CLAIMS

1. A method of manufacturing an AAV viral vector, comprising:

   a. culturing adherent cells;

   b. transfecting the adherent cells with plasmid(s) to enable production of the AAV viral vector;

   c. lysing the adherent cells to isolate the AAV viral vector;

   d. acidifying and clarifying the cell lysate of (c);

   e. purifying the product of (d) using cation exchange chromatography (CEX);

   f. filtering the product of (e) using tangential flow filtration (TFF);

   g. ultracentrifuging the product of (f) in a cesium chloride (CsCl) buffer; and

   h. collecting the AAV viral vectors from the product of (g).

2. The method of claim 1, wherein the AAV is AAV9.

3. The method of claims 1 or 2, wherein the AAV is self-complementary (scAAV).

4. The method of any one of claims 1-3, wherein the adherent cells are HEK293 cells.

5. The method of claim 4, wherein step (a) comprises seeding the cells in a large-scale bioreactor that can provide continuous circulation of cell culture media.

6. The method of claim 5, wherein the seeding density is about 8,000-12,000 cells/cm^2.

7. The method of any one of claims 1-6, wherein the transfecting step (b) comprises contacting the adherent cell with an adenovirus helper plasmid (pHELP), and a plasmid encoding AAV rep and cap genes.

8. The method of claim 7, wherein the AAV rep gene is rep2 and the AAV cap gene is cap9.

9. The method of any one of claims 1-8, wherein the transfecting step (b) comprises contacting the adherent cells with a plasmid comprising a polynucleotide encoding a survival motor neuron (SMN) protein, a polynucleotide encoding MeCP2 or a polynucleotide encoding a SOD1 shRNA.

10. The method of claim 9, wherein the plasmid comprising the polynucleotide encoding SMN1 protein comprises a modified AAV2 ITR, a chicken beta-actin (CB) promoter,

a cytomegalovirus (CMV) immediate/early enhancer, a modified SV40 late 16s intron, a Bovine growth hormone (BGH) polyadenylation signal, and an AAV2 ITR.

11. The method of claim 9 or 10, wherein the polynucleotide encoding SMN encodes the SMN protein of SEQ ID NO: 2.

12. The method of any one of claims 1-11, wherein the AAV viral vector comprises SEQ ID NO: 1.

13. The method of any one of claims 1-12, wherein the transfecting step comprises contacting the adherent cell with the transfection agent polyethylenimine (PEI).

14. The method of any one of claims 1-13, wherein the transfecting step comprises contacting the adherent cells with a transfection medium that does not contain serum, calcium, or glutamine.

15. The method of any one of claims 1-14, wherein the lysing step comprises a lysis buffer supplemented with benzonase and Tween.

16. .The method of any one of claims 1-15, further comprising freezing the cell lysate of step (c) prior to the acidification step of (d).

17. A method of purifying an AAV viral vector from a cell culture lysate, comprising the steps of:

    a. acidifying and clarifying the cell lysate;

    b. purifying the product of (a) using cation exchange chromatography (CEX);

    c. filtering the product of (b) through a tangential flow filtration;

    d. ultracentrifuging the product of (c) using a 2 – 4 M cesium chloride (CsCl) buffer;

    e. collecting the AAV viral vectors from the product of (d);

    f. filtering the product of (e) through a tangential flow filtration.

18. The method of any one of claims 1-17, wherein the acidification step comprises acidifying the cell lysate to a pH of about 3.0 – 4.0.

19. The method of any one of claims 1-18, wherein the ultracentrifugation is performed between 40,000 – 50,000 rpm.

20. The method of any one of claims 1-19, wherein the CsCl buffer comprises about 3 M CsCl, Tris, $MgCl_2$, and Poloxamer 188, and is between about pH 7.5 and 8.5.

21. The method of any one of claims 1-20, wherein the cell lysate is incubated with Tween prior to the acidification step.

22. The method of any one of claims 1-21, wherein the clarification step comprises filtering the cell lysate through a depth filter.

