

WHAT IS CLAIMED IS:

1. A method of producing a recombinant adeno-associated virus (rAAV) rAAVrh74.MHCK7.microdystrophin in adherent mammalian cells by a hybrid seed-train expansion process, comprising:
 - (a) culturing the adherent cells under adherent conditions with a first growth medium comprising serum in a N-2 container;
 - (b) removing the adherent cells from the first medium;
 - (c) inoculating the adherent cells from step (b) into a second medium comprising no serum or serum at a concentration less than the first medium in a N-1 container;
 - (d) culturing the adherent cells in the N-1 container under suspension conditions; and
 - (e) inoculating a third medium in an adherent bioreactor with the cells from step (d).
2. The method of claim 1, wherein the rAAV comprises the human micro-dystrophin nucleotide sequence of SEQ ID NO:1.
3. The method of claim 2, wherein the rAAV comprises the MHCK7 promoter sequence of SEQ ID NO: 7.
4. The method of any one of claims 1 to 3, wherein the rAAV comprises the human micro-dystrophin nucleotide sequence of SEQ ID NO:1 and the MHCK7 promoter sequence of SEQ ID NO: 7.
5. The method of any one of claims 1 to 4, wherein the hybrid seed-train expansion process further comprises:
 - (f) transfecting the adherent cells with a transgene plasmid comprising a rAAVrh74.MHCK7.microdystrophin construct, a plasmid comprising an AAV rep gene and an AAV cap gene, and an adenovirus helper plasmid.
6. The method of claim 5, wherein the transgene plasmid comprising a rAAVrh74.MHCK7.microdystrophin construct comprises:
 - the nucleic acid sequence of SEQ ID NO: 9;

nucleotides 55-5021 of SEQ ID NO: 3; or
nucleotides 1-4977 of SEQ ID NO: 8.

7. The method of claim 5 or 6, wherein the plasmid comprising an AAV rep gene and an AAV cap gene comprises an AAV2 rep gene and an rAAVrh74 cap gene.
8. The method of any one of claims 5 to 7, wherein the adenovirus helper plasmid comprises an adenovirus 5 E2A, E4ORF6, and a VA RNA gene.
9. The method of any one of claims 1 to 8, wherein the hybrid seed-train expansion process further comprises:
 - (g) lysing the adherent cells, wherein the adherent cells are lysed by freeze-thaw, solid shear, hypertonic and/or hypotonic lysis, liquid shear, sonication, high-pressure extrusion, detergent lysis, or combinations thereof.
10. The method of any one of claims 1 to 9, wherein the hybrid seed-train expansion process further comprises:
 - (h) purifying the rAAV by at least one column chromatography step, wherein the at least one column chromatography step comprises an anion exchange chromatography, a size exclusion chromatography, or a combination thereof.
11. The method of any one of claims 1 to 10, wherein the hybrid seed-train expansion process further comprises culturing cells with the first growth medium in a N-3 or N-4 container.
12. The method of claim 11, wherein the suspension seed process further comprises culturing cells with the first growth medium in a N-4 container.
13. The method of any one of claims 1 to 12, wherein the rAAV is purified from the culture produced in the adherent bioreactor.
14. The method of claim 13, wherein the third medium in the bioreactor comprises at least one factor that promotes cell adherence, wherein the at least one factor that promotes cell adherence is selected from the group consisting of serum, FBS, fibronectin, collagen, laminin, calcium ions, proteoglycans or non-proteoglycan polysaccharides of the extracellular matrix, and combinations thereof.

15. The method of claim 14, wherein the third medium in the bioreactor comprises DMEM and 10% FBS.
16. The method of any one of claims 1 to 15, wherein the adherent cells are cultured under suspension conditions for about 48-72 hours.
17. The method of any one of claims 1 to 16, wherein the adherent cells are selected from the group consisting of HeLa cells, CHO cells, HEK-293 cells, VERO cells, BHK cells, MDCK cells, MDBK cells, and COS cells.
18. The method of claim 17, wherein the adherent cells are HeLa cells or HEK-293 cells.
19. The method of any one of claims 1 to 18, wherein the adherent cells are not suspension-adapted.
20. The method of any one of claims 1 to 19, wherein the culturing does not alter the cells to create a new cell line.
21. A composition comprising a recombinant adeno-associated virus (rAAV) rAAVrh74.MHCK7.microdystrophin, wherein the rAAV is made by the method of any one of claims 1 to 20.
22. The composition of claim 21, wherein the composition comprises:
 - a) rAAV particles comprising the nucleic acid sequence of SEQ ID NO: 9;
 - b) rAAV particles comprising nucleotides 55-5021 of SEQ ID NO: 3; and/or
 - c) rAAV particles comprising nucleotides 1-4977 of SEQ ID NO: 8.
23. Use of the composition of claim 21 or 22 in the manufacture of a medicament for the treatment of muscular dystrophy.
24. The use of claim 23, wherein the composition is to be administered to a human subject in need thereof.
25. The use of claims 23 or 24, wherein the muscular dystrophy is Duchenne muscular dystrophy or Becker's muscular dystrophy.
26. The use of claim 25, wherein the muscular dystrophy is Duchenne muscular dystrophy.

27. The use of any one of claims 23 to 26, wherein the rAAV is to be administered using a systemic route of administration and at a dose of about 5.0×10^{12} vg/kg to about 1.0×10^{15} vg/kg.
28. The use of claim 27, wherein the systemic route of administration is an intravenous route and the dose of the rAAV to be administered is about 2×10^{14} vg/kg.
29. The use of claim 27 or 28, wherein the dose of rAAV is to be administered at a concentration of about 10 mL/kg.
30. The use of any one of claims 23 to 29, wherein the rAAV is to be administered by injection, infusion, or implantation.
31. The use of claim 30, wherein the rAAV is to be administered by infusion over approximately one hour.
32. The use of any one of claims 23 to 31, wherein the rAAV is to be administered by an intravenous route through a peripheral limb vein.
33. The use of any one of claims 23 to 32, 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.
34. The use of claim 33, wherein expression of the micro-dystrophin gene in the cell is detected by measuring the micro-dystrophin protein level by Western blot in muscle biopsied before and after administration of the rAAV.
35. The use of claim 34, wherein the expression is at least 55.4% after administration of the rAAV, as compared to before.
36. The use of any one of claims 23 to 35, wherein the mean percentage of micro-dystrophin positive fibers in the muscle tissue of the subject is increased after administration of the rAAV, as compared to the number of micro-dystrophin positive fibers before administration of the rAAV.

37. The use of claim 36, wherein the mean percentage of micro-dystrophin positive fibers is at least 70.5% and the mean intensity is at least 116.9% as detected by immunofluorescence (IF) in muscle biopsies before and after administration of the rAAV.
38. The use of any one of claims 23 to 37, wherein micro-dystrophin transduction by vector genome count is at least 3.87 mean vector genome copies per nucleus.
39. The use of any one of claims 23 to 38, wherein the composition is to be administered to a genotyped patient, wherein the patient is genotyped for at least one mutation in exons 18-79 of the human dystrophin (*DMD*) gene.
40. The use of claim 39, further comprising genotyping the *DMD* gene of the human patient prior to the administration of the composition to said human patient.
41. The use of claim 40, wherein the genotyping detects at least one mutation in exons 18 to 79 of the *DMD* gene.
42. The use of claim 41, wherein the at least one mutation is a frameshift deletion, a frameshift duplication, a premature stop, or other pathogenic variant resulting in the absence of expression of the human dystrophin protein.