

Claims

~~What is claimed is:~~

1. Use of a recombinant adeno-associated virus (rAAV) scAAVrh74.MHCK7.hSGCG in the manufacture of a medicament for ~~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.hSGCG to the subject~~, wherein

the rAAV is to be administered using a systemic route of administration and at a dose of about 2×10^{12} vg/kg to about 5.0×10^{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 the serum CK level before administration of the rAAV.

2. Use of a recombinant adeno-associated virus (rAAV) scAAVrh74.MHCK7.hSGCG in the manufacture of a medicament for ~~A method of~~ restoring dystrophin-associated protein complex in a subject in need thereof ~~comprising the step of administering a recombinant adeno-associated virus (rAAV) scAAVrh74.MHCK7.hSGCG~~, wherein the rAAV is to be administered using a systemic route of administration and at a dose of about 2×10^{12} vg/kg to about 5.0×10^{14} vg/kg based on a linearized plasmid as the quantitation standard.

3. The ~~method-use~~ of claim 1 or 2, wherein the level of gamma-sarcoglycan gene expression in a cell of the subject is increased after administration of the rAAV as compared to the level of gamma-sarcoglycan gene expression before administration of the rAAV;

wherein the average rAAV copy number in a muscle cell of the treated subject is at least 0.01 copy per nucleus;

wherein the centralized nuclei percentage and/or the fibrosis is reduced in the muscle of the treated subject as compared to the level before administration of the rAAV;

wherein motor function is improved in said subject as compared to the motor function of said subject before administration of the rAAV;

wherein the NSAD are increased in in said subject as compared to the NSAD of said subject before administration of the rAAV; and/or

wherein the ALT and AST levels are reduced in in said subject as compared to the ALT and AST levels of said subject before administration of the rAAV.

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

5. The ~~method-use~~ of any one of claims 1-4, wherein the rAAV is to be administered using an intravenous route.
6. The ~~method-use~~ of any one of claims 1-5, wherein the rAAV is to be administered at 4.63×10^{12} vg/kg, about 1.85×10^{13} vg/kg or 7.41×10^{13} vg/kg based on a linearized plasmid as the quantitation standard or about 1.25×10^{13} vg/kg, about 5.0×10^{13} vg/kg or about 2.0×10^{14} vg/kg based on a supercoiled plasmid as the quantitation standard.
7. The ~~method-use~~ of any one of claims 1-6, wherein rAAV is to be administered at a concentration of about 10 mL/kg.
8. The ~~method-use~~ of any one of claims 1-7, wherein the rAAV is to be administered by injection, infusion or implantation.
9. The ~~method-use~~ of any one of claims 1-8, wherein the rAAV is to be administered by infusion over approximately 1 to 2 hours.
10. The ~~method-use~~ of any one of claims 1-8, wherein the rAAV is to be administered by an intravenous route through a peripheral limb vein.
11. The ~~method-use~~ of any one of claims 1-10, wherein the rAAV comprises the human γ -sarcoglycan nucleotide sequence of SEQ ID NO: 1.
12. The ~~method-use~~ of any one of claims 1-11, wherein the rAAV comprises the MHCK7 promoter sequence of SEQ ID NO: 2.
13. The ~~method-use~~ of any one of claims 1-12, wherein the rAAV is of serotype AAVrh.74.
14. The ~~method-use~~ 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: 1 or SEQ ID NO: 7.
15. The ~~method-use~~ of any one of claims 1-14, wherein the rAAV comprises an intron sequence of SEQ ID NO: 6.
16. The ~~method-use~~ of any one of claims 1-15, wherein the rAAV comprises a polyA sequence of SEQ ID NO: 5.

17. The ~~method-use~~ of any one of claims 1-16, wherein the rAAV comprises a 5' inverted terminal repeat (ITR) sequence of SEQ ID NO: 3.
18. The ~~method-use~~ of any one of claims 1-17, wherein the rAAV comprises a 3' inverted terminal repeat (ITR) sequence of SEQ ID NO: 4.
19. The ~~method-use~~ of any one of claims 1-18, wherein the muscular dystrophy is limb-girdle muscular dystrophy.
20. The ~~method-use~~ of any one of claims 1-18, wherein the muscular dystrophy is limb-girdle muscular dystrophy type 2C.
21. ~~Use of 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 4.63×10^{12} vg/kg, about 1.85×10^{13} vg/kg or 7.41×10^{13} vg/kg based on a linearized plasmid as the quantitation standard or about 1.25×10^{13} vg/kg, about 5.0×10^{13} vg/kg or about 2.0×10^{14} vg/kg based on a supercoiled plasmid as the quantitation standard, and wherein the rAAV comprises a nucleotide sequence of SEQ ID NO: 1 or SEQ ID NO: 7, in the manufacture of a medicament for treating a limb-girdle muscular dystrophy in a subject in need.
22. ~~A method of expressing gamma-sarcoglycan gene in a cell of a subject comprising administering to the subject~~ Use of the scAAVrh74.MHCK7.hSGCG construct that comprises a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 7 in the manufacture of a medicament for expressing gamma-sarcoglycan gene in a cell of a subject.
23. ~~A method of increasing gamma-sarcoglycan positive fibers and/or decreasing CK level in a subject's muscle tissue comprising administering to the subject~~ Use of the scAAVrh74.MHCK7.hSGCG construct nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 7 in the manufacture of a medicament for increasing gamma-sarcoglycan positive fibers and/or decreasing CK level in a subject's muscle tissue.
24. The ~~method-use~~ of claim 22 or 23, wherein expression of the gamma-sarcoglycan gene or the number of positive gamma-sarcoglycan positive fibers is detected by measuring the gamma-sarcoglycan protein level on a Western blot in muscle biopsies before and after administration of the rAAV.

