

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) AAVrh74.tMCK.hSCGA, 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^{15} vg/kg based on a supercoiled DNA or plasmid as the quantitation standard.
2. The method of claim 1, wherein the rAAV is administered at a dose of about 1.0×10^{12} vg/kg to about 2.0×10^{15} vg/kg, about 5×10^{12} vg/kg to about 1.0×10^{15} vg/kg, about 1.0×10^{13} vg/kg to about 5.0×10^{14} vg/kg, about 2.0×10^{13} vg/kg to about 3.0×10^{14} vg/kg, or about 5×10^{13} vg/kg to about 2×10^{14} vg/kg based on a supercoiled DNA or plasmid as the quantitation standard.
3. The method of claim 1, wherein the rAAV is administered at a dose about 5×10^{13} vg/kg to about 2×10^{14} vg/kg based on a supercoiled DNA or plasmid as the quantitation standard.
4. The method of claim 1, wherein the rAAV is administered at a dose about 5×10^{13} vg/kg, about 1×10^{14} vg/kg, or about 2×10^{14} vg/kg based on a supercoiled DNA or plasmid as the quantitation standard.
5. A method of treating muscular dystrophy in a subject in need thereof comprising the step of administering a recombinant adeno-associated virus (rAAV) AAVrh74.tMCK.hSCGA, wherein the rAAV is administered using a systemic route of administration and is administered at a dose about 1.85×10^{13} vg/kg or 7.41×10^{13} vg/kg based on a linearized DNA or plasmid as the quantitation standard.
6. The method of any one of claims 1-5, wherein the level of alpha-sarcoglycan gene expression in a cell of the subject is increased after administration of the rAAV as compared to the level of alpha-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; wherein the locomotor activity and specific-force generation are increased; wherein fibrosis is reduced; wherein the resistance to contraction-induced injury in tibialis anterior muscle is increased; and/or wherein the number of alpha-sarcoglycan positive fibers in the muscle tissue of the subject is increased after administration of the rAAV as compared to the number of alpha-sarcoglycan positive fibers before administration of the rAAV.
7. The method of any one of claims 1-6, wherein the systemic route of administration is an intravenous route.
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.
10. The method of any one of claims 1-9, 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 a nucleotide sequence with at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 1.
12. The method of claim 11, wherein the rAAV comprises a nucleotide sequence of SEQ ID NO: 1.
13. The method of any one of claims 1-12, wherein the rAAV comprises a nucleotide sequence encoding a polypeptide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical with SEQ ID NO: 2.
14. The method of any one of claims 1-12, wherein the rAAV comprises a nucleotide sequence encoding a polypeptide sequence set forth in SEQ ID NO: 2.
15. The method of any one of claims 1-14, wherein the rAAV comprises the scAAVrh74.tMCK.hSGCA construct nucleotide sequence with at least at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 4.
16. The method of claim 15, wherein the rAAV comprises the scAAVrh74.tMCK.hSGCA construct nucleotide sequence of SEQ ID NO: 4.
17. The method of any one of claims 1-16, wherein the rAAV comprises a tMCK promoter.
18. The method of any one of claims 1-16, wherein the tMCK promoter comprises a nucleotide sequence set forth in SEQ ID NO: 3.
19. The method of any one of claims 1-18, wherein the rAAV comprises a 5' inverted terminal repeat sequence of SEQ ID NO: 5.
20. The method of any one of claims 1-19, wherein the rAAV comprises a 3' inverted terminal repeat sequence of SEQ ID NO: 6.
21. The method of any one of claims 1-20, wherein the rAAV comprises a poly A sequence of SEQ ID NO: 7.
22. The method of any one of claims 1-21, wherein the rAAV is of the serotype AAVrh.74.
23. The method of any one of claims 1-22, wherein the muscular dystrophy is limb-girdle muscular dystrophy.
24. The method of any one of claims 1-23, wherein the muscular dystrophy is limb-girdle muscular dystrophy type 2D (LGMD2D).
25. The method of any one of claims 1-24, wherein the subject is suffering from limb-girdle muscular dystrophy, and the rAAV is administered by intravenous infusion at a dose of about 5×10^{13} vg/kg to about 2×10^{14} vg/kg based on a supercoiled DNA or plasmid as the quantitation standard, and

wherein the rAAV comprises the scAAVrh74.tMCK.hSGCA construct nucleotide sequence of SEQ ID NO: 4.

