the beta-sarcoglycan gene in the cell is detected by measuring the beta-sarcoglycan protein level on a Western blot or immunohistochemistry in muscle biopsied before and after administration of the rAAV.

[0055] In any of the provided methods, uses or compositions, the level of beta-sarcoglycan protein is increased by at least 25%, or at least 26%, or at least 27%, or at least 28%, or at least 29%, or at least 30%, or at least 31%, or at least 32%, or at least 33%, or at least 34%, or at least or 35% or at least 36%, or at least 37%, or at least 38%, or at least 39%, or at least 40%, or at least 41%, or at least 42%, or at least 43%, or at least 44%, or at least 45% or at least 46%, or at least 47%, or at least 48%, or at least 49%, or at least 50%, or at least 51%, or at least 52%, or at least 53%, or at least 54%, or at least 55% or at least 56%, or at least 57%, or at least 58%, or at least 59%, or at least 60%, or at least 63%, or at least 65%, or at least 70%, or at least 75%, or at least 80%, or at least 85%, or at least 90% or at least 95%, or at least 98% after administration of rAAV. For example, the level of the level of beta-sarcoglycan protein is increased by at least 33% as detected by measuring the beta-sarcoglycan protein level on a Western blot in muscle biopsied before and after administration of the rAAV, or the level of beta-sarcoglycan protein is increased by at least 38% or at least 39% as detected by measuring the beta-sarcoglycan protein level by immunohistochemistry in muscle biopsies before and after administration of the rAAV

[0056] In any of the methods, uses or compositions provided herein, the serum CK level in the subject is decreased after administration of the rAAV as compared to serum CK level before administration of the rAAV. For example, the serum level CK level in the

subject is decreased by at least 50%, or at least 51%, or at least 52%, or at least 53%, or at least 54%, or at least 55% or at least 56%, or at least 57%, or at least 58%, or at least 59%, or at least 60%, or at least 63%, or at least 65%, or at least 70%, or at least 75%, or at least 80%, or at least 81%, or at least 82%, or at least 83%, or at least 84%, or at least 85%, or at least 86 %or at least 87%, or at least 88%, or at least 89%, or at least 90% or at least 95%, or at least 98% by 60 to 90 days or 60 days or 90 days after administration of rAAV as compared to the serum CK level before administration of the rAAV.

[0057] In any of the methods, uses or compositions provided herein, the number of beta-sarcoglycan positive fibers in the muscle tissue of the subject is increased after administration of the rAAV as compared to the number of beta-sarcoglycan positive fibers before administration of the rAAV. For example, the number of beta-sarcoglycan positive fibers is detected by measuring the beta-sarcoglycan protein level by Western blot or immunohistochemistry on muscle biopsies before and after administration of the rAAV. For example, the number of beta-sarcoglycan positive fibers in the muscle tissue of the subject is increased by at least 25%, or at least 26%, or at least 27%, or at least 28%, or at least 29%, or at least 30%, or at least 31%, or at least 32%, or at least 33%, or at least 34%, or at least 35% or at least 36%, or at least 37%, or at least 38%, or at least 39%, or at least 40%, or at least 41%, or at least 42%, or at least 43%, or at least 44%, or at least 45% or at least 46%, or at least 47%, or at least 48%, or at least 49%, or at least 50%, or at least 51%, or at least 52%, or at least 53%, or at least 54%, or at least 55% or at least 56%, or at least 57%, or at least 58%, or at least 59%, or at least 60%, or at least 63%, or at least 65%, or at least 70%, or at least 75%, or at least 80%, or at least 85%, or at least 90% or at least 95%, or at least 98% after administration of rAAV.

[0058] In any of the methods, compositions and uses provided herein, the level of alpha-sarcoglycan in the subject is increased after administration of the rAAV as compared to the level of alpha-sarcoglycan before administration of the rAAV. The level of alpha-sarcoglycan is detected by measuring the alpha-sarcoglycan protein level by immunohistochemistry or Western blot on muscle biopsies before and after administration of the rAAV.

[0059] Another embodiment provides for methods expressing beta-sarcoglycan gene in a cell comprising administering to the subjects the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 3 or SEQ ID NO: 19 or comprising the nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19.

[0060] Also provided are compositions for expressing beta-sarcoglycan gene in a cell, wherein the composition comprises the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 3 or SEQ ID NO: 19 or comprising the nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19.

[0061] The disclosure also provides for uses of the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence for the preparation of a medicament for the expressing beta-sarcoglycan gene in a cell, wherein the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence is at least 90%, 95%, or 99% identical to SEQ ID NO: 3 or SEQ ID NO: 19 or comprising the nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19.

[0062] In any of the provided methods, uses or compositions for expressing beta-sarcoglycan gene in a cell, expression of the beta-sarcoglycan gene in the cell is detected by measuring the beta-sarcoglycan protein level on a Western blot or immunohistochemistry in muscle biopsies before and after administration of the scAAVrh74.MHCK7.hSGCB construct. For example, the cell has more than one AAV viral copy number. In addition, the beta-sarcoglycan gene is measured in the subject by detecting greater than 1 rAAV vector genome copy per nucleus.

[0063] Also provided are compositions for decreasing serum CK levels in a subject in need thereof, wherein the composition comprises the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 3 or SEQ ID NO: 19 or comprising the nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19.

[0064] The disclosure also provides for uses of the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence for the preparation of a medicament for decreasing serum CK levels in a subject in need thereof, wherein the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence is at least 90%, 95%, or 99% identical to SEQ ID NO: 3 or SEQ ID NO: 19 or comprising the nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19.

[0065] In any of these methods, uses, and compositions, the serum CK level in the subject is decreased by at least 82% by 60 days after administration of the rAAV as compared to the serum CK level before administration of the rAAV.

[0066] Methods of increasing beta-sarcoglycan positive fibers in a muscle tissue of a subject comprising administering to the subject the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 3

or SEQ ID NO: 19 or comprising the nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19 are provided.

[0067] Also provided are compositions for increasing beta-sarcoglycan positive fibers in muscle tissue of a subject, wherein the composition comprises the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 3 or SEQ ID NO: 19 or comprising the nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19.

[0068] The disclosure also provides for uses of the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence for the preparation of a medicament for increasing beta-sarcoglycan positive fibers in muscle tissue of a subject, wherein the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence is at least 90%, 95%, or 99% identical to SEQ ID NO: 3 or SEQ ID NO: 19 or comprising the nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19.

[0069] In any of these methods, uses, and compositions, the number of beta-sarcoglycan positive fibers is detected by measuring the beta-sarcoglycan protein level by Western blot or immunohistochemistry on muscle biopsies before and after administration of the rAAV. In addition, in any of the methods, uses and compositions, the number of beta-sarcoglycan positive fibers is measured by detecting greater than 1 rAAV vector genome copy per nucleus.

[0070] Another embodiment provides for methods of increasing the expression of alpha-sarcoglycan in a subject in need thereof comprising administering to the subject the scAAVrh74.MHCK7.hSGCB construct that comprises a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 3 or SEQ ID NO: 19 or comprising the nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19.

[0071] Also provided are compositions for increasing the expression of alpha-sarcoglycan in a subject in need thereof, wherein the composition comprises the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 3 or SEQ ID NO: 19 or comprising the nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19.

[0072] The disclosure also provides for uses of the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence for the preparation of a medicament for increasing the

expression of alpha-sarcoglycan in a subject, wherein the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence is at least 90%, 95%, or 99% identical to SEQ ID NO: 3 or SEQ ID NO: 19 or comprising the nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19.

[0073] Also provided are methods of increasing localization of alpha-sarcoglycan to a cell membrane in a subject in need thereof comprising administering to the subject the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 3 or SEQ ID NO: 19 or comprising the nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19.

[0074] Also provided are compositions for increasing localization of alpha-sarcoglycan to a cell membrane in a subject in need thereof, wherein the composition comprises the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 3 or SEQ ID NO: 19 or comprising the nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19.

[0075] The disclosure also provides for uses of the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence for the preparation of a medicament for increasing localization of alpha-sarcoglycan to a cell membrane in a subject in need thereof, wherein the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence is at least 90%, 95%, or 99% identical to SEQ ID NO: 3 or SEQ ID NO: 19 or comprising the nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19.

[0076] In any of these methods, uses and compositions the level of alpha-sarcoglycan is detected by measuring the alpha-sarcoglycan protein level by Western blot or immunohistochemistry on muscle biopsies before and after administration of the rAAV. In addition, in any of the provided methods, uses and compositions, alpha-sarcoglycan is colocalized to the membrane of a cell expressing a beta-sarcoglycan encoded by scAAVrh74.MHCK7.hSGCB.

[0077] Another embodiment provides for methods of increasing sarcoglycan expression in muscle tissue of a subject in need thereof, comprising administering to the subject the scAAVrh74.MHCK7.hSGCB construct that comprises a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 or

SEQ ID NO: 19 or comprising the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 19.

[0078] Also provided are compositions for increasing the expression of sarcoglycan expression in muscle tissue of a subject in need thereof, wherein the composition comprises the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 19 or comprising the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 19.

[0079] The disclosure also provides for uses of the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence for the preparation of a medicament for increasing sarcoglycan expression in muscle tissue of a subject in need thereof, wherein the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence is at least 90%, 95%, or 99% identical to SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 19 or comprising the nucleotide sequence of SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 5 or SEQ ID NO: 19.

[0080] In any of these methods, uses and compositions for increasing sarcoglycan expression in muscle tisse, the subject suffers from a genetic mutation in a gene encoding a sarcoglycan or a muscular dystrophy. For example in any of these methods, usese or compositions, the sarcoglycan is β-sarcoglycan (SGCB), α-sarcoglycan (SGCA), γ-sarcoglycan (SGCG), or δ-sarcoglycan (SGCD).

[0081] Methods of producing a recombinant AAV vector particle comprising culturing a cell that is transferred with a plasmid described herein and recovering recombinant AAV particles from the supernatant of the transfected cells are also provided. Viral particles comprising any of the recombinant AAV vectors described herein are also contemplated. In one embodiment, the method of generating the rAAV comprising transferring an AAV vector plasmid to a host cell. In another embodiment, the plasmid comprises a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 24. In another aspect, the disclosure provides a cell that comprising an AAV vector plasmid that comprises a nucleotide sequence of SEQ ID NO: 24. The cell described herein comprises an insect cell, e.g., a *Drosophila* cell (e.g., an S2 cell or Kc cell), a silkworm cell (e.g., a Bme21 cell), or a mosquito cell (e.g., a C6/36 cell);or a mammalian cell (preferably a human cell,

e.g., a human primary cell or an established cell line). In one embodiment, the mammalian cell comprises a 293 cell, a COS cell, a HeLa cells, or a KB cell.

[0082] In another embodiment, the plasmid comprises a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 1, 3, 5, or 19. In some embodiments, the the vector plasmid comprises a nucleotide sequence of any one of SEQ ID NO: 1, 3, 5, or 19. In some embodiments, the AAV vector plasmid is stably expressed in the host cell. The host cell stably harboring the AAV vector plasmid can be used to generate rAAV. In one embodiment, the AAV vector plasmid is a pAAV.MHCK7.hSGCB. KAN plasmid.

[0083] The method of producing recombinant AAV vector particles provided herein may futher comprise a step of transferring a packaging plasmid and/or a helper virus to the host cell. For example, the methods further comprise a step wherein the packaging cell comprises a stably integrated AAV cap gene and/or wherein the packaging cell comprises a stably integrated AAV rep gene. The invention also provides for a cell comprising a plasmid that comprises a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 24 or an plasmid that comprises a nucleotide sequence of SEQ ID NO: 24. Also provides is a cell comprising a nucleotide sequence of SEQ ID NO: 1, 3, 5, or 19.

[0084] Methods of reducing fibrosis in a mammalian subject in need thereof is also provided. In this regard, the method comprises administering a therapeutically effective amount of an AAV vector described herein (or composition comprising an AAV vector described herein) to the mammalian subject. In some embodiments, the mammalian subject suffers from muscular dystrophy. In some embodiments, administration of an AAV vector described herein (or composition comprising an AAV vector described herein) reduces fibrosis in skeletal muscle or in cardiac muscle of the subject.

[0085] The term “muscular dystrophy” as used herein refers to a disorder in which strength and muscle bulk gradually decline. Non-limiting examples of muscular dystrophy diseases may include Becker muscular dystrophy, tibial muscular dystrophy, Duchenne muscular dystrophy, Emery-Dreifuss muscular dystrophy, facioscapulohumeral muscular dystrophy, sarcoglycanopathies, congenital muscular dystrophy such as congenital muscular dystrophy due to partial LAMA2 deficiency, merosin-deficient congenital muscular dystrophy, type 1D congenital muscular dystrophy, Fukuyama congenital muscular dystrophy, limb-girdle type 1A muscular dystrophy, limb-girdle type 2A muscular dystrophy, limb-girdle type 2B muscular dystrophy, limb-girdle type 2C

muscular dystrophy, limb-girdle type 2D muscular dystrophy, limb-girdle type 2E muscular dystrophy, limb-girdle type 2F muscular dystrophy, limb-girdle type 2G muscular dystrophy, limb-girdle type 2H muscular dystrophy, limb-girdle type 2I muscular dystrophy, limb-girdle type 2I muscular dystrophy, limb-girdle type 2J muscular dystrophy, limb-girdle type 2K muscular dystrophy, limb-girdle type IC muscular dystrophy, rigid spine muscular dystrophy with epidermolysis bullosa simplex, oculopharyngeal muscular dystrophy, Ullrich congenital muscular dystrophy, and Ullrich scleroatonic muscular dystrophy. In some embodiments, the subject is suffering from limb-girdle muscular dystrophy. In some embodiments, the subject is suffering from limb-girdle muscular dystrophy type 2E (LGMD2E).

[0086] The term "fibrosis" as used herein refers to the excessive or unregulated deposition of extracellular matrix (ECM) components and abnormal repair processes in tissues upon injury including skeletal muscle, cardiac muscle, liver, lung, kidney, and pancreas. The ECM components that are deposited include collagen, e.g. collagen 1, collagen 2 or collagen 3, and fibronectin.

[0087] In another aspect, described herein is a method of increasing muscular force and/or muscle mass in a mammalian subject comprising administering a therapeutically effective amount of an AAV vector described herein (or composition comprising an AAV vector described herein) to the mammalian subject. In one embodiment, the subject is a human.

[0088] In any of the methods of the invention, the subject may be suffering from muscular dystrophy such as limb-girdle muscular dystrophy or any other dystrophin-associated muscular dystrophy.

[0089] Also provided is a method of treating muscular dystrophy in a mammalian subject comprising administering a therapeutically effective amount of an AAV vector described herein (or composition comprising an AAV vector described herein) to the mammalian subject. In some embodiments, the muscular dystrophy is limb-girdle muscular dystrophy.

[0090] In any of the methods of the invention, the rAAV is administered by intramuscular injection or intravenous injection. In addition, in any of the method of the invention, the rAAV is administered systemically, such as parental administration by injection, infusion or implantation.

[0091] The compositions of the invention are formulated for intramuscular injection or intravenous injection. In addition, the compositions of the invention are formulated for systemic administration, such as parental administration by injection, infusion or implantation.

[0092] In addition, any of the compositions formulated for administration to a subject suffering from muscular dystrophy (such as limb-girdle muscular dystrophy or any other dystrophin-associated muscular dystrophy). In some embodiments, the composition may further comprise a second recombinant AAV vector comprising a polynucleotide sequence set forth in SEQ ID NO: 9 or SEQ ID NO: 8.

