CLAIMS

What is claimed:

1. A method of treating muscular dystrophy in a human subject in need thereof comprising the step of administering a recombinant adeno-virus associated (rAAV) and an anti-inflammatory steroid, wherein the rAAV is selected from the group consisting of rAAV.MHCK7.microdystrophin, AAVrh.74.tMCK.CAPN3, rAAVrh.74.MHCK7.DYSF, scAAVrh.74.MHCK7.hSGCG, AAVrh74.tMCK.hSCGA, scAAVrh74.MHCK7.HSGCB, and rAAVrh.74.MHCK7.huAN05.

2. The method of claim 1 wherein the muscular dystrophy is Duchenne Muscular Dystrophy and the rAAV is rAAV.MHCK7.microdystrophin.

3. The method of claim 1 wherein the muscular dystrophy is Limb Girdle Muscular Dystrophy and wherein the rAAV is selected from the group consisting of: AAVrh.74.tMCK.CAPN3, rAAVrh.74.MHCK7.DYSF, scAAVrh.74.MHCK7.hSGCG, AAVrh74.tMCK.hSCGA, scAAVrh74.MHCK7.HSGCB, and rAAVrh.74.MHCK7.huAN05.

4. The method of any one of claims 1-3, wherein the anti-inflammatory steroid is administered about 24 hours prior to administration of the rAAV.

5. The method of any one of claims 1-3, wherein the anti-inflammatory steroid is administered at least once a day for about 14 days prior to administration of the rAAV.

6. The method of any one of claims 1-5 wherein the anti-inflammatory steroid is administered prior to administration of the rAAV and the anti-inflammatory steroid is administered at least once a day from day 1 to 30 days after administration of the rAAV or at least once a day from 1 to 60 days after administration of the rAAV.

7. The method of any one of claims 1-6 wherein the anti-inflammatory steroid is administered orally.

8. The method of any one of claims 1-7, wherein the anti-inflammatory steroid is a glucocorticoid.

9. The method of any one of claims 1-7 wherein the anti-inflammatory steroid is prednisone, prednisolone, betamethasone, dexamethasone, hydrocortisone, methylprednisolone or deflazacort.

10. The method of any one of claims 1-9 further comprising the step of administering an anti-CD20 specific antibody prior to administration of the rAAV.

11. The method of claim 10 wherein the anti-CD-20 specific antibody is administered at least 7 days prior to administration of the rAAV.

12. The method of claim 10 or 11 wherein the anti-CD20 specific antibody is administered at least 14 days prior to administration of the rAAV.

13. The method of claim 10 wherein the anti-CD20 specific antibody is administered about 14 days prior to administration of the rAAV, about 7 days prior to administration of the rAAV and within about 24 hours of the administration of the rAAV.

14. The method of any one of claims 10-13 further comprising the step of administering an anti-CD20 specific antibody after administration of the rAAV.

15. The method of claims 10-14 wherein the anti-CD20 specific antibody is rituximab, ocrelizumab or ofatumumab.

16. The method of any one of claims 1-15 further comprising the step of administering an immunosuppressing macrolide.

17. The method of clam 16 wherein the immunosuppressing macrolide is administered at least once a day for at least three days prior to administration of the rAAV.

18. The method of claim 16 or 17 wherein the immunosuppressing macrolide is administered after administration of the rAAV.

19. The method of any one of claims 16-18 wherein the immunosuppressing macrolide is administered orally.

20. The method of any one of claims 16-19 wherein the immunosuppressing macrolide is tacrolimus, pinecrolimus or sirolimus.

21. A method of treating a muscular dystrophy in a human subject in need thereof comprising administering a recombinant adeno-virus associated (rAAV); and an immunosuppressing regimen, wherein the immunosuppressing regimen comprises administering one or more of an anti-inflammatory steroid, an anti-CD20 antibody, and an immunosuppressing macrolide and wherein the rAAV is selected from the group consisting of: AAVrh.74.MHCK7.microdystrophin, AAVrh.74.tMCK.CAPN3, rAAVrh.74.MHCK7.DYSF, scAAVrh.74.MHCK7.hSGCG, AAVrh74.tMCK.hSCGA, scAAVrh74.MHCK7.HSGCB, and rAAVrh.74.MHCK7.huAN05.