26. The method of any one of claims 1-25, wherein the level of alpha-sarcoglycan gene expression in a cell of the subject is increased after administration of the rAAV as compared to the level of alpha-sarcoglycan gene expression before administration of the rAAV.

27. The method of any one of claims 1-26, wherein fibrosis is reduced in the subject after administration of the rAAV as compared to before administration of the rAAV.

28. The method of any one of claims 1-26, wherein the fibrosis, the central nucleation, the CK level, and/or collagen deposition in the subject is reduced after administration of the rAAV as compared to before administration of the rAAV.

29. The method of any one of claims 1-26, wherein the specific force, the fiber diameter size, and/or the eccentric contraction in the muscle of the subject are increased after administration of the rAAV as compared to before administration of the rAAV.

30. The method of claim 26, wherein the alpha-sarcoglycan gene expression is detected by measuring the alpha-sarcoglycan protein level by Western blot, and/or immunohistochemistry.

31. A method of expressing alpha-sarcoglycan gene in a cell comprising administering to a subject the scAAVrh74.tMCK.hSGCA construct comprising a nucleotide sequence of SEQ ID NO: 4.

32. The method of claim 31, wherein expression of the alpha-sarcoglycan gene in the cell of the subject is detected by measuring the alpha-sarcoglycan protein level on a Western blot in muscle biopsies.

33. The method of claim 31, wherein expression of the alpha-sarcoglycan gene in the cell is detected by measuring the alpha-sarcoglycan protein level by immunohistochemistry in muscle biopsies.

34. The method of claim 31, wherein expression of the alpha-sarcoglycan gene is measured in the subject by detecting the number of vector genome per microgram of genomic DNA.

35. A method of decreasing a serum CK level in a subject in need thereof, the method comprising administering to the subject the scAAVrh74.tMCK.hSGCA construct nucleotide sequence of SEQ ID NO: 4.

36. A method of increasing alpha-sarcoglycan positive fibers in a muscle tissue of a subject comprising administering to the subject the scAAVrh74.tMCK.hSGCA construct nucleotide sequence of SEQ ID NO: 4.

37. A method of increasing the expression of alpha-sarcoglycan in a subject in need thereof comprising administering to the subject an effective amount of the scAAVrh74.tMCK.hSGCA construct nucleotide sequence of SEQ ID NO: 4.
38. A composition for treating muscular dystrophy in a subject in need thereof, wherein the composition comprises a recombinant adeno-associated virus (rAAV) AAVrh74.tMCK.hSCGA at a dose of about 1.0×10^{12} vg/kg to about 5.0×10^{15} vg/kg based on a supercoiled DNA or plasmid as the quantitation standard, and wherein the composition is formulated for a systemic route of administration.
39. The composition for treating muscular dystrophy in a subject in need thereof, wherein the composition comprises a recombinant adeno-associated virus (rAAV) AAVrh74.tMCK.hSCGA at a dose of about 1.85×10^{13} vg/kg or 7.41×10^{13} vg/kg based on a linearized DNA or plasmid as the quantitation standard and wherein the composition is formulated for a systemic route of administration.
40. A composition comprises a recombinant adeno-associated virus (rAAV), wherein the rAAV comprises the scAAVrh74.tMCK.hSGCA construct nucleotide sequence with at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 4.
41. The composition of claim 38 or 40, wherein the rAAV is at a dose about 5×10^{13} vg/kg to about 2×10^{14} vg/kg based on a supercoiled DNA or plasmid as the quantitation standard.
42. The composition of claim 38 or 40, wherein the rAAV is at a dose about 5×10^{13} vg/kg, about 1×10^{14} vg/kg, or about 2×10^{14} vg/kg based on a supercoiled DNA or plasmid as the quantitation standard.
43. The composition of any one of claims 38-42, wherein the level of alpha-sarcoglycan gene expression in a cell of the subject is increased after administration of the composition as compared to the level of alpha-sarcoglycan gene expression before administration of the composition; or wherein the serum CK level in the subject is decreased after administration of the composition as compared to serum CK level before administration of the composition; or wherein the number of alpha-sarcoglycan positive fibers in the muscle tissue of the subject is increased after administration of the composition as compared to the number of alpha-sarcoglycan positive fibers before administration of the composition.
44. The composition of any one of claims 38-43, wherein the systemic route of administration is an intravenous route.
45. The composition of any one of claims 38-44, wherein the composition is formulated for administration by injection, infusion or implantation.
46. The composition of any one of claims 38-45, wherein the composition is formulated for administration by infusion.