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

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

28. The ~~method-use~~ of claim 23, wherein the number of gamma-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 gamma-sarcoglycan positive fibers before administration of the rAAV.

29. The ~~method-use~~ of claim 22, wherein at least one cell has more than one AAV viral copy number.

30. The ~~method-use~~ 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-use~~ 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-use~~ of any one of claims 1-31, wherein the level of alpha-sarcoglycan and/or beta-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 and/or beta-sarcoglycan in a subject in need thereof comprising administering to the subject~~ Use of an rAAV comprising a scAAVrh74.MHCK7.hSGCG construct with a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 1 or SEQ ID NO: 7 in the manufacture of a

medicament for increasing the expression of alpha-sarcoglycan and/or beta-sarcoglycan in a subject in need thereof.

34. ~~A method of increasing localization of alpha-sarcoglycan and/or beta-sarcoglycan to a cell membrane in a subject in need thereof comprising administering to the subject~~ Use of the scAAVrh74.MHCK7.hSGCG construct nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 1 or SEQ ID NO: 7 in the manufacture of medicament for increasing localization of alpha-sarcoglycan and/or beta-sarcoglycan to a cell membrane in a subject in need thereof.

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

36. The ~~method-use~~ of claim 33 or 34, wherein the alpha-sarcoglycan and/or beta-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-use~~ of any one of claims 34-36, wherein said alpha-sarcoglycan and/or beta-sarcoglycan is colocalized to the membrane of a cell expressing a gamma-sarcoglycan encoded by scAAVrh74.MHCK7.hSGCG.

38. ~~A method of increasing sarcoglycan expression in muscle tissue or improving muscle function of a subject comprising administering to the subject~~ Use of an rAAV comprising a nucleotide sequence that is at least 90%, 95%, or 99% identical to SEQ ID NO: 7 in the manufacture of a medicament for increasing sarcoglycan expression in muscle tissue or improving muscle function of a subject.

39. The ~~method-use~~ 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-use~~ of claim 38, wherein the sarcoglycan is one or more of β -sarcoglycan (SGCB), α -sarcoglycan (SGCA), γ -sarcoglycan (SGCG), and δ -sarcoglycan (SGCD).

41. The ~~method-use~~ of claim 38, wherein the nucleotide sequence comprises a polynucleotide sequence of SEQ ID NO: 7.

42. ~~A method of increasing sarcoglycan expression in muscle tissue of a subject comprising administering to the subject~~ Use of 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 the manufacturement of a medicament for increasing sarcoglycan expression in muscle tissue of a subject.

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

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

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

48. The ~~method~~-use 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~~-use 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.hSGCG 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 1.0×10^{12} vg/ml to about 5.0×10^{14} vg/ml, or about 5.0×10^{12} vg/ml to about 1.0×10^{14} vg/ml.

52. The composition of claim 50, wherein the rAAV is at a concentration of about 2.0×10^{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 (NH₄Cl), ammonium acetate, magnesium chloride (MgCl₂), magnesium acetate, magnesium sulfate, manganese chloride (MnCl₂), 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 MgCl₂ 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 MgCl₂ 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 MgCl₂ 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.hSGCG, wherein the scAAVrh74.MHCK7.hSGCG comprises nucleotide sequence that is at least 95% or 99% identical to SEQ ID NO: 7.
66. The pharmaceutical composition of claim 65, wherein the scAAVrh74.MHCK7.hSGCG comprises a nucleotide sequence of SEQ ID NO: 7.
67. A method of generating a recombinant AAV scAAVrh74.MHCK7.hSGCG, 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: 8.
68. The method of claim 83, wherein the plasmid comprises a nucleotide sequence of SEQ ID NO: 8.
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, or 7.
70. The method of any one of claims 67-69, wherein the plasmid comprises a nucleotide sequence of SEQ ID NO: 7.
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: 8.
75. The cell of claim 74, wherein the plasmid that comprises a nucleotide sequence of SEQ ID NO: 8.
76. The cell of claim 74 or 75, comprising a nucleotide sequence of SEQ ID NO: 7 or 10.
77. The cell of any one of claims 74-76, wherein the cell is an insect cell, a mosquito cell, or a mammalian cell.
78. A method of measuring SGCG expression in a cell or a subject, wherein the method comprises a PCR analysis with the primers of SEQ ID NO: 11 and SEQ ID NO: 12.

79. The method of claim 78, wherein the subject suffers from a LGMD.
80. The method of claim 78 or 79, wherein the method comprises measuring expression of the scAAVrh74.MHCK7.hSGCG vector in the cell or the subject.
81. A method of measuring the titer of an AAV vector, comprising titrating the AAV vector with a PCR analysis with a first primer of SEQ ID NO: 13 and a second primer of SEQ ID NO: 14.
82. A method of measuring the titer of an AAV vector, comprising titrating the AAV vector with a probe comprising a sequence of SEQ ID NO: 15.
83. The method of claim 82, wherein the probe comprise 5'-FAM-TGG ATC CCC-Zen-TGC ATG CGA AGA TC-3IABKFQ.
84. The method of any one of claims 81-83, the AAV vector is an scAAVrh74.MHCK7.hSGCG vector.