22. The method of claim 21 wherein the muscular dystrophy is Duchenne Muscular Dystrophy and the rAAV is rAAV.MHCK7.microdystrophin.

23. The method of claim 21, wherein the muscular dystrophy is Limb-Girdle muscular dystrophy and the rAAV is AAVrh.74.tMCK.CAPN3, rAAVrh.74.MHCK7.DYSF, scAAVrh.74.MHCK7.hSGCG, AAVrh74.tMCK.hSCGA, scAAVrh74.MHCK7.HSGCB, and rAAVrh.74.MHCK7.huAN05.

24. The method of any one of claims 21-23 wherein the immunosuppressing regimen comprises administering an anti-inflammatory steroid, an anti-CD20 antibody, and an immunosuppressing macrolide.

25. The method of any one of claims 21-24 wherein the anti-inflammatory steroid is administered about 24 hours prior to administration of the rAAV.

26. The method of any one of claims 21-25 wherein the anti-inflammatory steroid is administered prior to administration of the rAAV and the anti-inflammatory steroid is administered at least once a day from day 1 to 30 days after administration of the rAAV.

27. The method of any one of claims 21-26 wherein the anti-inflammatory steroid is administered orally.

28. The method of any one of claims 21-27, wherein the anti-inflammatory steroid is a glucocorticoid.

29. The method of any one of claims 21-28 wherein the anti-inflammatory steroid is prednisone, prednisolone, betamethasone, dexamethasone, hydrocortisone, methylprednisolone or deflazacort.

30. The method of any one of claims 21-295 wherein the anti-CD20 specific antibody prior to administration of the rAAV.

31. The method of claim 30 wherein the anti-CD20 specific antibody is administered at least 14 days prior to administration of the rAAV.

32. The method of claim 30 wherein the anti-CD20 specific antibody is administered about 14 days prior to administration of the rAAV, about 7 days prior to administration of the rAAV and within about 24 hours of the administration of the rAAV.

33. The method of any one of claims 21-32, wherein the immunosuppressing regimen further comprises administering an anti-CD20 specific antibody after administration of the rAAV.

34. The method of any one of claims 21-33 wherein the anti-CD20 antibody is administered by intravascular infusion.

35. The method of any one of claims 21-34 wherein the anti-CD20 specific antibody is rituximab, ocrelizumab or ofatumumab.

36. The method of any one of claims 21-35 wherein the immunosuppressing macrolide is administered at least once a day for at least three days prior to administration of the rAAV.

37. The method of anyone of claims 21-36 wherein the immunosuppressing regimen further comprises administering an immunosuppressing macrolide after administration of the rAAV.

38. The method of any one of claims 21-37 wherein the immunosuppressing macrolide is administered orally.

39. The method of any one of claims 21-38 wherein the immunosuppressing macrolide is tacrolimus, pinecrolimus or sirolimus.

40. A method of treating a muscular dystrophy in a human subject in need thereof comprising administering a recombinant adeno-virus associated (rAAV) selected from the group consisting of : AAVrh.74.MHCK7.microdystrophin, AAVrh.74.tMCK.CAPN3, rAAVrh.74.MHCK7.DYSF, scAAVrh.74.MHCK7.hSGCG, AAVrh74.tMCK.hSCGA,

scAAVrh74.MHCK7.HSGCB, and rAAVrh.74.MHCK7.huAN05; and an immunosuppressing regimen, wherein the immunosuppressing regimen comprises the steps of

i) orally administering an anti-inflammatory steroid about 24 hours prior to administration of the rAAV, and administering an anti-inflammatory steroid at least once a day from day 1 to 30 days after administration of the rAAV or from day 1 to 60 days after administration of the rAAV,

ii) intravenously administering an anti-CD20 antibody about 14 days prior to administration of the rAAV, about 7 days prior to administration of the rAAV and within about 24 hours of the administration of the rAAV, and optionally administering the anti-CD20 antibody after administration of the rAAV,

iii) orally administering an immunosuppressing macrolide at least once a day for at least three days prior to administration of the rAAV, and optionally administering the an immunosuppressing macrolide after administration of the rAAV.