[0093] In any of the uses of the invention, the medicament is formulated for intramuscular injection or intravenous injection. In addition, in any of the uses of the invention, the medicament is formulated for systemic administration, such as parental administration by injection, infusion or implantation. In addition, any of the medicaments may be prepared for administration to a subject suffering from muscular dystrophy (such as limb-girdle muscular dystrophy or any other dystrophin associated muscular dystrophy). In some embodiments, the medicament may further comprise a second recombinant AAV vector comprising a polynucleotide sequence set forth in SEQ ID NO: 9 or SEQ ID NO: 8.

[0094] The invention also provides for a formulation or composition, comprising an rAAV virion that comprises an AAVrh74 derived capsid, a buffer agent, an ionic strength agent, and a surfactant. In the provided formulation or composition, the rAAV is at a concentration of about 1.0×10^{12} vg/ml to about 5.0×10^{14} vg/ml or at a concentration of about 5.0×10^{12} vg/ml to about 1.0×10^{14} vg/ ml. In addition, the rAAV is at a concentration of of about 2.0×10^{13} vg/ml, 4×10^{13} vg/ml, or 5×10^{13} vg/ml. In the provided formations or compositions, the rAAV may be an scAAVrh74.MHCK7.hSGCB viral particle or an scAAVrh74.MHCK7.hSGCB vector. For example, in any of the provided formulations or compositions scAAVrh74.MHCK7.hSGCB comprises a nucleotide sequence of SEQ ID NO: 19.

[0095] In any of the provided formulations or compositions, the buffer agent comprises one or more of tris, tricine, Bis-tricine, HEPES, MOPS, TES, TAPS, PIPES, and CAPS. For example, the buffer agent comprises the tris with pH 8.0 at concentration of about 5 mM to about 40 mM or the buffer agent comprises the tris with pH 8.0 at about 20 mM.

[0096] In any of the provided formulations or compositions, the ionic strength agent comprises one or more of potassium chloride (KCl), potassium acetate, potassium sulfate, ammonium sulfate, ammonium chloride (NH_4Cl), ammonium acetate, magnesium chloride ($MgCl_2$), magnesium acetate, magnesium sulfate, manganese chloride ($MnCl_2$), manganese acetate, manganese sulfate, sodium chloride (NaCl), sodium acetate, lithium chloride (LiCl), and lithium acetate. For example, the ionic strength agent comprises $MgCl_2$ at a concentration of about 0.2 mM to about 4 mM or the ionic strength agent comprises NaCl at a concentration of about 50 mM to about 500 mM, or the ionic strength agent comprises $MgCl_2$ at a concentration of about 0.2 mM to about 4 mM and NaCl at a concentration of about 50 mM to about 500 mM, or the ionic strength agent comprises $MgCl_2$ at a concentration of about 1 mM and NaCl at a concentration of about 200 mM.

[0097] In any of the provided formulations or compositions, the surfactant comprises one or more of a sulfonate, a sulfate, a phosphonate, a phosphate, a Poloxamer, and a cationic surfactant. For example, the Poloxamer comprises one or more of Poloxamer 124, Poloxamer 181, Poloxamer 184, Poloxamer 188, Poloxamer 237, Poloxamer 331, Poloxamer 338, and Poloxamer 407. The Poloxamer may be at a concentration of about 0.00001% to about 1%. An exemplary surfactant is Poloxamer 188 at a concentration of about 0.001%.

[0098] The foregoing paragraphs are not intended to define every aspect of the invention, and additional aspects are described in other sections, such as the Detailed Description. The entire document is intended to be related as a unified disclosure, and it should be understood that all combinations of features described herein are contemplated, even if the combination of features are not found together in the same sentence, or paragraph, or section of this document. The invention includes, as an additional aspect, all embodiments of the invention narrower in scope in any way than the variations defined by specific paragraphs above. For example, where certain aspects of the invention that are described as a genus, it should be understood that every member of a genus is, individually, an aspect of the invention.

BRIEF DESCRIPTION OF THE FIGURES

[0099] **Figure 1** provides a schematic of therapeutic β-sarcoglycan transgene cassette. Self-complementary AAV vector containing the codon-optimized human β-sarcoglycan

gene (hSGCB). A muscle specific MHCK7 promoter drives expression. The cassette also contains a chimeric intron to augment processing and polyadenylation signal for stability.

[00100] **Figure 2A-2B** demonstrates human β-sarcoglycan expression in skeletal muscle, A) Immunofluorescence imaging of skeletal muscles, diaphragm, and heart from $SGCB^{-/-}$ mice intravenously injected with 3e12 vg total dose scAAVrh.74.MHCK7.hSGCB. Representative images with all muscles displaying ≥98% transduction. 20X images are shown. B) Western blotting showing expression of hSGCB transgene (43 kDA) in clinical dose (#716) and high dose (#785, 786) treated muscles. N=6 for treatment group, 100 kDa corresponds to α-Actinin loading control..

[00101] **Figure 3** demonstrates the effect of systemic treatment with high dose scAAVrh74.MHCK7.hSGCB on muscle pathology. (A) H&E stain of quadriceps and diaphragm muscle from C57BL/6 WT, SGCB-/-, and scAAVrh.74.MHCK7.hSGCB treated mice, (B) Quantification of reduction in centrally nucleated fibers, (C) normalization of fiber distribution, and (D) increase in average fiber size. N=6 for each group.*=$p<0.05$; **=$p<0.01$; ***=$p<0.001$; ****=$p<0.0001$.

[00102] **Figure 4** demonstrates correction of force deficits in the diaphragm of $SGCB^{-/-}$ mice. Following 24 weeks of treatment diaphragm muscle strips were harvested from mice to measure force production following stimulus. Treatment restored force to WT levels and provided a greater restoration compared to the previously studied dose (1e12 vg total dose) (WT: n=5; KO: n=4; Low Dose: n=6; High Dose: n=6).**=$p<0.01$.

[00103] **Figure 5** demonstrates overall ambulation in x and y planes is significantly decreased in KO mice and slightly improved in MHCK7 treated mice. Vertical activity rearing onto hindlimbs was slightly improved in MCHK7 treated mice (n=6).

[00104] **Figure 6** provides biodistribution westerns on muscles and organs from two high dose ($2.0x10^{14}$ vg/kg) scAAVrh.74.MHCK7.hSGCB systemically injected $SGCB^{-/-}$ mice. 43 kDa corresponds to β-sarcoglycan protein. 125 kDa corresponds to Vinculin loading control.

[00105] **Figure 7** provides β-sarcoglycan protein expression in muscle biopsies in human subjects after systemic administration of 5.0×10^{13} vg/kg scAAVrh.74.MHCK7.hSGCB as detected and quantitated by immunohistochemistry.

[00106] **Figure 8** provides β-sarcoglycan protein expression in muscle biopsies in human subjects 90 days after systemic administration of 5.0×10^{13} vg/kg scAAVrh.74.MHCK7.hSGCB as detected and quantitated by Western Blot.

[00107] **Figure 9** demonstrates that β-sarcoglycan protein expression upregulated expression of the sarcoglycan complex as indicated by detection and quantification of alpha-sarcoglycan by immunohistochemistry.

[00108] **Figure 10** shows the restoration of expression of β–sarcoglycan, α-sarcoglycan, and the colocalization of both β-sarcoglycan and α-sarcoglycan at the membrane for patient #3 in the trial.

[00109] **Figure 11** shows the mean percent change from baseline or improvement on the 100 meter timed test in three subjects over the first three months post-gene transfer with scAAVrh.74.MHCK7.hSGCB.

[00110] **Figure 12A-C** provide the change from baseline in the North Star Assessment for Limb Girdle Muscular Dystrophies (NSAD) for the natural history control group (Figure 12A) and for the test subjects after administration of 5.0×10^{13} vg/kg scAAVrh.74.MHCK7.hSGCB (Figure 12B). The NSAD data for six individual natural history control patients are shown in Figure 12C.

[00111] **Figure 13** provides a schematic map of pAAV.MHCK7.hSGCB. KAN AAV vetor plasmid.

DETAILED DESCRIPTION

[00112] The present disclosure is based on the discovery that administration of an AAV vector comprising a polynucleotide expressing β-sarcoglycan results in a reduction or complete reversal of muscle fibrosis in a limb-girdle muscular dystrophy animal model. As demonstrated in the Examples, administration of the AAV vector described herein resulted in the reversal of dystrophic features including fewer degenerating fibers, reduced inflammation and improved functional recovery by protection against eccentric contraction with increased force generation.

[00113] As used herein, the term "AAV" is a standard abbreviation for adeno-associated virus. Adeno-associated virus is a single-stranded DNA parvovirus that grows only in cells in which certain functions are provided by a co-infecting helper virus. There are currently thirteen serotypes of AAV that have been characterized. General information and reviews

of AAV can be found in, for example, Carter, 1989, Handbook of Parvoviruses, Vol. 1, pp. 169-228, and Berns, 1990, Virology, pp. 1743-1764, Raven Press, (New York). However, it is fully expected that these same principles will be applicable to additional AAV serotypes since it is well known that the various serotypes are quite closely related, both structurally and functionally, even at the genetic level. (See, for example, Blacklowe, 1988, pp. 165-174 of Parvoviruses and Human Disease, J. R. Pattison, ed.; and Rose, Comprehensive Virology 3:1-61 (1974)). For example, all AAV serotypes apparently exhibit very similar replication properties mediated by homologous rep genes; and all bear three related capsid proteins such as those expressed in AAV2. The degree of relatedness is further suggested by heteroduplex analysis which reveals extensive cross-hybridization between serotypes along the length of the genome; and the presence of analogous self-annealing segments at the termini that correspond to "inverted terminal repeat sequences" (ITRs). The similar infectivity patterns also suggest that the replication functions in each serotype are under similar regulatory control.

[00114] An "AAV vector" as used herein refers to a vector comprising one or more polynucleotides of interest (or transgenes) that are flanked by AAV terminal repeat sequences (ITRs). Such AAV vectors can be replicated and packaged into infectious viral particles when present in a host cell that has been transfected with a vector encoding and expressing rep and cap gene products.

[00115] An "AAV virion," or "AAV viral particle" or "AAV vector particle" refers to a viral particle composed of at least one AAV capsid protein and an encapsidated polynucleotide AAV vector. If the particle comprises a heterologous polynucleotide (i.e. a polynucleotide other than a wild-type AAV genome such as a transgene to be delivered to a mammalian cell), it is typically referred to as an "AAV vector particle" or simply an "AAV vector". Thus, production of AAV vector particle necessarily includes production of AAV vector, as such a vector is contained within an AAV vector particle.

AAV

[00116] Recombinant AAV genomes of the invention comprise nucleic acid molecule of the invention and one or more AAV ITRs flanking a nucleic acid molecule. AAV DNA in the rAAV genomes may be from any AAV serotype for which a recombinant virus can be derived including, but not limited to, AAV serotypes AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAV-6, AAV-7, AAV-8, AAV-9, AAV-10, AAV-11, AAV-12, AAV-13 and

AAV rh.74. Production of pseudotyped rAAV is disclosed in, for example, WO 01/83692. Other types of rAAV variants, for example rAAV with capsid mutations, are also contemplated. See, for example, Marsic et al., Molecular Therapy, 22(11): 1900-1909 (2014). As noted in the Background section above, the nucleotide sequences of the genomes of various AAV serotypes are known in the art. To promote skeletal muscle specific expression, AAV1, AAV5, AAV6, AAV8 or AAV9 may be used.

[00117] DNA plasmids of the invention comprise rAAV genomes. The DNA plasmids are transferred to cells permissible for infection with a helper virus of AAV (e.g., adenovirus, E1-deleted adenovirus or herpesvirus) for assembly of the rAAV genome into infectious viral particles. Techniques to produce rAAV particles, in which an AAV genome to be packaged, rep and cap genes, and helper virus functions are provided to a cell are standard in the art. Production of rAAV requires that the following components are present within a single cell (denoted herein as a packaging cell): a rAAV genome, AAV rep and cap genes separate from (*i.e.*, not in) the rAAV genome, and helper virus functions. The AAV rep and cap genes may be from any AAV serotype for which recombinant virus can be derived and may be from a different AAV serotype than the rAAV genome ITRs, including, but not limited to, AAV serotypes AAV-1, AAV-2, AAV-3, AAV-4, AAV-5, AAV-6, AAV-7, AAV-8, AAV-9, AAV-10, AAV-11, AAV-12, AAV-13 and AAV rh.74. Production of pseudotyped rAAV is disclosed in, for example, WO 01/83692 which is incorporated by reference herein in its entirety.

[00118] A method of generating a packaging cell is to create a cell line that stably expresses all the necessary components for AAV particle production. For example, a plasmid (or multiple plasmids) comprising a rAAV genome lacking AAV rep and cap genes, AAV rep and cap genes separate from the rAAV genome, and a selectable marker, such as a neomycin resistance gene, are integrated into the genome of a cell. AAV genomes have been introduced into bacterial plasmids by procedures such as GC tailing (Samulski et al., 1982, Proc. Natl. Acad. S6. USA, 79:2077-2081), addition of synthetic linkers containing restriction endonuclease cleavage sites (Laughlin et al., 1983, Gene, 23:65-73) or by direct, blunt-end ligation (Senapathy & Carter, 1984, J. Biol. Chem., 259:4661-4666). The packaging cell line is then infected with a helper virus such as adenovirus. The advantages of this method are that the cells are selectable and are suitable for large-scale production of rAAV. Other examples of suitable methods employ

adenovirus or baculovirus rather than plasmids to introduce rAAV genomes and/or rep and cap genes into packaging cells.

[00119] General principles of rAAV production are reviewed in, for example, Carter, 1992, Current Opinions in Biotechnology, 1533-539; and Muzyczka, 1992, Curr. Topics in Microbial. and Immunol., 158:97-129). Various approaches are described in Ratschin et al., Mol. Cell. Biol. 4:2072 (1984); Hermonat et al., Proc. Natl. Acad. Sci. USA, 81:6466 (1984); Tratschin et al., Mo1. Cell. Biol. 5:3251 (1985); McLaughlin et al., J. Virol., 62:1963 (1988); and Lebkowski et al., 1988 Mol. Cell. Biol., 7:349 (1988). Samulski et al. (1989, J. Virol., 63:3822-3828); U.S. Patent No. 5,173,414; WO 95/13365 and corresponding U.S. Patent No. 5,658.776 ; WO 95/13392; WO 96/17947; PCT/US98/18600; WO 97/09441 (PCT/US96/14423); WO 97/08298 (PCT/US96/13872); WO 97/21825 (PCT/US96/20777); WO 97/06243 (PCT/FR96/01064); WO 99/11764; Perrin et al. (1995) Vaccine 13:1244-1250; Paul et al. (1993) Human Gene Therapy 4:609-615; Clark et al. (1996) Gene Therapy 3:1124-1132; U.S. Patent. No. 5,786,211; U.S. Patent No. 5,871,982; and U.S. Patent. No. 6,258,595. The foregoing documents are hereby incorporated by reference in their entirety herein, with particular emphasis on those sections of the documents relating to rAAV production.

[00120] The invention thus provides packaging cells that produce infectious rAAV. In one embodiment packaging cells may be stably transformed cancer cells such as HeLa cells, 293 cells and PerC.6 cells (a cognate 293 line). In another embodiment, packaging cells are cells that are not transformed cancer cells, such as low passage 293 cells (human fetal kidney cells transformed with E1 of adenovirus), MRC-5 cells (human fetal fibroblasts), WI-38 cells (human fetal fibroblasts), Vero cells (monkey kidney cells) and FRhL-2 cells (rhesus fetal lung cells).

[00121] Recombinant AAV (*i.e.*, infectious encapsidated rAAV particles) of the invention comprise a rAAV genome. Embodiments include, but are not limited to, the rAAV named pAAV.MHCK7.hSCGB which comprises the polynucleotide sequence set forth in SEQ ID NO: 3 or SEQ ID NO: 19; and pAAV.tMCK.hSCGB which comprises the polynucleotide sequence set forth in SEQ ID NO: 5.