47. The composition of any one of claims 38-46, wherein the composition is formulated for administration by an intravenous route through a peripheral limb vein.
48. The composition of any one of claims 38-47, wherein the rAAV comprises a nucleotide sequence with at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 1.
49. The composition of claim 48, wherein the rAAV comprises a nucleotide sequence of SEQ ID NO: 1.
50. The composition of any one of claims 38-49, wherein the rAAV comprises a nucleotide sequence encoding a polypeptide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical with SEQ ID NO: 2.
51. The composition of any one of claims 38-49, wherein the rAAV comprises a nucleotide sequence encoding a polypeptide sequence set forth in SEQ ID NO: 2.
52. The composition of any one of claims 38-49, wherein the rAAV comprises the scAAVrh74.tMCK.hSGCA construct nucleotide sequence with at least at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 4.
53. The composition of claim 52, wherein the rAAV comprises the scAAVrh74.tMCK.hSGCA construct nucleotide sequence of SEQ ID NO: 4.
54. The composition of any one of claims 38-53, wherein the rAAV comprises a tMCK promoter.
55. The composition of claim 54, wherein the tMCK promoter comprises a nucleotide sequence set forth in SEQ ID NO: 3.
56. The composition of any one of claims 38- 55, wherein the rAAV comprises a 5' inverted terminal repeat sequence of SEQ ID NO: 5.
57. The composition of any one of claims 38-56, wherein the rAAV comprises a 3' inverted terminal repeat sequence of SEQ ID NO: 6.
58. The composition of any one of claims 38-57, wherein the rAAV comprises a poly A sequence of SEQ ID NO: 7.
59. The composition of any one of claims 38-58, wherein the rAAV is of the serotype AAVrh.74.
60. The composition of any one of claims 38-59, wherein the muscular dystrophy is limb-girdle muscular dystrophy.
61. The composition of any one of claims 38-60, wherein the muscular dystrophy is limb-girdle muscular dystrophy type 2D (LGMD2D).

