CLAIMS

1. A recombinant adeno-associated virus (rAAV) which comprises:

(A) an AAVhu68 capsid comprising:

(1) a heterogenous population of AAVhu68 vp1 proteins,. AAVhu68 vp2 proteins, and AAVhu68 vp3 proteins produced by expression from a nucleic acid sequence which encodes the amino acid sequence of 1 to 736 of SEQ ID NO:2, or

(2) a heterogenous population of AAVhu68 vp1 proteins, AAVhu68 vp2 proteins, and AAVhu68 vp3 proteins produced by expression from a nucleic acid sequence at least 70% identical to SEQ ID NO:1 which encodes the amino acid sequence of 1 to 736 of SEQ ID NO:2;or

(3) a heterogenous population of AAVhu68 vp1 proteins, AAVhu68 vp2 proteins, and AAVhu68 vp3 proteins produced from a sequence comprising nucleotides 1 to 2211 of SEQ ID NO:1,

wherein: the heterogenous population of AAVhu68vp1, AAVhu68vp2 and AAVhu68 vp3 proteins of (1), (2) and (3) contain subpopulations with amino acid modifications comprising at least two highly deamidated asparagines (N) in positions N57, N329, N452 or N512 of SEQ ID NO: 2 as determined using mass spectrometry and further comprising subpopulations comprising other deamidated amino acids, and

wherein the heterogenous populations of AAVhu68 vp1 proteins, AAVhu68 vp2 proteins, and AAVhu68 vp3 proteins comprise asparagines deamidated to aspartic acid, isoaspartic acid, an interconverting aspartic acid/isoaspartic acid pair, or combinations thereof and wherein at least a subpopulation of the AAVhu68vp1 and AAVhu68vp2 proteins comprise a valine at position 157 and a glutamic acid at position 67 based on the numbering of the AAVhu68vp1 capsid of SEQ ID NO:2; and

(B) a vector genome in the AAVhu68 capsid, the vector genome comprising a nucleic acid molecule comprising AAV inverted terminal repeat sequences and a non-AAV nucleic acid sequence encoding a product operably linked to sequences which direct expression of the product in a mammalian target cell.

2. A recombinant adeno-associated virus (rAAV) which comprises:

(A) an AAVhu68 capsid comprising:

AAVhu68 vp1, AAVhu68vp2 and AAVhu68vp3 proteins comprising a heterogenous population of AAVhu68 vp1 proteins comprising amino acids 1 to 736 of SEQ ID No. 2 having a glutamic acid at position 67 and a valine at position 157 based on numbering of the vp1 protein of SEQ ID NO: 2, a heterogenous population of AAVhu68 vp2 proteins comprising amino acids 138 to 736 of SEQ ID NO:2 comprising a valine at position 157 based on the numbering of the vp1 capsid of SEQ ID NO:2, and a heterogenous population of AAVhu68vp3 proteins comprising amino acids 203 to 736 of SEQ ID NO:2,

wherein the heterogenous populations of AAVhu68 vp1, AAVhu68vp2 and AAVhu68vp3 proteins further comprise at least 50% to 100% of asparagines in positions N57, N329, N452 or N512 of SEQ ID NO: 2 deamidated to aspartic acid, isoaspartic acid, an interconverting aspartic acid/isoaspartic acid pair, or combinations thereof ; and

(B) a vector genome in the AAVhu68 capsid, the vector genome comprising a nucleic acid molecule comprising AAV inverted terminal repeat sequences and a non-AAV nucleic acid sequence encoding a product operably linked to sequences which direct expression of the product in a mammalian target cell.

3. The rAAV according to claim 1 or claim 2, wherein the AAVhu68 capsid

(i) comprises subpopulations having one or more of:

(a) at least 65% of asparagines (N) in asparagine - glycine pairs located at positions 57 of the AAVhu68vp1 proteins are deamidated, based on the numbering of SEQ ID NO:2, as determined using mass spectrometry;

(b) at least 75% of N in asparagine - glycine pairs in position 329 of the AAVhu68vp1, AAVhu68vp2 and AAVhu68vp3 proteins are deamidated, based on the residue numbering of the amino acid sequence of SEQ ID NO: 2, as determined using mass spectrometry;

(c) at least 50% of N in asparagine - glycine pairs in position 452 of the AAVhu68vp1, AAVhu68vp2 and AAVhu68vp3 proteins are deamidated, based on the residue numbering of the amino acid sequence of SEQ ID NO: 2, as determined using mass spectrometry; and/or

(d) at least 75% of Nin asparagine - glycine pairs in position 512 of the AAVhu68vp1, AAVhu68vp2 and AAVhu68vp3 proteins are deamidated, based on the residue numbering of the amino acid sequence of SEQ ID NO: 2, as determined using mass spectrometry;