[00122] The rAAV may be purified by methods standard in the art such as by column chromatography or cesium chloride gradients. Methods for purifying rAAV vectors from helper virus are known in the art and include methods disclosed in, for example, Clark *et*

al., Hum. Gene Ther., 10(6): 1031-1039 (1999); Schenpp and Clark, *Methods Mol. Med., 69* 427-443 (2002); U.S. Patent No. 6,566,118 and WO 98/09657.

[00123] In another embodiment, the invention contemplates compositions comprising rAAV of the present invention. Compositions described herein comprise rAAV in a pharmaceutically acceptable carrier. The compositions may also comprise other ingredients such as diluents and adjuvants. Acceptable carriers, diluents and adjuvants are nontoxic to recipients and are preferably inert at the dosages and concentrations employed, and include buffers such as phosphate, citrate, or other organic acids; antioxidants such as ascorbic acid; low molecular weight polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-formig counterions such as sodium; and/or nonionic surfactants such as Tween, pluronics or polyethylene glycol (PEG).

[00124] Titers of rAAV to be administered in methods of the invention will vary depending, for example, on the particular rAAV, the mode of administration, the treatment goal, the individual, and the cell type(s) being targeted, and may be determined by methods standard in the art. Titers of rAAV may range from about $1x10^6$, about $1x10^7$, about $1x10^8$, about $1x10^9$, about $1x10^{10}$, about $1x10^{11}$, about $1x10^{12}$, about $1x10^{13}$ to about $1x10^{14}$ or more DNase resistant particles (DRP) per ml. Dosages may also be expressed in units of viral genomes (vg). The titers of rAAV may be determined by the supercoiled plasmid quantitation standard or the linearized plasmid quantitation standard.

[00125] Methods of transducing a target cell with rAAV, *in vivo or in vitro*, are contemplated by the invention. The *in vivo* methods comprise the step of administering an effective dose, or effective multiple doses, of a composition comprising a rAAV of the invention to an animal (including a human being) in need thereof. If the dose is administered prior to development of a disorder/disease, the administration is prophylactic. If the dose is administered after the development of a disorder/disease, the administration is therapeutic. In embodiments of the invention, an effective dose is a dose that alleviates (eliminates or reduces) at least one symptom associated with the disorder/disease state being treated, that slows or prevents progression to a disorder/disease state, that slows or prevents progression of a disorder/disease state, that diminishes the extent of disease, that

results in remission (partial or total) of disease, and/or that prolongs survival. An example of a disease contemplated for prevention or treatment with methods of the invention is muscular dystrophy, such as limb-girdle muscular dystrophy. Thus, provided is a method of tranducing a target cell with an rAAV scAAVrh74.MHCK7.hSGCB, which comprises a nucleotide sequence of SEQ ID NO: 3 or 19.

[00126] Combination therapies are also contemplated by the invention. Combination as used herein includes both simultaneous treatment or sequential treatments. Combinations of methods of the invention with standard medical treatments (*e.g.,* steroids, corticosteroids, and/or glucocorticoids including but not limited to one or more of prednisone, prednisolone; and deflazacort) are specifically contemplated, as are combinations with novel therapies. In this regard, the combinations include administering to a subject one or more steroids, corticosteroids, and/or glucocorticoids including but not limited to one or more of prednisone, prednisolone; and deflazacort before administering an rAAV of the inventive methods to the subject, simultaneously with administering the rAAV to the subject, or after administering the rAAV to the subject.

[00127] In related embodiments of a combination therapy contemplated by the invention, the glucocorticoid includes, but is not limited to beclomethasone, betamethasone, budesonide, cortisone, dexamethasone, hydrocortisone, methylprednisolone, or triamcinolone.

[00128] It is recognized that an antigen specific T-cell response may occur in a subject administered with the rAAV vector. This is an expected response between 2 - 4 weeks following gene transfer. One possible consequence to such antigen specific T-cell responses is clearance of the transduced cells and loss of transgene expression. To dampen the host immune response to the rAAV based therapy, before the therapy, for example, twenty-four hours prior to the therapy procedure, subjects can be started on approximately 1mg/kg/day prophylactic prednisone or comparable glucocorticoid by mouth with a maximum dose of 60 mg/day. IV administration of a comparable glucocorticoid at the approximate dose of 1 mg/kg/day would also be allowable if needed. Treatment will continue for approximately one month. A tapering protocol for prednisone or comparable glucocorticoid can be implemented based on individual subjects' immune response to the gene transfer, assessed by ELISpot assay and also by liver function monitoring with GGT.

[00129] A therapeutically effective amount of the rAAV vector is a dose of rAAV ranging from about 1e13 vg/kg to about 5e14 vg/kg, or about 1e13 vg/kg to about 2e13 vg/kg, or about 1e13 vg/kg to about 3e13 vg/kg, or about 1e13 vg/kg to about 4e13 vg/kg, or about 1e13 vg/kg to about 5e13 vg/kg, or about 1e13 vg/kg to about 6e13 vg/kg, or about 1e13 vg/kg to about 7e13 vg/kg, or about 1e13 vg/kg to about 8e13 vg/kg, or about 1e13 vg/kg to about 9e13 vg/kg, or about 1e13 vg/kg to about 1e14 vg/kg, or about 1e13 vg/kg to about 2e14 vg/kg, or 1e13 vg/kg to about 3e14 vg/kg, or about 1e13 to about 4e14 vg/kg, or about 3e13 vg/kg to about 4e13 vg/kg, or about 3e13 vg/kg to about 5e13 vg/kg, or about 3e13 vg/kg to about 6e13 vg/kg, or about 3e13 vg/kg to about 7e13 vg/kg, or about 3e13 vg/kg to about 8e13 vg/kg, or about 3e13 vg/kg to about 9e13 vg/kg, or about 3e13 vg/kg to about 1e14 vg/kg, or about 3e13 vg/kg to about 2e14 vg/kg, or 3e13 vg/kg to about 3e14 vg/kg, or about 3e13 to about 4e14 vg/kg, or about 3e13 vg/kg to about 5e14 vg/kg, or about 5e13 vg/kg to about 6e13 vg/kg, or about 5e13 vg/kg to about 7e13 vg/kg, or about 5e13 vg/kg to about 8e13 vg/kg, or about 5e13 vg/kg to about 9e13 vg/kg, or about 5e13 vg/kg to about 1e14 vg/kg, or about 5e13 vg/kg to about 2e14 vg/kg, or 5e13 vg/kg to about 3e14 vg/kg, or about 5e13 to about 4e14 vg/kg, or about 5e13 vg/kg to about 5e14 vg/kg, or about 1e14 vg/kg to about 2e14 vg/kg, or 1e14 vg/kg to about 3e14 vg/kg, or about 1e14 to about 4e14 vg/kg, or about 1e14 vg/kg to about 5e14 vg/kg, 6e14 vg/kg, 7e14 vg/kg, 8e14 vg/kg, or 9e14 vg/kg. The invention also comprises compositions comprising these ranges of rAAV vector.

[00130] For example, a therapeutically effective amount of rAAV vector is a dose of 1e13 vg/kg, about 2e13 vg/kg, about 3e13 vg/kg, about 4e13 vg/kg, about 5e13 vg/kg, about 6e13 vg/kg, about 7e13 vg/kg, about 7.4e13 vg/kg ,about 8e13 vg/kg, about 9e13 vg/kg, about 1e14 vg/kg, about 2e14 vg/kg, about 3e14 vg/kg, about 4e14 vg/kg and 5e14 vg/kg. The titer or dosage of AAV vectors can vary based on the physical forms of plasmid DNA as a quantitation standard. For example, the value of titer or dosage may vary based off of a supercoiled standard qPCR titering method or a linear standard qPCR tittering method. In one embodiment, a therapeutically effective amount of rAAV is a dose of 5e13 vg/kg based on a supercoiled plasmid as the quantitation standard or a dose of 1.85e13 vg/kg based on a linearized plasmid as the quantitation standard. In another embodiment, a therapeutically effective amount of rAAV is a dose of 2e14 vg/kg based on the supercoiled plasmid as the quantitation standard or a dose of 7.41e13 vg/kg based on the linearized plasmid as the quantitation standard. In another embodiment, the

therapeutically effective amount of scAAVrh74.MHCK7.hSGCB is a dose ranging from about 1e13 vg/kg to about 5e14 vg/kg, or about 1e13 vg/kg to about 2e13 vg/kg, or about 1e13 vg/kg to about 3e13 vg/kg, or about 1e13 vg/kg to about 4e13 vg/kg, or about 1e13 vg/kg to about 5e13 vg/kg, or about 1e13 vg/kg to about 6e13 vg/kg, or about 1e13 vg/kg to about 7e13 vg/kg, or about 1e13 vg/kg to about 8e13 vg/kg, or about 1e13 vg/kg to about 9e13 vg/kg, or about 1e13 vg/kg to about 1e14 vg/kg, or about 1e13 vg/kg to about 2e14 vg/kg, or 1e13 vg/kg to about 3e14 vg/kg, or about 1e13 to about 4e14 vg/kg, or about 3e13 vg/kg to about 4e13 vg/kg, or about 3e13 vg/kg to about 5e13 vg/kg, or about 3e13 vg/kg to about 6e13 vg/kg, or about 3e13 vg/kg to about 7e13 vg/kg, or about 3e13 vg/kg to about 8e13 vg/kg, or about 3e13 vg/kg to about 9e13 vg/kg, or about 3e13 vg/kg to about 1e14 vg/kg, or about 3e13 vg/kg to about 2e14 vg/kg, or 3e13 vg/kg to about 3e14 vg/kg, or about 3e13 to about 4e14 vg/kg, or about 3e13 vg/kg to about 5e14 vg/kg, or about 5e13 vg/kg to about 6e13 vg/kg, or about 5e13 vg/kg to about 7e13 vg/kg, or about 5e13 vg/kg to about 8e13 vg/kg, or about 5e13 vg/kg to about 9e13 vg/kg, or about 5e13 vg/kg to about 1e14 vg/kg, or about 5e13 vg/kg to about 2e14 vg/kg, or 5e13 vg/kg to about 3e14 vg/kg, or about 5e13 to about 4e14 vg/kg, or about 5e13 vg/kg to about 5e14 vg/kg, or about 1e14 vg/kg to about 2e14 vg/kg, or 1e14 vg/kg to about 3e14 vg/kg, or about 1e14 to about 4e14 vg/kg, or about 1e14 vg/kg to about 5e14 vg/kg, 6e14 vg/kg, 7e14 vg/kg, 8e14 vg/kg, or 9e14 vg/kg, based on the supercoiled plasmid as the quantitation standard. The invention also comprises compositions comprising these doses of rAAV vector.

[00131] Administration of an effective dose of the compositions may be by routes standard in the art including, but not limited to, intramuscular, parenteral, intravenous, oral, buccal, nasal, pulmonary, intracranial, intraosseous, intraocular, rectal, or vaginal. Route(s) of administration and serotype(s) of AAV components of the rAAV (in particular, the AAV ITRs and capsid protein) of the invention may be chosen and/or matched by those skilled in the art taking into account the infection and/or disease state being treated and the target cells/tissue(s) that are to express the β-sarcoglycan.

[00132] The invention provides for local administration and systemic administration of an effective dose of rAAV and compositions of the invention. For example, systemic administration is administration into the circulatory system so that the entire body is affected. Systemic administration includes enteral administration such as absorption

through the gastrointestinal tract and parental administration through injection, infusion or implantation.

[00133] In particular, actual administration of rAAV of the present invention may be accomplished by using any physical method that will transport the rAAV recombinant vector into the target tissue of an animal. Administration according to the invention includes, but is not limited to, injection into muscle, the bloodstream and/or directly into the liver. Simply resuspending a rAAV in phosphate buffered saline has been demonstrated to be sufficient to provide a vehicle useful for muscle tissue expression, and there are no known restrictions on the carriers or other components that can be co-administered with the rAAV (although compositions that degrade DNA should be avoided in the normal manner with rAAV). Capsid proteins of a rAAV may be modified so that the rAAV is targeted to a particular target tissue of interest such as muscle. See, for example, WO 02/053703, the disclosure of which is incorporated by reference herein.

[00134] Pharmaceutical compositions can be prepared as injectable formulations or as topical formulations to be delivered to the muscles by transdermal transport. Numerous formulations for both intramuscular injection and transdermal transport have been previously developed and can be used in the practice of the invention. The rAAV can be used with any pharmaceutically acceptable carrier for ease of administration and handling. Thus, in another aspect, the application is directed to a formulation that comprises an rAAV that comprises an AAVrh74 derived capsid, a buffer agent, an ionic strength agent, and a surfactant. In one embodiment, the rAAV is at a concentration of about 1.0×10^{12} vg/ml to about 5.0×10^{14} vg/ml. In another embodiment, the rAAV is at a concentration of about 5.0×10^{12} vg/ml to about 1.0×10^{14} vg/ml based on a supercoiled plasmid as the quantitation standard. In another embodiment, the rAAV is at a concentration of about 2.0×10^{13}vg/ml based on a supercoiled plasmid as the quantitation standard. In one embodiment, the rAAV is an scAAVrh74.MHCK7.hSGCB vector. In one embodment, the concentration of rAAV in the composition or formulation is from 1×10^{13} vg/ml to 2×10^{14} vg/ml based on a supercoiled plasmid as the quantitation standard. In another embodiment, the concentration is 2×10^{13} vg/ml, 4×10^{13} vg/ml, or 5×10^{13} vg/ml based on a supercoiled plasmid as the quantitation standard. In one embodiment, the buffer agent comprises one or more of tris, tricine, Bis-tricine, HEPES, MOPS, TES, TAPS, PIPES, and CAPS. In another embodiment, the buffer agent comprises tris with pH 8.0 at concentration of about 5 mM to about 40 mM. In one embodiment, the buffer agent

comprises tris with pH 8.0 at about 20 mM. In one embodiment, the ionic strength agent comprises one of more of potassium chloride (KCl), potassium acetate, potassium sulfate, ammonium sulfate, ammonium chloride (NH_4Cl), ammonium acetate, magnesium chloride ($MgCl_2$), magnesium acetate, magnesium sulfate, manganese chloride ($MnCl_2$), manganese acetate, manganese sulfate, sodium chloride (NaCl), sodium acetate, lithium chloride (LiCl), and lithium acetate. In one embodiment, the ionic strength agent comprises $MgCl_2$ at a concentration of about 0.2 mM to about 4 mM. In another embodiment, the ionic strength agent comprises NaCl at a concentration of about 50 mM to about 500 mM. In another embodiment, the ionic strength agent comprises $MgCl_2$ at a concentration of about 0.2 mM to about 4 mM and NaCl at a concentration of about 50 mM to about 500 mM. In another embodiment, the ionic strength agent comprises $MgCl_2$ at a concentration of about 1 mM and NaCl at a concentration of about 200 mM. In one embodiment, the surfactant comprises one or more of a sulfonate, a sulfate, a phosphonate, a phosphate, a Poloxamer, and a cationic surfactant. In one embodiment, the Poloxamer comprises one or more of Poloxamer 124, Poloxamer 181, Poloxamer 184, Poloxamer 188, Poloxamer 237, Poloxamer 331, Poloxamer 338, and Poloxamer 407. In one embodiment, the surfactant comprises the Poloxamer at a concentration of about 0.00001% to about 1%. In another embodiment, the surfactant comprises Poloxamer 188 at a concentration of about 0.001%. For purposes of intramuscular injection, solutions in an adjuvant such as sesame or peanut oil or in aqueous propylene glycol can be employed, as well as sterile aqueous solutions. Such aqueous solutions can be buffered, if desired, and the liquid diluent first rendered isotonic with saline or glucose. Solutions of rAAV as a free acid (DNA contains acidic phosphate groups) or a pharmacologically acceptable salt can be prepared in water suitably mixed with a surfactant such as hydroxpropylcellulose. A dispersion of rAAV can also be prepared in glycerol, liquid polyethylene glycols and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms. In this connection, the sterile aqueous media employed are all readily obtainable by standard techniques well-known to those skilled in the art.