23. The method of any one of claims 1-22, wherein the CEX comprises a sulfonyl resin.

24. The method of any one of claims 1-23, wherein at least one TFF step comprises using cellulose membranes with a molecular weight cutoff of about 300 kDa MW.

25. The method of any one of claims 1-24, wherein the number of empty viral capsid is less than 7%, less than 5%, less than 3% or less than 1 % of the total viral capsids after collecting the AAV viral vectors from the ultracentrifuged cell lysate.

26. The method of any one of claims 17-25, wherein the AAV viral vectors collected after the second TFF step are stored in a solution comprising Tris, $MgCl_2$, NaCl, and Poloxamer 188, and the pH is between about 7.5 and 8.5.

27. The method of any one of claims 17-26, wherein the AAV viral vectors collected after the second TFF contain less than about 30 μg/g or less than about 20 μg/g of CsCl.

28. The method of any one of claims 17-27, wherein the concentration of AAV viral vectors collected after the second TFF is greater than or equal to about $3x10^{13}$ vg/ml.

29. The method of any one of claims 1-28, wherein the produced yield of AAV is more than 5×10^{15} vg, or more than 8×10^{15} vg or more than 1×10^{16} vg per manufacturing batch.

30. The method of any one of claims 1-29, wherein the AAV vector comprises a polynucleotide encoding a survival motor neuron (SMN) protein, a polynucleotide encoding MeCP2 or a polynucleotide encoding a SOD1 shRNA.

31. A pharmaceutical composition comprising an AAV9 viral vector manufactured according to the method of any one of claims 1-30.

32. The pharmaceutical composition of claim 31, comprising at least one of the following:

a. less than about 0.09 ng of benzonase per $1.0x10^{13}$ vg,

b. less than about 30 μg/g (ppm) of cesium,

c. about 20-80 ppm of Poloxamer 188,

d. less than about 0.22 ng of BSA per $1.0x10^{13}$ vg,

e. less than about $6.8x10^5$ pg of residual plasmid DNA per $1.0x10^{13}$ vg,

f. less than about $1.1x10^5$ pg of residual hcDNA per $1.0x10^{13}$ vg,

g. less than about 4 ng of rHCP per $1.0x10^{13}$ vg,

h. about pH 7.7-8.3,

i. about 390-430 mOsm/kg,

j. less than about 600 particles that are ≥ 25 μm in size per container,

k. less than about 6000 particles that are ≥ 10 μm in size per container,

l. about 1.7×10^{13} - 2.3×10^{13} vg/mL genomic titer,

m. infectious titer of about 3.9×10^{8} - 8.4×10^{10} IU per 1.0×10^{13} vg,

n. total protein of about 100-300 μg per 1.0×10^{13} vg,

o. relative potency of about 70-130%, and

p. less than about 5% empty capsid.

33. A pharmaceutical composition comprising:

a. between $1 – 8 \times 10^{13}$ AAV9 viral vector genomes/mL (vg/mL);

b. less than about 7% empty viral capsids;

c. less than about 100 ng/mL host cell protein per 1×10^{13} vg/mL;

d. less than about 5×10^{6} pg/mL residual host cell DNA per 1×10^{13} vg/mL;

and wherein at least about 80% of the $1 – 8 \times 10^{13}$ AAV9 viral vector genomes/mL are functional.

34. The composition of claim 33, wherein the AAV9 viral vector comprises a polynucleotide encoding a survival motor neuron (SMN) protein, a polynucleotide encoding MeCP2 or a polynucleotide encoding a SOD1 shRNA.

35. The composition of claim 33 or 34, wherein the AAV9 viral vector comprises a modified AAV2 ITR, a chicken beta-actin (CB) promoter, a cytomegalovirus (CMV) immediate/early enhancer, a modified SV40 late 16s intron, a bovine growth hormone (BGH) polyadenylation signal, and an unmodified AAV2 ITR.