41. The method of claim 40 wherein the muscular dystrophy is Duchenne muscular dystrophy and the rAAV is AAVrh.74.MHCK7.microdystrophin.

42. The method of claim 40 wherein the muscular dystrophy is Limb Girdle muscular dystrophy and the rAAV is AAVrh.74.tMCK.CAPN3, rAAVrh.74.MHCK7.DYSF, scAAVrh.74.MHCK7.hSGCG, AAVrh74.tMCK.hSCGA, scAAVrh74.MHCK7.HSGCB, or rAAVrh.74.MHCK7.huAN05.

43. The method of any one of claims 40-42 wherein the anti-inflammatory steroid is prednisone, prednisolone, betamethasone, dexamethasone, hydrocortisone, methylprednisolone or deflazacort.

44. The method of any one of claims 40-43 wherein the anti-CD20 specific antibody is rituximab, ocrelizumab or ofatumumabone.

45. The method of any one of claims 40-42 wherein the immunosuppressing macrolide is tacrolimus, pinecrolimus or sirolimus.

46. The method of any one of claims 40-42 wherein the anti-inflammatory steroid is prednisolone, the anti-CD20 antibody is rituximab, and the immunosuppressing macrolide is sirolimus.

47. A method of treating a muscular dystrophy in a human subject in need thereof comprising subjecting the subject's plasma to at least one therapeutic plasma exchange (TPE) prior to administration of a second dose of recombinant adeno-virus associated (rAAV) selected from the group consisting of: AAVrh.74.MHCK7.microdystrophin, AAVrh.74.tMCK.CAPN3, rAAVrh.74.MHCK7.DYSF, scAAVrh.74.MHCK7.hSGCG, AAVrh74.tMCK.hSCGA, scAAVrh74.MHCK7.HSGCB, and rAAVrh.74.MHCK7.huAN05, wherein the subject was administered a first dose of rAAV prior to being subjected to TPE.

48. A method of treating a muscular dystrophy in a human subject in need thereof comprising the steps of

a) administering a first dose of recombinant adeno-virus associated selected from the group consisting of: AAVrh.74.MHCK7.microdystrophin, AAVrh.74.tMCK.CAPN3, rAAVrh.74.MHCK7.DYSF, scAAVrh.74.MHCK7.hSGCG, AAVrh74.tMCK.hSCGA, scAAVrh74.MHCK7.HSGCB, and rAAVrh.74.MHCK7.huAN05;

b) subjecting the subject's plasma to at least one therapeutic plasma exchange (TPE), and c) administering a second dose or rAAV.

49. The method of claim 47 or 48 wherein the muscular dystrophy is Duchenne muscular dystrophy and the rAAV is AAVrh.74.MHCK7.microdystrophin.

50. The method of claim 47 or 48 wherein the muscular dystrophy is Limb Girdle muscular dystrophy and the rAAV is AAVrh.74.tMCK.CAPN3, rAAVrh.74.MHCK7.DYSF, scAAVrh.74.MHCK7.hSGCG, AAVrh74.tMCK.hSCGA, scAAVrh74.MHCK7.HSGCB, and rAAVrh.74.MHCK7.huAN05.

51. The method of any one of claim 47-50, wherein the subject's plasmas is subject to at least two TPE or at least three TPE prior to administration of the 2nd dose or rAAV.

52. A method of treating muscular dystrophy in a human subject in need thereof comprising the steps of

a) subjecting the subject's plasma to at least one therapeutic plasma exchange (TPE) prior to administering recombinant adeno-virus associated (rAAV)

b) administering rAAV, and

wherein the rAAV is rAAV.MHCK7.microdystrophin, AAVrh.74.tMCK.CAPN3,

rAAVrh.74.MHCK7.DYSF, scAAVrh.74.MHCK7.hSGCG, AAVrh74.tMCK.hSCGA, scAAVrh74.MHCK7.HSGCB, or rAAVrh.74.MHCK7.huAN05.