[00135] The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of

manufacture and storage and must be preserved against the contaminating actions of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol and the like), suitable mixtures thereof, and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of a dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal and the like. In many cases it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by use of agents delaying absorption, for example, aluminum monostearate and gelatin.

[00136] Sterile injectable solutions are prepared by incorporating rAAV in the required amount in the appropriate solvent with various other ingredients enumerated above, as required, followed by filter sterilization. Generally, dispersions are prepared by incorporating the sterilized active ingredient into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and the freeze drying technique that yield a powder of the active ingredient plus any additional desired ingredient from the previously sterile-filtered solution thereof.

[00137] Transduction with rAAV may also be carried out *in vitro*. In one embodiment, desired target muscle cells are removed from the subject, transduced with rAAV and reintroduced into the subject. Alternatively, syngeneic or xenogeneic muscle cells can be used where those cells will not generate an inappropriate immune response in the subject.

[00138] Suitable methods for the transduction and reintroduction of transduced cells into a subject are known in the art. In one embodiment, cells can be transduced *in vitro* by combining rAAV with muscle cells, *e.g.,* in appropriate media, and screening for those cells harboring the DNA of interest using conventional techniques such as Southern blots and/or PCR, or by using selectable markers. Transduced cells can then be formulated into pharmaceutical compositions, and the composition introduced into the subject by various techniques, such as by intramuscular, intravenous, subcutaneous and intraperitoneal injection, or by injection into smooth and cardiac muscle, using *e.g.*, a catheter.

[00139] Transduction of cells with rAAV of the invention results in sustained expression of β-sarcoglycan. The present invention thus provides methods of administering/delivering rAAV which express β-sarcoglycan to a mammalian subject, preferably a human being. These methods include transducing tissues (including, but not limited to, tissues such as muscle, organs such as liver and brain, and glands such as salivary glands) with one or more rAAV of the present invention. Transduction may be carried out with gene cassettes comprising tissue specific control elements. For example, one embodiment of the invention provides methods of transducing muscle cells and muscle tissues directed by muscle specific control elements, including, but not limited to, those derived from the actin and myosin gene families, such as from the myoD gene family [See Weintraub *et al., Science, 251*: 761-766 (1991)], the myocyte-specific enhancer binding factor MEF-2 [Cserjesi and Olson, *Mol Cell Biol* 11: 4854-4862 (1991)], control elements derived from the human skeletal actin gene [Muscat *et al., Mol Cell Biol, 7*: 4089-4099 (1987)], the cardiac actin gene, muscle creatine kinase sequence elements [See Johnson *et al., Mol Cell Biol, 9*:3393-3399 (1989)] and the murine creatine kinase enhancer (mCK) element, control elements derived from the skeletal fast-twitch troponin C gene, the slow-twitch cardiac troponin C gene and the slow-twitch troponin I gene: hypoxia-inducible nuclear factors (Semenza *et al., Proc Natl Acad Sci USA, 88*: 5680-5684 (1991)), steroid-inducible elements and promoters including the glucocorticoid response element (GRE) (See Mader and White, *Proc. Natl. Acad. Sci. USA* 90: 5603-5607 (1993)), and other control elements.

[00140] Muscle tissue is an attractive target for *in vivo* DNA delivery, because it is not a vital organ and is easy to access. The invention contemplates sustained expression of miRNAs from transduced myofibers.

[00141] By "muscle cell" or "muscle tissue" is meant a cell or group of cells derived from muscle of any kind (for example, skeletal muscle and smooth muscle, *e.g.* from the digestive tract, urinary bladder, blood vessels or cardiac tissue). Such muscle cells may be differentiated or undifferentiated, such as myoblasts, myocytes, myotubes, cardiomyocytes and cardiomyoblasts.

[00142] The term "transduction" is used to refer to the administration/delivery of a polynucleotide of interest (e.g., a polynucleotide sequence encoding β-sarcoglycan) to a recipient cell either *in vivo* or *in vitro*, via a replication-deficient rAAV described resulting in expression of β-sarcoglycan by the recipient cell.

[00143] Thus, also described herein are methods of administering an effective dose (or doses, administered essentially simultaneously or doses given at intervals) of rAAV that encode β-sarcoglycan to a mammalian subject in need thereof.

[00144] All publications and patents mentioned herein are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference. In case of conflict, the present application, including any definitions herein, will control.

[00145] The invention is further described in the following Examples, which do not limit the scope of the invention described in the claims.

[00146] In another embodiment, the disclosure provides a method of generating the rAAV pAAV.MHCK7.hSCGB, which comprises transferring an AAV vector plasmid to a host cell. The methods of transferring a DNA to a host cell are known in the art, which include but are not limited to transfection, infection, transformation, electroporation, and transduction. In one embodiment, the vector plasmid comprises a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 24. In another embodiment, the vector plasmid comprises a nucleotide sequence of SEQ ID NO: 24. In another aspect, the disclosure provides a host cell comprising an AAV vector plasmid that comprises a nucleotide sequence of SEQ ID NO: 24. In some embodiment, the AAV vector plasmid is stably expressed in the host cell. The host cell stably harboring the AAV vector plasmid can be used to generate rAAV. In one embodiment, the AAV vector plasmid is a pAAV.MHCK7.hSGCB. KAN plasmid. The pAAV.MHCK7.hSGCB. KAN plasmid is illustrated in Figure 11.

[00147] In one embodiment, the vector plasmid comprises a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 1, 3, 5, or 19. In one embodiment, the vector plasmid comprises a nucleotide sequence of SEQ ID NO: 1, 3, 5, or 19. The method of generating rAAV, in one embodiment, further comprises transferring a packaging plasmid and/or a helper virus to the host cell. The packaging plasmid, in some embodiments, comprises an AAV rep and/or cap gene that is operably linked to a promoter. The promoter, in one embodiment, is an AAV transcription promoter. In one embodiment, the host cell is a packaging cell. In one embodiment, the packaging cell comprises a stably integrated AAV cap gene. In another embodiment, the packaging cell comprises a stably integrated AAV rep gene.

[00148] As used herein, the term "host cell" refers to a cell that can be used to express an exogenous DNA sequence. Non-limiting examples of a host cell comprise a microorganism, a yeast cell, an insect cell, and/or a mammalian cell. The host cell can be used as a recipient for an AAV helper construct, a packaging plasmid, an AAV vector plasmid, an accessary function vector, or other DNA. The term as used here encompasses the progeny of the original cell after expressing the exogenous DNA sequence in the original host cell. Non-limiting examples of host cells for AAV production include Sf9 insect cells and HEK 293T cells. In one embodiment, the cell described herein comprises an insect cell, e.g., a *Drosophila* cell (e.g., an S2 cell or Kc cell), a silkworm cell (e.g., a Bme21 cell), or a mosquito cell (e.g., a C6/36 cell);or a mammalian cell (preferably a human cell, e.g., a human primary cell or an established cell line). In one embodiment, the mammalian cell comprises a 293 cell, a COS cell, a HeLa cells, or a KB cell. The AAV vector plasmid can be introduced to the host cells, e.g., Sf9 or 293T, by infection (virus or baculovirus), transient transfection using reagents (e.g., liposomal, calcium phosphate) or physical means (e.g., electroporation), or other means know in the art. In another embodiment, the host cell lines are stably integrated with the rAAV plasmids into their genomes. Such stable cell lines can be established by incorporating a selection marker into the vector plasmid.

[00149] In one embodiment, the host cell is a packaging cell for production of AAV viral particles. Thus, in another aspect, the disclosure provides a host cell that comprises an AAV vector plasmid that comprises a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 24. In one embodiment, the AAV vector plasmid that comprises a nucleotide sequence of SEQ ID NO: 24. In another embodiment, the host cell comprises a nucleotide sequence of SEQ ID NO: 1, 3, 5, or 19.

EXAMPLES

[00150] Preclinical studies using scAAVrh74.MHCK7.hSGCB are described in International Patent Publication No. WO 2017/180976, which is incorporated by reference herein in its entirety.

Example 1

Materials and Methods

[00151] *Animal models* - All procedures were approved by The Research Institute at Nationwide Children's Hospital Institutional Animal Care and Use Committee (protocol

AR12-00040). B6.129-Sgcb$^{tm1Kcam/1J}$ heterozygous mice were purchased from the Jackson Laboratory (Bar Harbor, ME, USA; Strain # 006832). Sgcb$^{-/-}$ mice were generated by breeding heterozygous mice. KO mice were bred and maintained as homozygous animals in standardized conditions in the Animal Resources Core at the Research Institute at Nationwide Children's Hospital. Mice were maintained on Teklad Global Rodent Diet (3.8z5 fiber, 18.8% protein, 5% fat chow) with a 12:12-h dark:light cycle. Identification of SGCB$^{-/-}$ mice was performed by genotyping using PCR. All animals were housed in standard mouse cages with food and water ad libitum.

[00152] *Beta-sarcoglycan gene construction.* The full-length human beta-sarcoglycan cDNA (GenBank Accession No. NM_0034994.3) was codon optimized and synthesized by GenScript Inc, Piscataway, NJ, USA. Codon optimization through GenScript uses an algorithm that takes into account parameters that include transcription, mRNA processing and stability, translation and protein folding to design a cDNA sequence that results in maximum expression in muscle tissue (www.genscript.com).

[00153] For the pAAV.tMCK.hSGCB construct, the cDNA was then cloned into a plasmid containing AAV2 ITRs and the cassette included a consensus Kozak sequence (CCACC), an SV40 chimeric intron and a synthetic polyadenylation site (53 bp). The recombinant tMCK promoter was a gift from Dr Xiao Xiao (University of North Carolina). It is a modification of the previously described CK6 promoter27 and includes a modification in the enhancer upstream of the promoter region containing transcription factor binding sites. The enhancer is composed of two E-boxes (right and left). The tMCK promoter modification includes a mutation converting the left E-box to a right E-box (2R modification) and a 6-bp insertion (S5 modification). The pAAV.tMCK.hSGCB vector was constructed by ligation of 1040 bp KpnI/XbaI fragment from pUC57-BSG (Genscript Inc.) into the KpnI/XbaI sites of pAAV. tMCK.hSGCA.26

[00154] The pAAV.MHCK7.hSGCB vector was constructed by removing the tMCK promoter and SV40 chimeric intron with NotI/KpnI sites and inserting a PCR amplified fragment containing the MHCK7 promoter and identical SV40 chimeric intron with NotI/KpnI sites. MHCK7 is an MCK based promoter which utilizes a 206-bp enhancer taken from ~1.2kb 5' of the transcription start site within the endogenous muscle creatine kinase gene with a proximal promoter (enh358MCK, 584-bp)[3,12] . The MHCK7 promoter itself contains this modified CK7 cassette from the MCK family of genes ligated to a 188-bp α-MyHC (α-myosin heavy chain) enhancer 5' of the CK portion to enhance cardiac

expression[12]. The creatine kinase portion of the promoter (CK) is 96% identical between tMCK and MHCK7. Finally, the pAAV.MHCK7.hSGCB vector was constructed by ligation of the 960 bp NotI/KpnI MHCK7+Intron fragment from pAAV.MHCK7.DYSF5'DV44 into the NotI/KpnI sites of pAAV.tMCK.hSGCB (Pozgai et al., *Gene Ther.* 23: 57-66, 2016)

[00155] *rAAV production.* A modified cross-packaging approach, previously reported by Rodino-Klapac et al. (*J. Trans. Med.* 5:45, 2007), was used to produce the rAAV vector. Here, a triple transfection method with $CaPO_4$ precipitation in HEK293 cells allows for AAV2 ITRs to be packaged into a different AAV capsid serotype. (28,29) The production plasmids were (i) pAAV.tMCK.hSGCB or pAAV.MHCK7.hSGCB, (ii) rep2-caprh.74 modified AAV helper plasmids encoding cap serotype 8-like isolate rh.74 and (iii) an adenovirus type 5 helper plasmid (pAdhelper) expressing adenovirus E2A, E4 ORF6 and VA I/II RNA genes. Vectors were purified and encapsidated vg titer (utilizing a Prism 7500 Taqman detector system; PE Applied Biosystems, Carlsbad, CA, USA) was determined as previously described. 30 The primer and fluorescent probe targeted the tMCK promoter and were as follows: tMCK forward primer, 5′-ACC CGA GAT GCC TGG TTA TAA TT-3′ (SEQ ID NO: 10); tMCK reverse primer, 5′-TCC ATG GTG TAC AGA GCC TAA GAC-3′ (SEQ ID NO: 11); and tMCK probe, 5′-FAM-CTG CTG CCT GAG CCT GAG CGG TTA C-TAMRA-3′ (SEQ ID NO: 12). The primer and fluorescent probe targeted the MHCK7 promoter and were as follows: MHCK7 forward primer, 5'-CCA ACA CCT GCT GCC TCT AAA-3' (SEQ ID NO: 16); MHCK7 reverse primer, 5'-GTC CCC CAC AGC CTT GTT C-3' (SEQ ID NO: 17); and MHCK7 probe, 5'-FAM-TGG ATC CCC-Zen-TGC ATG CGA AGA TC-3IABKFQ-3' (SEQ ID NO: 18).

[00156] *Systemic Gene Delivery:* Systemic delivery was achieved through injection of vector into the tail vein of *sgcb*$^{-/-}$ mice. Mice were injected with $3x10^{12}$ vg of scAAVrh.74.MHCK7.hSGCB (2.0×10^{14} vg/kg) diluted in saline using a 30 gauge ultra-fine insulin syringe. Mice were restrained in a holding tube placing the tail back through tail slot to warm it up in order dilate the blood vessels for ease of injection. After locating the artery down the center line of the tail, the injection was performed in one of the purple/blue lateral veins that run alongside the tail artery. All treated mice were injected at 4-5 weeks of age and euthanized 6-months post-injection.

[00157] *Immunofluorescence.* Cryostat sections (12 μm) were incubated with a monoclonal human beta-sarcoglycan primary antibody (Leica Biosystems, New Castle,

UK; Cat. No. NCL-L-b-SARC) at a dilution of 1:50 in a block buffer (1 × TBS, 10% Goat Serum, 0.1% Tween) for 1 h at room temperature in a wet chamber. Sections were then washed with TBS three times, each for 20 min and re-blocked for 30 min. AlexaFluor 594 conjugated goat anti-mouse secondary IgG1 antibody (Life Technologies, Grand Island, NY, USA; Cat. No. A21125) was applied at a 1:250 dilution for 45 min. Sections were washed in TBS three times for 20 min and mounted with Vectashield mounting medium (Vector Laboratories, Burlingame, CA, USA). Four random ×20 images covering the four different quadrants of the muscle section were taken using a Zeiss AxioCam MRC5 camera. Percentage of fibers positive for beta-sarcoglycan staining (450% of muscle membrane staining intensity) was determined for each image and averaged for each muscle.