36. The composition of claim 34 or 35, wherein the polynucleotide encodes the SMN protein of SEQ ID NO: 2.

37. The composition of any one of claims 33 - 36, wherein the AAV9 viral vector comprises SEQ ID NO: 1.

38. The composition of any one of claims 33-37, comprising between 1.7 – 2.3 x 10^{13} AAV9 vg/mL.

39. The composition of any one of claims 33-38, wherein the composition is an aqueous pharmaceutical formulation.

40. The composition of claim 39, wherein the aqueous pharmaceutical formulation comprises a Tris buffer, $MgCl_2$, NaCl, and Poloxamer 188.

41. The composition of claim 39 or 40, wherein the aqueous pharmaceutical formulation does not comprise a preservative.

42. The composition of claim 40 or 41, wherein:
(i) the Tris buffer concentration is about 10-30 nM;
(ii) the pH of the formulation is about 7.7 to about 8.3;
(iii) the $MgCl_2$ concentration is about 0.5-1.5 mM;
(iv) the NaCl concentration is about 100-300 mM;
(v) the formulation comprises about 0.005% w/v Poloxamer 188; or
(vi) the aqueous pharmaceutical formulation has an osmolality of 390-430 mOsm/kg.

43. The composition of any one of claims 34 -42 for use in the treatment of type I spinal muscular atrophy (SMA) in a patient in need thereof, wherein the patient:
    a. is nine months old or younger;
    b. has a body weight of at least about 2.6 kg;
    c. has bi-allelic *SMN1* null mutations or deletions; and
    d. has at least one functional copy of *SMN2*.

44. The composition of claim 43, wherein the viral vector is to be administered at a dose of about 1-2.5x 10^{14} vg/kg.

45. The composition of claim 43 or 44, wherein the patient has a body weight of no more than about 8.5 kg.

46. The composition of any one of claims 43-45, wherein the patient does not have a c.859G>C substitution in exon 7 of at least one copy of the *SMN2* gene.

47. The composition of any one of claims 43-46, wherein the composition is to be administered to the patient before the age of 6 months, or before the onset of one or more SMA symptoms selected from hypotonia, delay in motor skills, poor head control, round shoulder posture and hypermobility of joints.

48. The composition of any one of claims 43-47, wherein the viral vector is to be administered in about 5-20 mL/kg, about 10-20 mL/kg, or about 5.5-6.5 mL/kg of Tris-buffered saline.

49. The composition of any one of claims 43-48, wherein efficacy is determined using the CHOP-INTEND scale.

50. The composition of claim 43-49, wherein a dose volume to be administered is selected from the group consisting of a dose of 16.5 mL for a patient weighing 2.6-3.0 kg, a dose of 19.3 mL for a patient weighing 3.1-3.5 kg, a dose of 22.0 mL for a patient weighing 3.6 to 4.0 kg, a dose of 24.8 mL for a patient weighing 4.1-4.5 kg, a dose of 27.5 mL for a patient weighing 4.6-5.0 kg, a dose of 30.3 mL for a patient weighing 5.1-5.5 kg, a dose of 33.0 mL for a patient weighing 5.6-6.0 kg, a dose of 35.8 mL for a patient weighing 6.1-6.5 kg, a dose of 38.5 mL for a patient weighing 6.6-7.0 kg, a dose of 41.3 mL for a patient weighing 7.1-7.5 kg, a dose of 44.0 mL for a patient weighing 7.6-8.0 kg, and a dose of 46.8 mL for a patient weighing 8.1-8.5 kg.

51. The composition of any one of claims 43-50, wherein the composition is to be administered by intravenous or intrathecal infusion to a patient in need thereof.

52. The composition of claim 51, wherein the viral vector is to be infused over about 45 – 75 minutes.

53. Use of the composition of any one of claims 34-52 in the manufacture of a medicament for the treatment of type I spinal muscular atrophy (SMA) in a patient in need thereof.

54. A kit comprising vials containing about 5.5 mL or about 8.3 mL of an AAV9 viral vector comprising a polynucleotide encoding a survival motor neuron (SMN) protein,

and formulated at a concentration of about 2.0 x 10^{13} vg/mL in 20 mM Tris, 1 mM $MgCl_2$, 200 mM NaCl, 0.005% w/v Poloxamer 188 at pH 7.7-8.3.