62. The composition of any one of claims 38-61, wherein the subject is suffering from limb-girdle muscular dystrophy, and the composition is formulated for administration by intravenous infusion at a dose of about 5×10^{13} vg/kg to about 2×10^{14} vg/kg based on a supercoiled DNA or plasmid as the quantitation standard, and wherein the rAAV comprises the scAAVrh74.tMCK.hSGCA construct nucleotide sequence of SEQ ID NO: 4.
63. The composition of any one of claims 38-62, wherein the level of alpha-sarcoglycan gene expression in a cell of the subject is increased after administration of the composition as compared to the level of alpha-sarcoglycan gene expression before administration of the composition.
64. The composition of any one of claims 38-63, wherein fibrosis is reduced in the subject after administration of the composition as compared to before administration of the composition.
65. The composition of any one of claims 38-63, wherein fibrosis, the central nucleation, the CK level, and/or collagen deposition in the subject is reduced after administration of the composition as compared before administration of the composition.
66. The composition of any one of claims 38-65, wherein the specific force, the fiber diameter size, and/or the eccentric contraction in the muscle of the subject are increased after administration of the composition as compared to before administration of the composition.
67. The composition of claim 63, wherein the alpha-sarcoglycan gene expression is detected by measuring the alpha-sarcoglycan protein level by Western blot, and/or immunohistochemistry.
68. A composition for expressing alpha-sarcoglycan gene in a cell, wherein the composition comprises the scAAVrh74.tMCK.hSGCA construct nucleotide sequence of SEQ ID NO: 4.
69. The composition of claim 68, wherein expression of the alpha-sarcoglycan gene in the cell of the subject is detected by measuring the alpha-sarcoglycan protein level on a Western blot in muscle biopsies.
70. The composition of claim 68, wherein expression of the alpha-sarcoglycan gene in the cell is detected by measuring the alpha-sarcoglycan protein level by immunohistochemistry in muscle biopsies.
71. The composition of claim 68, wherein expression of the alpha-sarcoglycan gene is measured in the subject by detecting the number of vector genome per microgram of genomic DNA.
72. A composition for decreasing a serum CK level in a subject in need thereof, the composition comprising the scAAVrh74.tMCK.hSGCA construct comprising the nucleotide sequence of SEQ ID NO: 4.

73. A composition for increasing alpha-sarcoglycan positive fibers in a muscle tissue of a subject comprising the scAAVrh74.tMCK.hSGCA construct comprising the nucleotide sequence of SEQ ID NO: 4.
74. A composition for increasing the expression of alpha-sarcoglycan in a subject in need thereof comprising the scAAVrh74.tMCK.hSGCA construct comprising the nucleotide sequence of SEQ ID NO: 4.
75. Use of a recombinant adeno-associated virus (rAAV) AAVrh74.tMCK.hSCGA A for the preparation of a medicament for treating muscular dystrophy in a subject in need thereof, wherein the rAAV is at a dose of about 1.0×10^{12} vg/kg to about 5.0×10^{15} vg/kg based on a supercoiled DNA or plasmid as the quantitation standard, and wherein the medicament is formulated for a systemic route of administration.
76. The use of claim 75, wherein the rAAV is at a dose of about 1.0×10^{12} vg/kg to about 2.0×10^{15} vg/kg, about 5×10^{12} vg/kg to about 1.0×10^{15} vg/kg, about 1.0×10^{13} vg/kg to about 5.0×10^{14} vg/kg, about 2.0×10^{13} vg/kg to about 3.0×10^{14} vg/kg, or about 5×10^{13} vg/kg to about 2×10^{14} vg/kg based on a supercoiled DNA or plasmid as the quantitation standard.
77. The use of claim 75, wherein the rAAV is at a dose about 5×10^{13} vg/kg to about 2×10^{14} vg/kg based on a supercoiled DNA or plasmid as the quantitation standard.
78. The use of claim 75, wherein the rAAV is at a dose about 5×10^{13} vg/kg, about 1×10^{14} vg/kg, or about 2×10^{14} vg/kg based on a supercoiled DNA or plasmid as the quantitation standard.
79. Use of a recombinant adeno-associated virus (rAAV) AAVrh74.tMCK.hSCGA A for the preparation of a medicament for treating muscular dystrophy in a subject in need thereof, wherein the rAAV is at a dose of about 1.85×10^{13} vg/kg or 7.41×10^{13} vg/kg based on a linearized DNA or plasmid as the quantitation standard and wherein the medicament is formulated for a systemic route of administration.
80. The use of any one of claims 75-79, wherein the level of alpha-sarcoglycan gene expression in a cell of the subject is increased after administration of the medicament as compared to the level of alpha-sarcoglycan gene expression before administration of the medicament; or wherein the serum CK level in the subject is decreased after administration of the medicament as compared to serum CK level before administration of the medicament; or wherein the number of alpha-sarcoglycan positive fibers in the muscle tissue of the subject is increased after administration of the medicament as compared to the number of alpha-sarcoglycan positive fibers before administration of the medicament.
81. The use of any one of claims 75-80, wherein the systemic route of administration is an intravenous route.
82. The use of any one of claims 75-81, wherein the medicament is formulated for administration by injection, infusion or implantation.