[00158] *Western blot analysis.* Tissue sections or muscle biopsies were collected into a micro-centrifuge and homogenized with 100 µl homogenization buffer (125 mM Tris-HCl, 4% SDS, 4 M urea) in the presence of 1 protease inhibitor cocktail tablet (Roche, Indianapolis, IN, USA). After homogenization, the samples were centrifuged at 10,000 rpm for 10 min at 4°C. Protein was quantified on NanoDrop (Thermo Scientific, Waltham, MA, USA). Protein samples (20 µg) were electrophoresed on a 3–8% polyacrylamide Tris-acetate gel (NuPage, Invitrogen, Carlsbad, CA, USA) for 1 h 5 min at 150 V and then transferred onto a PVDF membrane (Amersham Biosciences, Piscataway, NJ, USA) for 1 h 15 min at 35 V. The membrane was blocked in 5% non-fat dry milk in TBST for 1 h, and then incubated with a rabbit polyclonal human beta-sarcoglycan antibody (Novus Biologicals, Littleton, CO, USA; Cat. No. NBP-1–90300 1:100 or 1:250 dilution) and a 1:5000 of a monoclonal mouse gamma-tubulin antibody (Sigma-Aldrich, St Louis, MO, USA; Cat. No. T6557) or a 1:5000 dilution of a mouse monoclonal mouse α-actinin antibody (Sigma-Aldrich, St Louis, MO, USA; Cat. No. A7811). A 1:500 dilution of a rabbit polyclonal mouse cardiac troponin I antibody (Abcam, Cambridge, MA; Cat. No. ab47003) and a 1:1000 dilution of a rabbit monoclonal mouse vinculin antibody (Invitrogen, Frederick, MD; Cat. No. 70062) were used. Anti-mouse (Millipore, Billerica, MA, USA; Cat. No. AP308P) and anti-rabbit (Life Technologies; Cat. No. 656120) secondary-HRP antibodies were used for ECL immunodetection.

[00159] *Biodistribution qPCR analysis.* Taqman quantitative PCR was performed to quantify the number of vector genome copies present in targeted and untargeted contralateral muscle as previously described.(18,30) A vector-specific primer probe set

was used to amplify a sequence of the intronic region directly downstream from the tMCK promoter that is unique and located within the scAAVrh.74.tMCK.hSGCB transgene cassette. The following primers and probe were used in this study: tMCK and MHCK7 intron Forward Primer 5′-GTG AGG CAC TGG GCA GGT AA -3′ (SEQ ID NO: 13); tMCK and MHCK7 intron Reverse Primer 5′-ACC TGT GGA GAG AAA GGC AAA G -3′ (SEQ ID NO: 14); and tMCK and MHCK7 intron Probe 5′-6FAM-ATC AAG GTT ACA AGA CAG-GTT TAA GGA GAC CAA TAG AAA -tamra-3′ (IDT) (SEQ ID NO: 15). Copy number is reported as vector genomes per microgram of genomic DNA. *Immunohistochemistry for immune cell staining*. Immunohistochemistry was used to identify immune cells. Frozen tissue sections on Fisherbrand Superfrost charged microscope slides were incubated with rat anti-mouse monoclonal antibodies using an anti-rat Ig HRP Detection kit (BD Pharmagen, San Jose, CA, USA; Cat: 551013): CD3 (Cat: 555273), CD4 (Cat: 550280), CD8 (Cat: 550281) and Mac-3 for macrophages (Cat: 550292). All primary antibodies were diluted at 1:20 with phosphate-buffered saline. Positive immune staining was visualized using DAB chromagen diluted in DAB buffer with Streptavidin-HRP peroxidase ectastain ABC Peroxidase. Ten random × 40 images were taken for each muscle and each corresponding stain. The number of mono-nuclear cells was counted and expressed as total number per mm^2.

[00160] *Immunofluorescence*: Cryostat sections (12µm) from the tibialis anterior (TA), gastrocnemius (GAS), quadriceps (QUAD), psoas major (PSOAS), gluteal (GLUT), triceps (TRI), and diaphragm muscles along with the heart were subjected to immunofluorescence staining for the hSGCB transgene via our previously used protocol as described in Pozgai et al., Gene Therap. 23: 57-66, 2016. Sections were incubated with a mouse monoclonal human beta-sarcoglycan primary antibody (Leica Biosystems, New Castle, UK; Cat. No. NCL-L-b-SARC) at a dilution of 1:100. Four random 20X images covering the four different quadrants of the muscle section were taken using a Zeiss AxioCam MRC5 camera. Percentage of fibers positive for beta-sarcoglycan staining (>50% of muscle membrane staining) was determined for each image and averaged for each muscle.

[00161] *Morphometric Analysis*: Hematoxylin and eosin (H&E) staining was performed on 12µm thick cryosections of muscle from 7 month old C57BL6 WT mice (n=5), *sgcb*$^{-/-}$ mice (n=5), and rAAV.MHCK7.hSGCB 6 month treated *sgcb*$^{-/-}$ mice (n=5) for analysis. The percentage of myofibers with central nuclei was determined in the TA, GAS, QUAD,

PSOAS, GLUT, TRI, and diaphragm muscles. Additionally, muscle fiber diameters were measured in the GAS, PSOAS, and TRI muscles. Four random 20X images per muscle per animal were taken with a Zeiss AxioCam MRC5 camera. Centrally nucleated fibers were quantified using the NIH ImageJ software and fiber diameters were measured using Zeiss Axiovision LE4 software.

[00162] *XLaser Monitoring of Open Field Cage Activity*: An open-field activity chamber was used to determine overall activity of experimental mice. Mice at 7 months old from the C57BL6 WT (n=6) and untreated *sgcb*$^{-/-}$ (n=6) control groups along with the rAAV.MHCK7.hSGCB 6 month treated *sgcb*$^{-/-}$ mice (n=6) were subjected to analysis following a previously described protocol (Kobayashi et al., *Nature* 456: 511-5, 2008, Beastrom et al., *Am. J. Pahol.* 179: 2464-74, 2011) with several modifications. All mice were tested at the same time of day in the early morning near then end of the night cycle when mice are most active. All mice were tested in an isolated room, under dim light and with the same handler each time. To reduce anxiety and keep behavioral variables at a minimum, which could potentially affect normal activity of the mice and consequently the results of the assay, the mice tested were not individually housed (Voikar et al., *Genes Brain Behav.* 4: 240-52, 2005). Mice were activity monitored using the Photobeam Activity System (San Diego Instruments, San Diego, CA). This system uses a grid of invisible infrared light beams that traverse the animal chamber front to back and left to right to monitor the position and movement of the mouse within an X-Y-Z plane. Activity was recorded for 1 hour cycles at 5-minute intervals. Mice were acclimatized to the activity test room for an initial 1 hour session several days prior to beginning data acquisition. Mice were tested in individual chambers in sets of 4. Testing equipment was cleaned between each use to reduce mouse reactionary behavioral variables that could alter our results. Data collected was converted to a Microsoft Excel worksheet and all calculations were done within the Excel program. Individual beam breaks for movement in the X and Y planes were added up for each mouse to represent total ambulation, and beam breaks in the Z plane were added up to obtain vertical activity within the 1 hour time interval.

Example 2
scAAVrh.74.MHCK7.hSGCB construction

[00163] The transgene cassette containing a codon-optimized full-length human SCGB cDNA as shown in Figure 1 was constructed. The cassette includes a consensus Kozak

sequence (CCACC), an SV40 chimeric intron, a synthetic polyadenylation site, and the muscle-specific MHCK7 used to drive expression of the cassette. This is an MCK based promoter which utilizes a 206-bp enhancer taken from ~1.2kb 5' of the transcription start site within the endogenous muscle creatine kinase gene with a proximal promoter (enh358MCK, 584-bp)[3,12]. The cassette was packaged into a self-complementary (sc) AAVrh.74 vector that is 93% homologous to AAV8. AAVrh.74 has been shown in mice and non-human primates to be safe and effective, particularly in crossing the vascular barrier when delivered to muscle through the circulation.(17, 18, 21)

Example 3

Long-Term Efficacy of High Dose scAAVrh.74.MHCK7.hSGCB Systemic Delivery

[00164] Following the strong results of the previous studies with at a dose of $1.0x10^{12}$ vg total dose ($5.0x10^{13}$ vg/kg) scAAVrh.74.MHCK7.hSGCB, vector was delivered through a tail vein injection to 6 $SGCB^{-/-}$ mice at a high dose of $3.0x10^{12}$ vg total dose ($2.0x10^{14}$ vg/kg) to assess transgene expression and efficacy of the vector when delivered systemically at a long-term time point of 24 weeks. Mice were injected at 4-5 weeks of age and a full necropsy on all 6 mice was performed at 24 weeks post-injection. The following muscles were extracted for analysis: TA, gastrocnemius, quadriceps, gluteal, PSOAS major, tricep, diaphragm and heart. Organs were also removed for toxicology and biodistribution studies. In short, hSGCB transgene expression was as high (98.77% across all muscles) following 24 weeks treatment at this high dose compared to our previously studied dose (98.10% across all muscles) and all muscles from treated mice were again almost fully transduced. This was accompanied by improved muscle histopathology and improved function.

β-Sarcoglycan Expression

[00165] Immunofluorescence (IF) staining for human β-sarcoglycan was used to determine hSGCB transgene expression in six skeletal muscles, in additional to the diaphragm and heart of all the KO mice given a systemic injection of hSGCB vector. These muscles included the TA, gastrocnemius (GAS), quadriceps (QUAD), gluteal (GLUT), psoas major (PSOAS), and triceps (TRI). For the purposes of expression analysis and transduction efficiency, images for the muscles from six treated mice were utilized for quantification. Four 20X images were taken of each muscle and the percent of hSGCB positive fibers was determined for each image resulting in the average percent transduction

for each muscle from each mouse, and these data are presented in Appendix C. The results shown in the panel below in Figure 2A are representative images from the treated mice and demonstrate once again ≥98% transduction in all muscles analyzed including the diaphragm and heart. Finally, the Western blot also depicted in Figure 2B shows expression of hSGCB in the TA and TRI muscle similar to what was achieved following delivery of the initial clinical dose.

Histopathology of Treated Muscle

[00166] As it was discussed previously, muscles from $SGCB^{-/-}$ mice, both skeletal and cardiac, exhibit widespread myopathy including pronounced myofiber atrophy and hypertrophy with multiple focal areas of necrosis. Also present are increasing numbers of mononuclear cell inflammation (lymphocytes and macrophages, with scattered neutrophils) and increased dystrophic calcification, fatty infiltration, central nucleation, and fibrosis. Hematoxylin & eosin staining in Figure 3 below illustrates this dystrophic phenotype in $SGCB^{-/-}$ mice when compared to normal wild type mice and the improvement of muscle pathology following treatment. Quantification of histological parameters shows a reduction in central nucleation (CN) in numerous different skeletal muscles as a result of β-sarcoglycan gene transfer. A more in depth analysis of muscle histopathology reveals a normalization of fiber size distribution accompanied by an increase in average fiber diameter in diseased mice treated with vector in all three muscles examined (gastrocnemius, psoas, and tricep) (Figure 3).

Functional Assessment of Systemic Delivery

[00167] To determine whether high dose hSGCB gene transfer provides an even greater functional benefit to diseased muscle, the functional properties of the diaphragm muscle from $SGCB^{-/-}$ mice treated with high dose scAAVrh.74.MHCK7.hSCGB. Histopathology was demonstrated and established a functional deficit in diaphragms and hearts of $SGCB^{-/-}$ mice. β-sarcoglycan KO diaphragms demonstrated a 50.9% reduction in specific force output compared to BL6 WT mice (116.24 mN/mm^2 vs. 236.67 mN/mm^2). Tail vein delivery of high dose scAAVrh.74.MHCK7.hSGCB resulting in nearly 100% hSGCB expression in the diaphragm lead to restoration of diaphragm specific force output which improved to 259.97 mN/mm^2 (n=6) (Figure 4). These data show that high dose hSGCB gene transfer does provide a greater functional benefit to diseased muscle deficient for β-sarcoglycan.

[00168] In order to determine if high dose AAV.hSGCB therapy provides an overall functional benefit to diseased $SGCB^{-/-}$ mice as occurred with delivery of our clinical dose and ultimately improves the phenotype of $SGCB^{-/-}$ mice, laser-monitoring of open-field cage activity was performed on all groups of mice. The graphs in Figure 5 below depict a significant decrease by 58.6% in total ambulation in x and y planes along with a 48.9% decrease in hindlimb vertical rearing in KO mice compared to WT. High dose scAAVrh.74.MHCK7.hSGCB treated mice were overall more active compared to KO by qualitative observation and that is illustrated in the quantification of activity where total ambulation increased by 36.2% and hindlimb vertical rearing increased by 39.0% in MCHK7 treated mice (n=6 per group) (Figure 5).

[00169] Intravenous injection of scAAVrh.74.MHCK7.hSGCB at a higher dose of $3.0x10^{12}$ vg total dose ($2.0x10^{14}$ vg/kg) lead to nearly complete transduction and restoration of hSGCB expression in limb skeletal muscles, diaphragm, and importantly cardiac muscle (≥98%) (Figure 2). The fact that high levels of transduction was achieved in all muscles throughout the body using relatively low doses ($5.0x10^{13}$ vg/kg and $2.0x10^{14}$ vg/kg) due to the self-complementary AAV vector and rh.74 serotype gives this therapy great promise for translation to LGMD2E patients. The severe dystrophic pathology seen in all muscles in the absence of β-sarcoglycan was significantly improved following treatment (Figure 3). These results led to the observed increase in specific force output in the diaphragm and increased open-field cage activity (Figures 4, 5).

Example 4

Toxicology & Vector Biodistribution

[00170] The purpose of this study was to assess any potential toxicity or safety concerns of SGCB gene therapy in male and female $SGCB^{-/-}$ mice at 24 weeks after delivery of the test article scAAVrh.74.MHCK7.hSGCB. Test article was given at 3.0x1012 vg total dose ($2.0x10^{14}$ vg/kg) by the intravenous (IV) route to 4-5 week old $SGCB^{-/-}$ mice in a total volume 520 μL split into two injections of 260 μL each 5 hours apart to achieve the desired dose. To assess the safety of our vector, hematoxylin & eosin staining was performed on cryosections of muscle tissue and all offsite organs harvested from a group of six $SGCB^{-/-}$ mice treated with vector along with two WT and two KO controls injected with LRS (Table 1).

Table 1: scAAVrh.74.MHCK7.hSGCB Safety Study Design

Group	Genotype	Vector Titer (vg Total Dose)	Mouse No.	Sex	Age at Injection	Age at Necropsy
1	SGCB-/-	$3.0x10^{12}$	785	Male	4 weeks	28 weeks
			786	Female	4 weeks	28 weeks
			787	Female	4 weeks	28 weeks
			788	Male	4 weeks	28 weeks
			789	Male	4 weeks	28 weeks
			790	Male	4 weeks	28 weeks
2	SGCB-/-	None	1	Male	N/A	28 weeks
			2	Male	N/A	28 weeks
3	Wild-type	None	1	Male	N/A	28 weeks
			2	Male	N/A	28 weeks

[00171] These sections were then formally reviewed for toxicity by a veterinary pathologist and no adverse effects were detected in most samples from any of the mice with the exception of a few focal areas of hepatitic lesions in the livers of two treated mice (#789 and 790). Protein expression and vector biodistribution were also assessed using qPCR and Western blotting, and these data indicate no hSGCB transgene expression in any non-muscle tissue except for the livers of mice #785 and #787.

Histopathology Review of Vector Transduced Tissue

[00172] In order to determine the safety and toxicology profile of $2.0x10^{14}$ vg/kg scAAVrh.74.MHCK7.hSGCB using systemic delivery, a variety of skeletal muscles including the diaphragm, along with the heart and five other organs were harvested from a group of vector dosed $SGCB^{-/-}$ mice and controls and H&E sections of each tissue were formally reviewed by an independent veterinary pathologist. Group details and study design are shown in Table 1.