53. The method of claim 52 wherein the subject's plasma is subjected to at least two TPE, at least three TPE, at least four TPE, at least five TPE rAAV, at least six TPE or at least seven TPE prior to administering.

54. The method of claim 52 or 53 wherein the subject's plasma is subjected to TPE for at least 9 days prior to administration of the rAAV, at least 7 days prior to administration, 5 days prior to administration, or 2 days prior to administration.

55. The method of any one of claims 52-54 wherein the subject plasma is subjected to TPE on the day the rAAV is administered.

56. The method of any one of claims 52-55, wherein the subject's plasma is subjected to at least two TPE, wherein there is about 48 hours in between the TPE.

57. The method of any one of claims 52-56, wherein the subject is administered an anti-inflammatory steroid about 24 hours prior to administration of the rAAV.

58. The method of any one of claims 52-57, wherein the subject is administered an anti-inflammatory steroid at least once a day from day 1 to 60 days after administration of the rAAV.

59. The method of claim 57 or 58 wherein the anti-inflammatory steroid is administered orally.

60. The method of any one of claims 57-59, wherein the anti-inflammatory steroid is a glucocorticoid.

61. The method of any one of claims 57-60, wherein the anti-inflammatory steroid is prednisone, prednisolone, betamethasone, dexamethasone, hydrocortisone, methylprednisolone or deflazacort.

62. The method of claim any one of claims 1-61 wherein the rAAV is administered by systemic route of administration.

63. The method of claim 62 wherein the systemic route of administration is an intravenous route and the dose of the rAAV administered is about 2x 10^{14} vg/kg.

64. The method of any one of claims 1-63 wherein the dose of rAAV is administered at a concentration of about 10 mL/kg.

65. The method of any one of claims 1-64 wherein the rAAV is administered by injection, infusion or implantation.

66. The method of any one of claims 1-65 wherein the rAAV is administered by infusion over approximately one hour.

67. The method of any one of claims 1-65 wherein the rAAV is administered by an intravenous route through a peripheral limb vein.

68. The method of any one of claims 1-67 wherein the rAAV is of the serotype AAVrh.74.

69. The method of any one of claims 1-68 wherein the rAAV comprises the human micro-dystrophin nucleotide sequence of SEQ ID NO: 1.

70. The method of any one of claims 1-69 wherein the rAAV comprises the MHCK7 promoter sequence of SEQ ID NO: 2 or SEQ ID NO:7

71. The method of any one of claims 1-70 wherein the rAAV comprises the nucleotide sequence of SEQ ID NO: 44.

72. The method of any one of claims 1-68 wherein the rAAV comprises the AAVrh74.MHCK7.micro-dystrophin construct nucleotide sequence of SEQ ID NO: 9 or nucleotides 55-5021 of SEQ ID NO: 3.

73. The method of any one of claims 1-68 wherein the human subject is suffering from Duchenne muscular dystrophy, and the rAAV is administered by intravenous infusion over approximately one hour at a dose of about 2x 10^{14} vg/kg, and wherein the rAAV comprises the AAVrh74.MHCK7.micro-dystrophin construct nucleotide sequence of SEQ ID NO: 9 or of nucleotides 55-5021 of SEQ ID NO: 3.

74. The method of any one of claims 1-68 wherein the muscular dystrophy is LGMD2E and the rAAV is scAAVrh74.MHCK7.HSGCB.

75. The method of any one of claims 1-68 wherein the human subject is suffering from LGMD2E, and the rAAV is administered by intravenous infusion at a dose of about 2x

10^{14} vg/kg, and wherein the rAAV comprises the rAAV is scAAVrh74.MHCK7.HSGCB comprising the nucleotide sequence of SEQ ID NO: 44.

76. The method of any one of claims 1-75 wherein the level of micro-dystrophin gene expression in a cell of the subject is increased after administration of the rAAV as compared to the level of micro-dystrophin gene expression before administration of the rAAV.

77. The method of any one of claims 1-76, further comprising the step of determining the presence of anti-AAVrh.74 antibodies in serum or plasma of said subject.

78. The method of claim 77, wherein the determining step is performed prior to the step of administering said administering of an immunosuppressing regimen or TPE.