83. The use of any one of claims 75-82, wherein the medicament is formulated for administration by infusion.
84. The use of any one of claims 75-83, wherein the medicament is formulated for administration by an intravenous route through a peripheral limb vein.
85. The use of any one of claims 75-84, wherein the rAAV comprises a nucleotide sequence with at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 1.
86. The use of claim 85, wherein the rAAV comprises a nucleotide sequence of SEQ ID NO: 1.
87. The use of any one of claims 75-86, wherein the rAAV comprises a nucleotide sequence encoding a polypeptide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical with SEQ ID NO: 2.
88. The use of any one of claims 75-86, wherein the rAAV comprises a nucleotide sequence encoding a polypeptide sequence set forth in SEQ ID NO: 2.
89. The use of any one of claims 75-86, wherein the rAAV comprises the scAAVrh74.tMCK.hSGCA construct nucleotide sequence with at least at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NO: 4.
90. The use of claim 89, wherein the rAAV comprises the scAAVrh74.tMCK.hSGCA construct nucleotide sequence of SEQ ID NO: 4.
91. The use of any one of claims 75-90, wherein the rAAV comprises a tMCK promoter.
92. The use of claim 91, wherein the tMCK promoter comprises a nucleotide sequence set forth in SEQ ID NO: 3.
93. The use of any one of claims 75-92, wherein the rAAV comprises a 5' inverted terminal repeat sequence of SEQ ID NO: 5.
94. The use of any one of claims 75-93, wherein the rAAV comprises a 3' inverted terminal repeat sequence of SEQ ID NO: 6.
95. The use of any one of claims 73-94, wherein the rAAV comprises a poly A sequence of SEQ ID NO: 7.
96. The use of any one of claims 73-95, wherein the rAAV is of the serotype AAVrh.74.
97. The use of any one of claims 73-96, wherein the muscular dystrophy is limb-girdle muscular dystrophy.

98. The use of any one of claims 73-97, wherein the muscular dystrophy is limb-girdle muscular dystrophy type 2D (LGMD2D).
99. The use of any one of claims 73-98, wherein the subject is suffering from limb-girdle muscular dystrophy, and the medicament is formulated for intravenous infusion and the rAAV is at a dose of about 5×10^{13} vg/kg to about 2×10^{14} vg/kg based on a supercoiled DNA or plasmid as the quantitation standard, and wherein the rAAV comprises the scAAVrh74.tMCK.hSGCA construct nucleotide sequence of SEQ ID NO: 4.
100. The use of any one of claims 73-99, wherein the level of alpha-sarcoglycan gene expression in a cell of the subject is increased after administration of the medicament as compared to the level of alpha-sarcoglycan gene expression before administration of the medicament.
101. The use of any one of claims 73-100, wherein fibrosis is reduced in the subject after administration of the medicament as compared to before administration of the medicament.
102. The use of one of claims 73-100, wherein fibrosis, central nucleation, CK level, and/or collagen deposition in the subject is reduced after administration of the medicament as compared to the fibrosis before administration of the medicament.
103. The use of any one of claims 72-102, wherein the specific force, the fiber diameter size, and/or the eccentric contraction in the muscle of the subject are increased after administration of the medicament as compared to before administration of the medicament.
104. The use of claim 100, wherein the alpha-sarcoglycan gene expression is detected by measuring the alpha-sarcoglycan protein level by Western blot, and/or immunohistochemistry.
105. A use of scAAVrh74.tMCK.hSGCA construct for the preparation of a medicament for expressing alpha-sarcoglycan gene in a cell, wherein the scAAVrh74.tMCK.hSGCA construct comprises the nucleotide sequence of SEQ ID NO: 4.
106. The use of claim 105, wherein expression of the alpha-sarcoglycan gene in the cell of the subject is detected by measuring the alpha-sarcoglycan protein level on a Western blot in muscle biopsies.
107. The use of claim 105, wherein expression of the alpha-sarcoglycan gene in the cell is detected by measuring the alpha-sarcoglycan protein level by immunohistochemistry in muscle biopsies.
108. The use of claim 105, wherein expression of the alpha-sarcoglycan gene is measured in the subject by detecting the number of vector genome per microgram of genomic DNA.