[00173] Dosing cohorts for scAAVrh.74.MHCK7.hSGCB histopathology studies. Two BL6 WT mice and two $SGCB^{-/-}$ mice were injected with LRS to serve as appropriate age-matched controls. Six $SGCB^{-/-}$ were given 3.0x1012 vg total dose by IV. Mice were euthanized 24 weeks post-injection at and endpoint age of 28 weeks.

[00174] In summary, IV injection of high dose scAAVrh.74.MHCK7.hSGCB did not elicit any microscopic changes in myofibers of any skeletal muscles examined. Any changes noted in muscle were seen in both treated and control mice and were considered

incidental findings. In addition, no treatment-related lesions were seen in most of the non-muscle tissues evaluated histologically, with only the livers of mice #789 and #790 showing small focal hepatic lesions.

[00175] To further evaluate clinical liver function, the levels of liver enzymes in the serum of these mica was assessed. Two untreated BL6 WT mice and two untreated SGCB$^{-/-}$ mice along with six scAAVrh.74.MHCK7.hSGCB treated mice for analysis of Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) levels to determine if they are elevated compared to normal levels. Table 2 below indicates untreated SGCB-/- mice present with elevated ALT and AST levels at an average of 288 U/L and 784.5 U/L respectively, outside of the normal range in healthy mice. AAV dosed SGCB-/- mice however present with average ALT and AST levels that are not elevated and in the normal range at 89.5 U/L and 330.75 U/L for ALT and AST respectively.

[00176] Taken together, these data indicate that this test article was well tolerated by the test subjects. Furthermore, relative to reference specimens from two age-matched, untreated SGCB$^{-/-}$ mice, independent histopathology review indicated administration of scAAVrh.74.MHCK7.hSGCB substantially decreased myofiber atrophy and destruction in treated SGCB$^{-/-}$ mice, thus showing that the test article can ameliorate the degree of myopathy associated with profound deficiencies of SGCB.

Table 2. Liver Enzyme Level Analysis in Serum From SGCB$^{-/-}$ Mice Treated Systemically With scAAVrh.74.MHCK7.hSGCB

Animal Number	Strain	AAV Treated	ALT (U/L)	AST (U/L)	Average ALT (U/L)	Average AST (U/L)
785	BSG KO	Y	71	291	89.5	330.75
786	BSG KO	Y	N/A	N/A		
787	BSG KO	Y	N/A	N/A		
788	BSG KO	Y	55	267		
789	BSG KO	Y	182	563		
790	BSG KO	Y	50	202		
BL6 WT-1	BL6 WT	N	N/A	N/A	69	132
BL6 WT-2	BL6 WT	N	69	132		
BSG KO-1	BSG KO	N	480	862	288	784.5
BSG KO-2	BSG KO	N	96	707		

Normal ALT Range: 27-195 U/L
Normal AST Range: 43-397 U/L

[00177] Table 2 provides analysis of Alanine Aminotransferase and Aspartate Aminotransferase levels in the serum of untreated BL6 WT (n=2) and SGCB$^{-/-}$ (n=2) along

with scAAVrh.74.MHCK7.hSGCB treated SGCB$^{-/-}$mice (n=6). Averages reported in far right two columns are for each of the three cohorts. Reported in Units/L. N/A indicates samples were hemolyzed and unable to be analyzed.

Vector Genome Biodistribution

[00178] The presence of test article-specific DNA sequences was examined using a real time, quantitative PCR assay (qPCR). Biodistribution analysis was performed on tissue samples collected from four vector dosed SGCB$^{-/-}$ animals. A positive signal is anything equal to or greater than 100 single-stranded DNA copies/µg genomic DNA detected. Tissues were harvested at necropsy and vector specific primer probe sets specific for sequences of the MHCK7 promoter were utilized. Table 3 depicts the vector genome copies detected in each tissue sample from high dose ($3.0x10^{12}$ vg total dose) scAAVrh.74.MHCK7.hSGCB injected mice (#785, 786, 789, 790) along with the vg copy numbers from the same tissue samples in our previously studied clinical dose ($1.0x10^{12}$ vg total dose) treated mice (#712, 713).

[00179] scAAVrh.74.MHCK7.hSGCB transcript was detected at varying levels in all collected tissues. As expected, the highest levels were seen in skeletal muscle and the heart. The lowest levels were detected in gonad, lung, kidney, and spleen. Of note, the vector genome copy numbers were similar in each tissue when comparing the original clinical dose ($5.0x10^{13}$ vg/kg) with this high dose ($2.0x10^{14}$ vg/kg) cohort. These data indicate that the test article was efficiently delivered into all investigated tissues of vector dosed mice.

Table 3. Quantitative PCR Results Following High Dose scAAVrh.74.MHCK7.hSGCB Systemic Delivery in SGCB-/- Mice

Tissue	Vector genome copies/ug					
	$1.0x10^{12}$ vg dose		$3.0x10^{12}$ vg dose			
	#712	#713	#785	#786	#789	#790
Gonad	1.54e+004	2.31e+004	7.27E+04	2.43E+06	2.32E+05	2.02E+05
Heart	9.81e+005	1.23e+006	2.07E+06	3.59E+06	2.04E+06	4.60E+06
Lung	2.34e+005	3.21e+005	4.54E+05	9.19E+05	2.55E+06	1.08E+06
Kidney	1.30e+005	9.16e+004	5.46E+05	1.48E+06	2.63E+06	5.91E+05
Liver	3.51e+007	4.07e+007	7.31E+07	3.46E+07	4.75E+05	1.84E+06
Spleen	3.30e+005	1.84e+005	5.39E+05	9.72E+05	9.87E+05	1.02E+06
Diaphragm	9.82e+005	1.29e+006	3.85E+06	4.11E+05	5.50E+06	2.57E+06
TRI	1.82e+006	1.29e+006	1.77E+06	2.21E+06	5.41E+06	2.52E+06
QD	9.20e+005	1.14e+006	1.47E+06	3.45E+06	3.79E+06	3.65E+06
GAS	1.37e+006	8.04e+005	2.06E+06	1.35E+06	7.09E+06	2.35E+06
TA	1.80e+006	1.11e+006	2.02E+06	1.15E+06	2.23E+06	2.51E+06

[00180] Table 3 provides vector genome copy numbers organs and muscles from four high dose treated SGCB$^{-/-}$mice. Values are shown in vg/µg genomic DNA

[00181] As the qPCR results above indicate, intravenous delivery of high dose scAAVrh.74.MHCK7.hSGCB results in distribution of vector transcript to varying levels in most tissues, however with the highest levels occurring in muscle. Therefore, the objective of this portion of the study was to determine the protein expression of the human β-sarcoglycan transgene in these tissues to ensure the functionality of the muscle specific MHCK7 promoter. Western blotting was used to detect β-sarcoglycan expression in the tissue samples from four of the treated mice (#785, 787, 789, and 790).

[00182] β-sarcoglycan protein expression was observed in varying amounts in all skeletal muscle samples as well as heart samples, and was detected in the livers of mice #785, and 787. (Table 4, Figure 6). To investigate the expression in liver further, Western blotting for β-sarcoglycan protein expression was performed on liver tissue from all six treated mice (#785, 786, 787, 788, 789, and 790). The results of this Western shown in Figure 6 indicate we do see β-Sarcoglycan protein expression in four of the six livers from vector dosed mice (#785, 786, 787, and 788). Below in Table 5 lists the detailed β-sarcoglycan protein expression results from all six mice in which full biodistribution westerns were performed (#785, 796, 787, 788, 789, and 790).

Table 4. β-Sarcoglycan Protein Biodistribution Following High Dose scAAVrh.74.MHCK7.hSGCB Systemic Delivery in SGCB-/- Mice

Tissue	Mouse #					
	785	786	787	788	789	790
TA	X	N/A	X	N/A	X	X
GAS	X	N/A	X	N/A	X	X
DIA	X	N/A	X	N/A	X	X
HEART	X	N/A	X	N/A	X	X
GONADS		N/A		N/A		
LUNGS		N/A		N/A		
KIDNEY		N/A		N/A		
LIVER	X	X	X	X		
SPLEEN		N/A		N/A		

[00183] Table 4 provides β-Sarcoglycan protein expression in individual tissues from six SGCB-/- mice treated systemically with $2.0x10^{14}$ vg/kg scAAVrh.74.MHCK7.hSGCB. An X indicates protein expression in the corresponding tissue. NA = assay not performed

[00184] This cardiac expression using the MHCK7 promoter is very encouraging at dosing levels that could be applied clinically, and given the high incidence of heart involvement in the β-sarcoglycan deficiency in the LGMD2E patients, systemic delivery would be most beneficial to these patients clinically. $SGCB^{-/-}$ mice given an intravenous tail vein injection of scAAVrh.74.MHCK7.hSGCB at this proposed high dose of $3.0x10^{12}$ vg total dose ($2.0x10^{14}$ vg/kg) were fully necropsied and all muscles and organs extracted were stained by H&E and sent to an independent veterinary pathologist for review. The livers from four vector dosed mice (#785, 786, 787, and 788) did show expression of the β-Sarcoglycan transgenic protein which has been demonstrated previously with systemic dosing (Salva et al., Mol Ther, 2007. **15**(2): p. 320-9). Two of six treated animals (#789 and 790) were reported to have minimal to mild focal hepatic lesions however all other organs and muscles reviewed from the six treated mice showed no adverse effects. To evaluate any clinical manifestations of the mild hepatic lesions in the livers from mice #789 and #790, we measured liver enzyme levels, Alanine Aminotransferase and Aspartate Aminotransferase, in the serum from all six treated mice. The results of this experiment shown in Table 4 depict the average AST and ALT levels from the six treated mice are within the normal range, indicating no clinical liver enzyme abnormalities. The livers from animals #789 and #790 presented with a lower vg copy number (Table 3) in addition to absent β-Sarcoglycan transgenic protein expression. Taken together, this data indicates that the transgenic β-Sarcoglycan protein may have been cleared from the liver in these two animals; however there was no impact on skeletal muscle expression or liver function.

[00185] At the high dose ($2.0x10^{14}$ vg/kg), there does appear to be persistent β-Sarcoglycan expression in liver which was not observed in lower dose treated animals ($5.0x10^{13}$ vg/kg). There has been no overt toxicity observed at any doses provided. The trial will be initiated at a NOAEL dose ($5.0x10^{13}$ vg/kg). Liver toxicity in patients was closely monitored, and liver enzyme elevations have been effectively managed using corticosteroids in another systemic delivery trial for spinal muscular atrophy using AAV (Mendell et al., N Engl J Med 2017; 377:1713-1722).

Example 5

LGMD2E Open-label Trial

[00186] Recombinant AAVrh74 carrying the human SGCB gene under control of the muscle-specific MHCK7 promoter (scAAVrh74.MHCK7.hSGCB) was delivered one-time via a systemic infusion through a peripheral vein. The vector is delivered in approximately 10 ml/kg Lactated Ringer's, if needed, to be infused over approximately 1-2 hours. Patient were Glucocorticosteroid adrenal suppression is at most very minimal after 30 days but in the interest of caution, the maximum dose for each enrollee will reduced by 50% for 1 week, and again by 50% for 1 week before stopping.

[00187] Cohort 1 included 3 treated subjects 4-15 years of age which had confirmed SGCB mutation in both allele, were negative for AAVrh74 antibodies and > 40% of normal 100 meter walk test. Each of the subjects received a dose of 5×10^{13} vg/kg. Sixty days after dosing, needle muscle biopsies were done on the tibialis anterior and biceps muscles with appropriate anesthesia under advisement of anesthesiologist (or anesthetist). The biopsies may be done under ultrasound guidance. Each subject received 1 mg/kg prednisone 1 day prior to gene transfer, taped the dose for 30 days.

[00188] Biopsies were read and if ≥50% of muscle fibers express SGCB in TA and biceps of all Cohort 1 subjects, there will be no dose escalation in Cohort 2. If these criteria are not met, the subjects in Cohort 2 will receive 2×10^{14} vg/kg. Three of the patients in Cohort 2 will receive placebo Lactated Ringers'. These placebo subjects will be treated with the same dose as the treated subjects in their cohort approximately one year later.

Baseline Measurements Prior to Injection (Day -60 to day -2)

[00189] After obtaining informed consent and completing the registration procedures, a baseline patient history was collected, including records of all medications and supplements that the patient is taking. Baseline functional testing to establish a stable baseline were compared to functional testing results gathered in a previous natural history study for consistency of the baseline testing. At the screening visit, the 100 m timed test must be ≥40% of predicted for age, height and weight matched healthy controls for inclusion. If a subject does not screen-in, he or she may continue to participate in an LGMD natural history study. The following assessments will be performed to confirm

subject eligibility for this study. Baseline tests which must be completed prior to treatment administration include the following:

Baseline

Day -60 to day -2 before gene transfer

- Informed Consent
- Medical History
- Physical exam/ vitals
- EKG
- Cardiac MRI (will be done without anesthesia but if the procedure is poorly tolerated and considering the importance of cardiac evaluations in this disease, we will discuss options with anesthesia using an acceptable protocol at Nationwide Children's protocol)
- Skeletal muscle MRI without anesthesia
- Antibody (IgG and IgM) testing for Hepatitis B, and C, and for HIV
- Safety Labs:
    - Complete blood count (CBC) with differential and platelets
    - Serum total protein
    - Serum gamma-glutamyl transferase (GGT)
        - GGT will be used to monitor liver enzymes rather than ALT or AST because of the source of these enzymes from damaged muscle, where levels can reach 9-10X ULN. ALT and AST can vary by 30-40% from day to day making interpretation difficult. GGT is not affected by muscle disease [22,23]
    - Serum total bilirubin
    - Glucose
    - Creatine kinase (CK) (CK levels will only be drawn preferably on 2 day visits but may be tested on a one day visit per PI discretion)
    - Creatinine/BUN
    - Cystatin C
    - Alkaline phosphatase
    - Amylase
    - AST
    - ALT
    - Prothrombin time (PT), partial thromboplastin time (PTT)
    - Electrolytes (sodium, potassium, chloride, C02
    - Urinalysis
- Serum binding antibody to rAAVrh74
- Serum binding antibody to β-sarcoglycan
- ELISpot assay to AAVrh74 capsid proteins and β-sarcoglycan
- Pregnancy test (if judged by the investigator to be of childbearing potential)
- Strength testing (handheld dynamometry) of knee and elbow flexors and extensors, hip adductors, and shoulder abductors
- PROMIS questionnaires
- Set up with equipment for activity monitoring
- Pulmonary function testing (PFTs), including spirometry

- Timed Functional Testing [100 meter timed test, Ascending 4 stairs, Timed Up and Go]
- Workspace volume
- North Star Assessment for Limb Girdle Muscular Dystrophies (NSAD)
- Baseline muscle biopsy, may use guided ultrasound, of upper and lower limb muscle; choice depending on clinical findings targeting a muscle that will be adequate for analysis with the least risk to the patient. Placebo delayed subjects will not have a second baseline muscle biopsy performed.
- Chest X-ray

Day -1

- Physical exam and vital signs
- Begin Prednisone or similar glucocorticoid
- Photographs of potential injection site
- Safety Labs:
    - Complete blood count (CBC) with differential and platelets
    - Serum total protein
    - Serum gamma-glutamyl transferase (GGT)
        - GGT will be used to monitor liver enzymes rather than ALT or AST because of the source of these enzymes from damaged muscle, where levels can reach 9-10X ULN. ALT and AST can vary by 30-40% from day to day making interpretation difficult. GGT is not affected by muscle disease [22,23]
    - Serum total bilirubin
    - Glucose
    - Creatine kinase (CK) (CK levels will only be drawn preferably on 2 day visits but may be tested on a one day visit per PI discretion)
    - Creatinine/BUN
    - Cystatin C
    - Alkaline phosphatase
    - Amylase
    - AST
    - ALT
    - Prothrombin time (PT), partial thromboplastin time (PTT)
    - Electrolytes (sodium, potassium, chloride, C02)
    - Urinalysis

Prophylactic Administration of Prednisone

[00190] An expected antigen specific T-cell response to the AAV vector was expected between 2 - 4 weeks following gene transfer. One possible consequence to such antigen specific T-cell responses was clearance of the transduced cells and loss of transgene expression. To dampen the host immune response to the AAV based therapy, twenty-four hours prior to the procedure subjects were started on approximately 1mg/kg/day prophylactic prednisone or comparable glucocorticoid by mouth with a maximum dose of 60

mg/day. IV administration of a comparable glucocorticoid at the approximate dose of 1 mg/kg/day was also be allowable if needed. Treatment continued for approximately one month. A tapering protocol for prednisone or comparable glucocorticoid was implemented based on individual subjects' immune response to the gene transfer, assessed by ELISpot assay and also by liver function monitoring with GGT.