79. The method of claim 78 wherein the determining step is performed prior to any administration of an AAV to said subject.

80. The method of claim 79 where the determining step is performed prior to administration of aAAVrh.74 to said subject.

81. The method of any one of claims 77-80, wherein said determination is quantitative.

82. The method of claim 81, further comprising a step of comparing the level of anti-AAVrh.74 antibodies in serum or plasma of said subject to a positive control.

83. The method of claim 82 wherein said positive control utilizes an anti-AAVrh.74 monoclonal antibody.

84. The method of any one of claims 77-83, wherein the determining step comprises utilizing an anti-AAVrh.74 monoclonal antibody.

85. The method of any one of claims 77-84, wherein said determination step comprises utilizing an immunofluorescence assay, an immunohistochemical assay, a Western blot, a direct enzyme-linked immunosorbent assay (ELISA), an indirect ELISA, a sandwich ELISA, a competitive ELISA, a reverse ELISA, a chemiluminescence assay, a radioimmunoassay, or an immunoprecipitation assay.

86. The method of any one of clams 83-85, wherein said monoclonal antibody comprises a VH CDR1 amino acid sequence selected from the group consisting of NYGMN (SEQ ID NO: 20), DYGMN (SEQ ID NO: 22), YTFTNYGMN (SEQ ID NO: 21), and YTFTKYGMN (SEQ ID NO: 23).

87. The method of any one of claims 83-85, wherein said monoclonal antibody comprises a VH CDR2 amino acid sequence selected from the group consisting of WINTYTGEPTYADDFKG (SEQ ID NO: 24), WINTNTGEPTYGDDFKG (SEQ ID NO: 25), and WMGWINTYTGEPTY (SEQ ID NO: 26).

88. The method of any one of claims 83-85, wherein said monoclonal antibody comprises a VH CDR3 amino acid sequence selected from the group consisting of GVAHYSDSRFAFDY (SEQ ID NO: 27), GNAHPGGSAFVY (SEQ ID NO: 28), RGSYYYDSSPAWFAY (SEQ ID NO: 29), RGVDSSGYGAFAY (SEQ ID NO: 30), and TRGTSTMISTFAFVY (SEQ ID NO: 31).

89. The method of any one of claims 83-85, wherein said monoclonal antibody comprises a VL CDR1 amino acid sequence selected from the group consisting of SVSSSVSYMH (SEQ ID NO: 32), SASSGVTYMH (SEQ ID NO: 33), SSVSYMH (SEQ ID NO: 34), and SSVRYMH (SEQ ID NO: 35).

90. The method of any one of claims 83-85, wherein said monoclonal antibody comprises a VL CDR2 amino acid sequence selected from the group consisting of YTSNLAS (SEQ ID NO: 36), RTSNLAS (SEQ ID NO: 37), LWIYSTSNLAS (SEQ ID NO: 38), and VWIYSTSNLAS (SEQ ID NO: 39).

91. The method of any one of claims 83-85, wherein said monoclonal antibody comprises a VH CDR3 amino acid sequence selected from the group consisting of QQRSSYPFT (SEQ ID NO: 40), QQRSTYPF (SEQ ID NO: 41), QQRSFYPF (SEQ ID NO: 42), and QQRTYYPF (SEQ ID NO: 43).

92. The method of any one of claims 83-85, wherein said monoclonal antibody comprises a variable heavy chain (VH) sequence set forth in SEQ ID NO: 10, 12, 14, 16, or 18.

93. The method of any one of claims 83-85, wherein said monoclonal antibody comprises a variable light chain (VL) sequence set forth in SEQ ID NO: 11, 13, 15, 17, or 19.

94. The method of any one of claims 83-85, wherein said monoclonal antibody comprises a variable heavy chain (VH) sequence set forth in SEQ ID NO: 10, 12, 14, 16, or 18, and a variable light chain (VL) sequence set forth in SEQ ID NO: 11, 13, 15, 17, or 19.

95. The method of any one of claims 83-85, wherein said determination is quantitative, wherein said subject is identified as seropositive for anti-AAVrh.74 antibodies based said quantitation, and wherein said immunosuppressing regimen or TPE is selectively administered to the seropositive subject.