109. Use of a scAAVrh74.tMCK.hSGCA construct for preparation of a medicament for decreasing a serum CK level in a subject in need thereof, wherein the scAAVrh74.tMCK.hSGCA construct comprises the nucleotide sequence of SEQ ID NO: 4.
110. Use of a scAAVrh74.tMCK.hSGCA construct for the preparation of a medicament for increasing alpha-sarcoglycan positive fibers in a muscle tissue of a subject, wherein the scAAVrh74.tMCK.hSGCA construct comprises the nucleotide sequence of SEQ ID NO: 4.
111. Use of scAAVrh74.tMCK.hSGCA construct for the preparation of a medicament for increasing the expression of alpha-sarcoglycan in a subject in need thereof, the scAAVrh74.tMCK.hSGCA construct comprises the nucleotide sequence of SEQ ID NO: 4.
112. The method, composition or use of any one of claims 1-111, wherein the subject is a human subject is 4 to 15 years of age.
113. The method, composition or use of any one of claims 1-111, wherein the subject is a pediatric subject, an adolescent subject or a young adult subject.
114. The method, composition or use of any one of claims 1-111, wherein the subject is a human subject that is 4-15 years of age, has a confirmed alpha-sarcoglycan (SGCA) mutation in both alleles, was negative for AAVrh74 antibodies and/or had >40% or normal 100 meter walk test.
115. The method, composition or use of any one of claims 1-111, wherein the subject is a middle aged adult or elderly subject.
116. The method, use or composition of any one of claims 1-111, wherein the subject is a human subject is 25 to 55 years of age.
117. The method, composition or use of any one of claims 1-111, wherein the subject is a human subject is over 50 years of age.
118. A method of generating the rAAV administered in a method, composition or use of any one of claims 1-117, comprising transferring an AAV vector plasmid to a host cell, wherein the AAV vector plasmid comprises a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 8.
119. The method of claim 118, wherein the AAV vector plasmid comprises a nucleotide sequence of SEQ ID NO: 8.
120. The method of claim 118 or 119, wherein the vector plasmid comprises a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 1, 4, or 8.

121. The method of any one of claims 118 or 119, wherein the vector plasmid comprises a nucleotide sequence of SEQ ID NO: 1, 4, or 8.
122. The method of any one of claims 118-121, further comprising transferring a packaging plasmid and/or a helper virus to the host cell.
123. The method of any one of claims 118-122, wherein the packaging cell comprises a stably integrated AAV cap gene.
124. The method of any one of claims 118-123, wherein the packaging cell comprises a stably integrated AAV rep gene.
125. A host cell, comprising an AAV vector plasmid that comprises a nucleotide sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identical to SEQ ID NO: 1, 4, or 8.
126. The host cell of claim 125, wherein the AAV vector plasmid that comprises a nucleotide sequence of SEQ ID NO: 1, 4, or 8.