Protocol for Gene Transfer

[00191] The scAAVrh74.MHCK7.hSGCB gene vector was prepared by the research pharmacist according to the Manual of Operating Procedures (MOOP). Immediately prior to transportation to the clinical setting, appropriate dilutions of the test article were completed by the pharmacy. The vector was diluted using lactated Ringer's and drawn up in sterile 60 ml polypropylene syringes. Documentation of the dilution was completed by the pharmacy following standard pharmacy protocol.

[00192] The vector-containing syringes were delivered at room temperature and administered to the subject within 24 hours of preparation. Handling of scAAVrh74.MHCK7.hSGCB followed compliance standards for Biosafety Level 1 vectors. (NIH Guidelines for Research Involving recombinant or Synthetic Acid Molecules [NIH Guidelines], April 2016, Department of Health and Human Services, National Institutes of Health Office of Science Policy, Office of Biotechnology Activities.

[00193] Subjects were admitted for gene transfer, either PICU or Pulmonary PICU, the night before gene transfer and were examined by either the PI or Co-Is (DAY -1). Subjects were held NPO after midnight the night before the gene transfer procedure. Procedures were performed under sterile conditions in the hospital room.

[00194] An intravenous catheter with heparin lock was placed in a peripheral vein for delivery of vector. A second intravenous catheter was placed to be used in the event of a complication with the first site. Pictures were taken of these sites on the day of the gene transfer. The vector was delivered intravenously while the patient is awake. If deemed necessary by the study doctor, the patient received conscious sedation per protocol. The patient were dosed with scAAVrh74.MHCK7.hSGCB administered over approximately 1-2 hours through 60 mL polypropylene syringes using a syringe pump. The patient's vital signs were monitored during the infusion and every 15 minutes for 4 hours and every hour for the remaining 24 hours post-infusion.

Post-Gene Transfer Monitoring

[00195] The patient's vital signs were monitored every 15 minutes for 4 hours and every hour for the remaining 24 hours post-infusion. Safety labs and a urinalysis were checked the day after the procedure. Concomitant medications and all adverse events/serious adverse events were also be monitored and documented following injection. Subjects were discharged one day after gene transfer (if no side effects are observed that are a concern for safety). Subjects returned for follow up visits on days 7, 14, 30, 60, 90, and 180 and months 9,12,18, 24, 30 and 36. Toxicity monitoring on each of these dates included:

- Physical Exam and vital signs
- Safety Labs:
    - Complete blood count (CBC) with differential and platelets
    - Serum total protein
    - Serum gamma-glutamyl transferase (GGT)*
        - GGT will be used to monitor liver enzymes rather than ALT or AST because of the source of these enzymes from damaged muscle, where levels can reach 9-10X ULN. ALT and AST can vary by 30-40% from day to day making interpretation difficult. GGT is not affected by muscle disease [22,23]
    - Serum total bilirubin
    - Glucose
    - Creatine kinase (CK) (CK levels will only be drawn preferably on 2 day visits but may be tested on a one day visit per PI discretion)
    - Creatinine/BUN
    - Cystatin C
    - Alkaline phosphatase
    - Amylase
    - AST
    - ALT
    - Prothrombin time (PT), partial thromboplastin time (PTT)
    - Electrolytes (sodium, potassium, chloride, C02)
    - Urinalysis
- Immunology studies
- Physical Therapy assessments starting at Day 30 (100 meter timed test, strength testing, PROMIS questionnaires, North Star Assessment for Limb Girdle Muscular Dystrophies (NSAD), Ascending 4 stairs, Timed Up and Go and workspace volume)
- Urinalysis
- Photograph of injection site (Days -1, 0, 1, 7, 14, 30)
- Adverse events (collected at all study visits)
- EKG (Day 180, Months 12,24,36)
- Cardiac and skeletal muscle MRI (Months 12, 24, 36),
- Pulmonary Function Tests (Days 60, 180, Months 12, 24, 36)
- Post gene transfer muscle biopsy at 60 days for Cohort 1 and 2 and at 2 years post treatment for all subjects; choice of muscle will be the same as pre-treatment biopsy

sites. The post treatment biopsy will preferably be on the same side unless risks dictate biopsies be done on the opposite limb.

Long-Term Monitoring

[00196] The recent FDA guidelines are followed with regard to long-term subject follow-up following gene transfer. As discussed and based on prior experience with rAAV or transgene, there is a very low probability of gene transfer-related delayed adverse events. Short-term safety over a three-year period is evaluated that incorporates the active phase of the protocol. If newly identified risks are associated with the product, or if the subjects suffer any adverse events during this period, a long-term follow-up is initiated according to the FDA guidelines.

[00197] CBER is notified if there is any indication of need to extend follow-up period. All subjects will be provided with written instructions on how to contact the Principal Investigator or study coordinator if they experience any serious adverse event that they consider possibly related to study treatment or study participation. This information is included in the Informed Consent document. All subjects are instructed to notify the Principal Investigator of a change of address or contact information.

Post-Study Follow-up

[00198] The most recent FDA guidance are followed with regard to long-term subject follow-up post gene transfer. As indicated by the guidelines, the vector has a very low probability of gene transfer-related delayed adverse events. Safety is evaluated over a three-year period post-dosing that incorporates the active phase of the protocol. If newly identified risks are associated with our product, or if the subjects suffer any adverse events during this period, a long-term follow-up is initiated according to the FDA guidelines.

Primary Outcome for Clinical Trial

[00199] This is a Phase I clinical trial and safety is the primary outcome. Demonstration of β-SG protein expression, as judged by quantified immunofluorescent or immunoblot analysis (≥ 20% above baseline) on muscle biopsy at 8 weeks.

Exploratory Outcomes

- Improvement in 100 meter time ≥10% compared to baseline for each participant 3 years post gene transfer
- A decrease in CK following gene therapy will serve as an exploratory outcome. CK levels will only be drawn preferably on 2 day visits but may be tested on a one day visit per PI discretion

- Workspace volume
- Handheld dynamometry of knee and elbow extensors and flexors, hip adductors, and shoulder abductors
- Improvement in ejection fraction as measured by cMRI
- Skeletal MRI
- Pulmonary Function Testing (PFTs), including spirometry
- Timed Functional Testing [Ascending 4 stairs, Timed Up and Go]
- North Star Assessment for Limb Girdle Muscular Dystrophies (NSAD)
    - Activity level as determined by a Fitbit or similar activity monitoring device
    - Patient report of physical function using PROMIS Upper Extremity and Mobility questionnaires

Cohort 1 Results

[00200] All subjects in Cohort 1 were doing well at the time of testing (Subjects 1 and 2: tested 90 days post injection; Subject 3 tested 60 days post injection). All subjects continued to do well out to 9 months post injection. There was one serious adverse event in this study, in which one subject demonstrated elevated liver enzymes and bilirubin following discontinuation of steroids. This event was resolved with increased steroids. Two of the subjects had elevated liver enzymes that resolved with increased steroids and these level returned to baseline.

[00201] Muscle needle biopsies of the tibialis anterior and biceps were used to quantify transgene expression comparing baseline to day 60 in Cohort 1. The primary endpoint was $\geq$ 20% expression of SGCB protein. If expression of SGCB is $\geq$50% above baseline in all of the treated subjects there will be no increase in dosing. If expression of SGCB is <50% in all treated subjects, the dose will be escalated to $2x10^{14}$ vg/kg for Cohort 2 and the placebo subjects. The 2 year post-treatment biopsies will be done on the same muscle(s) as the baseline biopsies, when possible. All biopsy samples were blinded and coded by the laboratory director with a computer generated code. Quantification of expression was done using direct immunofluorescence and Western Blot studies of the muscle biopsies. Bioquant® automated software will be used to quantify the number of muscle fibers expressing SGCB. Baseline demographics are set out in Table 5.

Table 5: Baseline Demographics

Subject	Age (years)	CK Levels at Baseline (U/L)
1	**13**	**10,727**
2	**4**	**12,826**
3	**13**	**10,985**

[00202] Figure 7 provides representative images that demonstrate robust SGCB expression in the muscles of all three subjects 8 weeks after vector administration. Table 6 provides the mean intensity and percentage of SGCB-positive fibers in each subject. The mean intensity of immunohistochemistry staining for the entire cohort was 47% and the mean percentage of SGCB-positive fibers was 51%. Figure 8 provides a Western Blot demonstrating detection of β-sarcoglycan expression in the three subjects 90 days after vector administration. The Western Blot data demonstrates that the gene transfer delivers full length β-sarcoglycan. Quantification by Western Blot is provided in Table 7, which demonstrates a mean β-sarcoglycan protein expression is about 36.1% increased compared to normal.

Table 6 – Immunohistochemisty

Subject	Mean Intensity	Percentage of SCGB-Positive Fibers
1	47%	63%
2	57%	49%
3	38%	42%
MEAN	47%	51%

Table 7 – Western Blot

Subject	Mean Beta-Sarcoglycan Expression (N=3) vs. Normal
1	34.7%
2	39.2%
3	34.5%
MEAN	36.1%

[00203] The presence of test article-specific DNA sequences was examined using a real time, quantitative PCR assay (qPCR) on the collected muscle biopsies. A positive signal is anything equal to or greater than 100 single-stranded DNA copies/µg genomic DNA detected. A mean 8.4E+04 vector copies/µg DNA, and 0.6 copies per nucleus, was detected in the muscle biopsies.

[00204] The presence of the sarcoglycan complex in each subject was also investigated. As determined by Western Blot, mean micro-dystrophin expression was 36% of normal (n=3). In addition, alpha-sarcoglycan expression was quantified by

immunohistochemistry. Figure 9 demonstrates that beta-sarcoglycan expression in the subjects upregulated the sarcoglycan complex as indicated by alpha-sarcoglycan expression.

[00205] The creatine kinase (CK) levels in the blood of the subject were tested. As shown in Table 9, there was a mean reduction of about 82% in CK levels in the subjects.

Table 9

Subject	Age	CK Levels (U/L) at					
		Baseline	Day 30	Day 60	Day 90	Day 180	Day 270
1	13	10,727	619	2257	1135	1553	2300
2	4	12,826	4795	910	2159	5070	2665
3	13	10,985	687	2061	2392	10,055	1295

Example 6

β-Sarcoglycan Gene Transfer Restores Sarcoglycan Complex to the Membrane

[00206] Treatment with scAAVrh74.MHCK7.hSGCB restored sarcoglycan complex to the membrane (Figure 10). Figure 10 shows the restoration of expression of β-sarcoglycan, α-sarcoglycan, and the colocalization of both β-sarcoglycan and α-sarcoglycan at the membrane for a patient in the trial. Co-localization of β-sarcoglycan and α-sarcoglycan indicates that scAAVrh74.MHCK7.hSGCB restored the sarcoglycan complex.

Example 7

LGMD2E Patients Treated with β-Sarcoglycan Gene Transfer Improved on the 100 Meter Timed Test at Theee Months Post-Adminstration

[00207] Treatment with scAAVrh74.MHCK7.hSGCB provided patients with demonstrable improvement in the 100 meter timed test over only a 3-month period following gene transfer (Figure 11). Timed walking tests, like the 100 meter timed test, are used to measure function in subjects with a muscular dystrophy. The test in this study measured the patients baseline performance compared to their performance after treatment. Figure 11 shows the mean percent change from baseline in three subjects over the first three months post-gene transfer. The data show that there was a greater than 15% mean increase over baseline after 3 months, demonstrating the improvement in motor function after β-sarcoglycan gene transfer.

Example 8

LGMD2E Patients Treated with β-Sarcoglycan Gene Transfer Showed Improved Functional Measures at Nine Months Post-Administration

[00208] Treatment with scAAVrh74.MHCK7.hSGCB provided patients with demonstrable and improvement nine months following systemic administration of scAAVrh.74.MHCK7.hSGCB. Three patients participated the functional study. For example, in a 100 m timed test, at the baseline (before administration) one patient had limited hip extension and flexion when running the 100 m. However, at 9 months post-administration, the same patient showed improved hip extension and flexion and a faster speed when running. In addition, for the trunk control test, another patient showed an improvement in the time to rise test 9 months after post-administration. At baseline or before administration, the subjects showed poor trunk control but this was also improved 9 months post-administration. Also, in the sitting up test, the patients were asked to sit up from the sitting position. The remaining patient, for example, showed a shortened getting up time 9-month post administration as compared to that before administration. These data are summarized in Table 10.

Table 10

Subject	Assesssment	NSAD (Δ)	Time to Rise (sec)	4 Stairs Up (sec)	100 m (sec)	10 m (sec)
1	Baseline	40	5.0	2.4	49.3	5
	Day 270	41	4.1	2.3	43.2	4.5
2	Baseline	41	3.5	2.8	49.9	5.2
	Day 270	47	3.0	1.9	48.6	4.3
3	Baseline	48	1.5	1.6	59.3	3.4
	Day 270	54	1.2	1.3	48.4	3.2

[00209] An age matched natural history study compared the change from baseline in the NSAD, herein denoted as "North Star Assessment for Limb Girdle Muscular Dystrophies," for untreated subjects (denoted as natural history subjects; see Table 11) and subjects administered scAAVrh74.MHCK7.hSGCB. As shown in Figures 12A-C, the natural history subjects had a steady decreased in change in NSAD over 200 days, while the treated subjects showed a steady improvement in change in NSAD over 270 days (Figures 12A and 12C), while the treated subject showed a steady improvement in change in NSAD over 270 days (Figure 12B).

Table 11

Subject	Age (years)
1	5
2	12
3	10
4	9
5	9

Example 9

Formulations

[00210] scAAVrh74.MHCK7.hSGCB is formulated in a buffer containing 20 mM Tris (pH 8.0), 1mM magnesium chloride ($MgCl_2$), 200 mM sodium chloride (NaCl), and 0.001% Poloxamer 188. In one embodiment, the formulation information is summarized in Table 12.

Table 12

Formulation (as Frozen Liquid)	
Component	**Concentration**
scAAVrh.74.MHCK7.hSGCB	2×10^{13} vg/ml, 5×10^{13} vg/ml, or 4×10^{13} vg/ml [a]
Tris (pH 8.0)	20 mM
Magnesium Chloride (MgCl2)	1 mM
Sodium Chloride (NaCl)	200 mM
Poloxamer 188	0.001%

[00211] The drug product is stored as a frozen liquid at temperatures below -60°C. The frozen drug product must be thawed prior to clinical administration.

[00212] scAAVrh74.MHCK7.hSGCB is stored at temperatures below -60°C, under which the material is stable under the long-term storage condition. scAAVrh74.MHCK7.hSGCB vials are thawed at room temperature (20°C to 25°C). Thawed vector vials are wiped with alcohol and placed in the biosafety cabinet. The scAAVrh74.MHCK7.hSGCB formulation is prepared aseptically in a Class II biosafety cabinet under sterile conditions.

[00213] The scAAVrh74.MHCK7.hSGCB for intravenous (IV) infusion is supplied in a vial (2 mL per vial). The total vg dose is calculated based on the patient's body weight. The appropriate number of vials is determined for each patient based on body weight at the equivalent of 5×10^{13} vg/kg or 2×10^{14} vg/kg, as well as product titer for the scAAVrh74.MHCK7.hSGCB lot of 2×10^{13} vg/mL, 5×10^{13} vg/mL, or 4×10^{13} vg/ml.

[00214] The scAAVrh74.MHCK7.hSGCB is administered as a one-time IV infusion, delivered over approximately 1 to 2 hours via syringe pump into a peripheral limb vein.

Example 10

Elder patients and durability

[00215] scAAVrh74.MHCK7.hSGCB-mediated gene replacement has shown positive results in treating LGMD-2E and other associated diseases. The study is to test the ability of scAAVrh74.MHCK7.hSGCB to treat older more severely affected muscle, and the long-term durability of the AAV viral vector. First, for the long-term durability study, $sgcb^{-/-}$ mice at 4 weeks of age were treated systemically with scAAVrh74.MHCK7.hSGCB. More than 24 months post-treatment, high-level vector

genome copy numbers were detected with PCR across all transduced muscles. Moreover, immunofluorescence staining of treated muscle showed no decrease of protein expression levels in all muscles (>95%) compared to earlier timepoints, with hSGCB protein remaining correctly localized at the membrane.

[00216] Second, a mouse model of LGMD2E (β-sarcoglycan) is treated at older age (e.g., 12 month) with systemic delivery of an scAAVrh74.MHCK7.hSGCB vector. At the 6-month endpoint post treatment, the muscle from these mice are evaluated for protein expression, histological rescue, and functional improvement. In particular, the gene expression in muscles throughout the lower limb, upper limb, and proximal torso muscles, including the diaphragm and heart, is observed. Moreover, the level of fibrosis is compared to untreated controls. Further, a functional study involves evaluation of force output in the tibialis anterior (TA) and diaphragm (DIA) muscle and resistance to contraction-induced injury in the TA muscle.

[00217] While the present disclosure has been described in terms of specific embodiments, it is understood that variations and modifications will occur to those skilled in the art. Accordingly, only such limitations as appear in the claims should be placed on the disclosure.

[00218] All documents referred to in this application are hereby incorporated by reference in their entirety.

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Claims

What is claimed is:

1. A method of treating muscular dystrophy in a subject in need thereof comprising the step of administering a recombinant adeno-associated virus (rAAV) scAAVrh74.MHCK7.hSGCB to the subject, wherein

the rAAV is administered using a systemic route of administration and at a dose of about 1.0×10^{12} vg/kg to about 5.0×10^{14} vg/kg based on a supercoiled plasmid as the quantitation standard; wherein the serum creatine kinase (CK) level in the subject is decreased after administration of the rAAV as compared to the serum CK level before administration of the rAAV.

2. A method of treating muscular dystrophy in a subject in need thereof comprising the step of administering a recombinant adeno-associated virus (rAAV) scAAVrh74.MHCK7.hSGCB,

wherein the level of beta-sarcoglycan gene expression in a cell of the subject is increased after administration of the rAAV as compared to the level of beta-sarcoglycan gene expression before administration of the rAAV;

wherein the number of beta-sarcoglycan positive fibers in the muscle tissue of the subject is increased after administration of the rAAV as compared to the number of beta-sarcoglycan positive fibers before administration of the rAAV; or

wherein motor function is improved in said subject as compared to the motor function of said subject before administration of the rAAV, and wherein the motor function is determined by a 100 meter timed walk test.

3. The method of claim 2, wherein the motor function is improved by at least 5% in 1 month or thirty days post-gene transfer, at least 10 % in 2 months or sixty days post-gene transfer, or at least 15% in 3 months or ninety days post gene transfer.

4. The method of claim 2 or 3, wherein the motor function is improved by at least 5%, 10%, 15%, 20%, 25%, 30%, 40%, 45%, or 50%.

5. The method of any one of claims 1-4, wherein the rAAV is administered using an intravenous route.

6. The method of any one of claims 1-5, wherein the rAAV is administered at about 5.0 x 10^{13} vg/kg or about 2.0 x 10^{14} vg/kg based on a supercoiled plasmid as the quantitation standard, or about 1.85 x 10^{13} vg/kg or 7.41 x 10^{13} vg/kg based on a linearized plasmid as the quantitation standard.

7. The method of any one of claims 1-6, wherein rAAV is administered at a concentration of about 10 mL/kg.

8. The method of any one of claims 1-7, wherein the rAAV is administered by injection, infusion or implantation.

9. The method of any one of claims 1-8, wherein the rAAV is administered by infusion over approximately 1 to 2 hours.

10. The method of any one of claims 1-8, wherein the rAAV is administered by an intravenous route through a peripheral limb vein.

11. The method of any one of claims 1-10, wherein the rAAV comprises the human β-sarcoglycan nucleotide sequence of SEQ ID NO: 1.

12. The method of any one of claims 1-11, wherein the rAAV comprises the MHCK7 promoter sequence of SEQ ID NO: 4.

13. The method of any one of claims 1-12, wherein the rAAV is of serotype AAVrh.74.

14. The method of any one of claims 1-13, wherein the rAAV comprises a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 3 or SEQ ID NO: 19.

15. The method of any one of claims 1-14, wherein the rAAV comprises an intron sequence of SEQ ID NO: 20.

16. The method of any one of claims 1-15, wherein the rAAV comprises a polyA sequence of SEQ ID NO: 21.

17. The method of any one of claims 1-16, wherein the rAAV comprises a 5' inverted terminal repeat (ITR) sequence of SEQ ID NO: 22.

18. The method of any one of claims 1-17, wherein the rAAV comprises a 3' inverted terminal repeat (ITR) sequence of SEQ ID NO: 23.

19. The method of any one of claims 1-18, wherein the muscular dystrophy is limb-girdle muscular dystrophy.

20. The method of any one of claims 1-18, wherein the muscular dystrophy is limb-girdle muscular dystrophy type 2E.

21. A method of treating a limb-girdle muscular dystrophy in a subject in need, comprising administering to the subject an rAAV intravenous infusion over approximately 1 to 2 hours at a dose of about 5.0×10^{13} vg/kg or about 2.0×10^{14} vg/kg based on a supercoiled plasmid as the quantitation standard, or about 1.85×10^{13} vg/kg or 7.41×10^{13} vg/kg based on a linearized plasmid as the quantitation standard, and wherein the rAAV comprises comprises a nucleotide sequence of SEQ ID NO: 3 or SEQ ID NO: 19.

22. A method of expressing beta-sarcoglycan gene in a subject's cell comprising administering to the subject the scAAVrh74.MHCK7.hSGCB construct that comprises a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 19.

23. A method of increasing beta-sarcoglycan positive fibers and/or decreasing CK level in a subject's muscle tissue comprising administering to the subject the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 19.

24. The method of claim 22 or 23, wherein expression of the beta-sarcoglycan gene or the number of positive beta-sarcoglycan positive fibers is detected by measuring the beta-sarcoglycan protein level on a Western blot in muscle biopsies before and after administration of the rAAV.

25. The method of claim 22, wherein the expression of beta-sarcoglycan protein is increased by at least 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40 % after administration of rAAV.

26. The method of any one of claims 22-25, wherein expression of beta-sarcoglycan gene or number of beta-sarcoglycan positive muscle fibers is detected by measuring the beta-sarcoglycan protein level by immunohistochemistry in muscle biopsies before and after administration of the rAAV.

27. The method of claim 22, wherein the expression of beta-sarcoglycan protein is increased by at least 39 % after administration of rAAV.

28. The method of claim 23, wherein the number of beta-sarcoglycan positive fibers in the muscle tissue of the subject is increased by at least 40, 41, or 42% after administration of the rAAV as compared to the number of beta-sarcoglycan positive fibers before administration of the rAAV.

29. The method of claim 22, wherein the cell has more than one AAV viral copy number.

30. The method of any one of claims 22-29, wherein the serum CK level in the subject is decreased after administration of the rAAV as compared to serum CK level before this administration of the rAAV.

31. The method of claim 30, wherein the serum CK level in the subject is decreased by at least 82, 83, 84, 85, 86, 87, 88, 89, or 90% by 60 days to 90 days, 60 days, or 90 days after administration of the rAAV as compared to the serum CK level before administration of the rAAV.

32. The method of any one of claims 1-31, wherein the level of alpha-sarcoglycan in the subject is increased after administration of the rAAV as compared to the level of alpha-sarcoglycan before administration of the rAAV.

33. A method of increasing the expression of alpha-sarcoglycan in a subject in need thereof comprising administering to the subject an rAAV comprising a scAAVrh74.MHCK7.hSGCB construct with a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 3 or SEQ ID NO: 19.

34. A method of increasing localization of alpha-sarcoglycan to a cell membrane in a subject in need thereof comprising administering to the subject the scAAVrh74.MHCK7.hSGCB construct nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 3 or SEQ ID NO: 19.

35. The method of claim 33 or 34, wherein the alpha-sarcoglycan is detected by measuring the alpha-sarcoglycan protein level by immunohistochemistry on muscle biopsies before and after administration of the rAAV.

36. The method of claim 33 or 34, wherein the alpha-sarcoglycan is detected by measuring the alpha-sarcoglycan protein level by Western blot on muscle biopsies before and after administration of the rAAV.

37. The method of any one of claims 34-36, wherein said alpha-sarcoglycan is colocalized to the membrane of a cell expressing a beta-sarcoglycan encoded by scAAVrh74.MHCK7.hSGCB.

38. A method of increasing sarcoglycan expression in muscle tissue or improving muscle function of a subject comprising administering to the subject an rAAV comprising a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 19.

39. The method of claim 38, wherein the subject carries a genetic mutation in a gene encoding a sarcoglycan or suffers from a muscular dystrophy.

40. The method of claim 38, wherein the sarcoglycan is β-sarcoglycan (SGCB), α-sarcoglycan (SGCA), γ-sarcoglycan (SGCG), or δ-sarcoglycan (SGCD).

41. The method of claim 38, wherein the nucleotide sequence comprises a polynucleotide sequence of SEQ ID NO: 19.

42. A method of increasing sarcoglycan expression in muscle tissue of a subject comprising administering to the subject a construct comprising a nucleotide sequence encoding a first sarcoglycan, and detecting increased expression of at least a second sarcoglycan in the cell membrane of the cell expressing said first sarcoglycan.

43. The method of claim 42, wherein said first sarcoglycan is β-sarcoglycan (SGCB), and said second sarcoglycan is α-sarcoglycan (SGCA), γ-sarcoglycan (SGCG), and/or δ-sarcoglycan (SGCD).

44. The method of any one of claims 1-43, wherein the subject is a human subject that is 4 to 15 years of age.

45. The method of any one of claims 1-43, wherein the subject is a pediatric subject, an adolescent subject or a young adult subject.

46. The method of any one of claims 1-43, wherein the subject is a human subject that is 4-15 years of age, has confirmed beta-sarcoglycan (SGCB) mutation in both alleles, was negative for AAVrh74 antibodies and/or had >40% or normal 100 meter walk test.

47. The method of any one of claims 1-43, wherein the subject is a middle aged adult or elderly subject.

48. The method of any one of claims 1-43, wherein the subject is a human subject that is 25 to 55 years of age.

49. The method of any one of claims 1-43, wherein the subject is a human subject that is over 50 years of age.

50. A composition, comprising
an rAAV scAAVrh74.MHCK7.hSGCB vector ,
a buffer agent,
an ionic strength agent, and
a surfactant.

51. The composition of claim 50, wherein the rAAV is at a concentration of about 1.0 x 10^{12} vg/ml to about 5.0 x 10^{14} vg/ml, or about 5.0 x 10^{12} vg/ml to about 1.0 x 10^{14} vg/ ml.

52. The composition of claim 50, wherein the rAAV is at a concentration of about 2.0 x 10^{13} vg/ml, 4 × 10^{13} vg/ml, 5× 10^{13} vg/ml.

53. The composition of claim 50, wherein the buffer agent comprises one or more of tris, tricine, Bis-tricine, HEPES, MOPS, TES, TAPS, PIPES, and CAPS.

54. The composition of claim 53, wherein the buffer agent comprises the tris with pH 8.0 at concentration of about 5 mM to about 40 mM.

55. The composition of claim 53, where the buffer agent comprises the tris with pH 8.0 at about 20 mM.

56. The composition of claim 50, wherein the ionic strength agent comprises one or more of potassium chloride (KCl), potassium acetate, potassium sulfate, ammonium sulfate, ammonium chloride (NH_4Cl), ammonium acetate, magnesium chloride ($MgCl_2$), magnesium acetate, magnesium sulfate, manganese chloride ($MnCl_2$), manganese acetate, manganese sulfate, sodium chloride (NaCl), sodium acetate, lithium chloride (LiCl), and lithium acetate.

57. The composition of claim 50, wherein the ionic strength agent comprises $MgCl_2$ at a concentration of about 0.2 mM to about 4 mM.

58. The composition of claim 50, wherein the ionic strength agent comprises NaCl at a concentration of about 50 mM to about 500 mM.

59. The composition of claim 50, wherein the ionic strength agent comprises $MgCl_2$ at a concentration of about 0.2 mM to about 4 mM and NaCl at a concentration of about 50 mM to about 500 mM.

60. The composition of claim 50, wherein the ionic strength agent comprises $MgCl_2$ at a concentration of about 1 mM and NaCl at a concentration of about 200 mM.

61. The composition of claim 50, wherein the surfactant comprises one or more of a sulfonate, a sulfate, a phosphonate, a phosphate, a Poloxamer, and a cationic surfactant.

62. The composition of claim 61, wherein the Poloxamer comprises one or more of Poloxamer 124, Poloxamer 181, Poloxamer 184, Poloxamer 188, Poloxamer 237, Poloxamer 331, Poloxamer 338, and Poloxamer 407.

63. The composition of claim 61, wherein the surfactant comprises the Poloxamer at a concentration of about 0.00001% to about 1%.

64. The composition of claim 61, wherein the surfactant comprises Poloxamer 188 at a concentration of about 0.001%.

65. A pharmaceutical composition comprising a recombinant AAV (rAAV) scAAVrh74.MHCK7.hSGCB, wherein the scAAVrh74.MHCK7.hSGCB comprises nucleotide sequence that is at least 95% or 99% identical to SEQ ID NO: 19.

66. The pharmaceutical composition of claim 65, wherein the scAAVrh74.MHCK7.hSGCB comprises a nucleotide sequence of SEQ ID NO: 19.

67. A method of generating a recombinant AAV scAAVrh74.MHCK7.hSGCB, comprising transferring a plasmid to a cell, wherein the plasmid comprises a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 24.

68. The method of claim 83, wherein the plasmid comprises a nucleotide sequence of SEQ ID NO: 24.

69. The method of claim 67, wherein the plasmid comprises a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 1, 3, or 19.

70. The method of any one of claims 67-69, wherein the plasmid comprises a nucleotide sequence of SEQ ID NO: 19.

71. The method of any one of claims 67-70, further comprising transferring a packaging plasmid and/or a helper virus to the cell.

72. The method of any one of claims 67-70, wherein the cell comprises a stably integrated AAV cap gene.

73. The method of any one of claims 67-70, wherein the cell comprises a stably integrated AAV rep gene.

74. A cell, comprising a plasmid that comprises a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 24.

75. The cell of claim 74, wherein the plasmid that comprises a nucleotide sequence of SEQ ID NO: 24.

76. The cell of claim 74 or 75, comprising a nucleotide sequence of SEQ ID NO: 19.

77. The cell of any one of claims 74-76, wherein the cell is an insect cell, a mosquito cell, or a mammalian cell.