(ii) comprises a subpopulation of AAVhu68vp1 in which 75% to 100 % of the N at position 57 of the vp1 proteins are deamidated, as determined using mass spectrometry;

(iii) comprises subpopulations of AAVhu68vp1 proteins, vp2 proteins, and/or vp3 proteins in which 75% to 100% of the N at position 329, based on the numbering of SEQ ID NO:2, are deamidated as determined using mass spectrometry;

(iv) comprises subpopulations of AAVhu68vp1 proteins, vp2 proteins, and/or vp3 proteins in which 75% to 100% of the N at position 452, based on the numbering of SEQ ID NO:2, are deamidated as determined using mass spectrometry;

(v) comprises subpopulations of AAVhu68vp1 proteins, vp2 proteins, and/or vp3 proteins in which 75% to 100% of the N at position 512, based on the numbering of SEQ ID NO:2, are deamidated as determined using mass spectrometry;

(vi) comprises subpopulations of AAVhu68vp1, vp2 and/or vp3 proteins which further comprise

(a) 1% to 40% deamidation in at least one or more of positions N94, N113, N252, N253,Q259,N270,N303,N304, N305,N319, N328, N336, N409, N410,N477, N515, N598, Q599, N628, N651, N663, N709 of SEQ ID NO: 2, or combinations thereof; or

(b) one or more modifications selected from one or more modification in one or more of the following: acetylated lysine, phosphorylated serine and/or threonine, isomerized aspartic acid, oxidized tryptophan and/or methionine, or an amidated amino acid, as determined using mass spectrometry; and/or

(vii) further comprises at least subpopulations of AAVhu68vp1, vp2 and/or vp3 proteins having amino acid modifications from SEQ ID NO: 2 comprising at least 50 to 100% deamidation at
three or more of N57, 329, 452, and_512, as determined using mass spectrometry.

4. The rAAV according to claim 1 or 2, wherein the nucleic acid sequence encoding the proteins is SEQ ID NO: 1, or a sequence at least 80% to at least 99% identical to SEQ ID NO: 1 which encodes the amino acid sequence of SEQ ID NO:2.

5. The rAAV according to claim 1 or claim 2, wherein the AAVhu68 capsid comprises subpopulations of AAVhu68vp1, AAVhu68vp2 and/or AAVhu68vp3 proteins having amino acid modifications from SEQ ID NO: 2 comprising at least 50 to 100% deamidation at each of positions N57, 329, 452, and 512, wherein the deamidated asparagines are deamidated to aspartic acid, isoaspartic acid, an interconverting aspartic acid/isoaspartic acid pair, or combinations thereof, as determined using mass spectrometry, wherein the modified AAVhu68 vp1 and vp2 proteins comprise a valine at position 157 and a glutamic acid at position 67 based on the numbering of the vp1 capsid of SEQ ID NO:2, and

wherein the capsid further comprises subpopulations of AAVhu68 VP1, VP2 and VP3 proteins which comprise one or more further modifications, selected from one or more of: 1% to 40% deamidation in at least one or more of positions N94, N113, N252, N253, 0259, N270, N303, N304,N305, N319, N328,N336,N409, N410, N477, N515, N598,Q599,N628, N651, N663, N709, or combinations thereof; and/or one or more modifications of acetylated lysine, phosphorylated serine and/or threonine, isomerized aspartic acid, oxidized tryptophan and/or methionine, or an amidated amino acid, as determined using mass spectrometry.

6. A composition comprising a mixed population of recombinant adeno-associated virus hu68 (rAAVhu68), wherein each of the rAAVhu68 is independently selected from an rAAV according to any one of claims 1 to 5.

7. The composition according to claim 6, wherein the AAV ITR sequences are a 5' ITR and a 3' ITR from AAV2.

8. The composition according to claim 6, wherein the composition is formulated for

(i) intrathecal delivery and the vector genome comprises a nucleic acid sequence encoding a product for delivery to the central nervous system;

(ii) intravenous delivery; or

(iii) intranasal or intramuscular delivery.

9. A composition according to any one of claims 6 to 8 for use in therapy which further comprises an aqueous liquid, a buffer, a carrier and/or a preservative.

10. The composition for use according to claim 9, wherein the therapy is gene therapy.

11. An rAAV production system useful for producing a recombinant AAVhu68 according to claim 1 or claim 2, wherein the production system comprises:

(a) a nucleic acid molecule comprising_an AAVhu68 capsid nucleic acid sequence encoding the amino acid sequence of SEQ ID NO:2;

(b) a nucleic acid molecule suitable for packaging into the AAVhu68 capsid, said nucleic acid molecule comprising at least one AAV inverted terminal repeat (ITR) and a non-AAV nucleic acid sequence encoding a gene product operably linked to sequences which direct expression of the product in a host cell; and

(c) sufficient AAV rep functions and helper functions to permit packaging of the nucleic acid molecule into the recombinant AAVhu68 capsid.

12. The system according to claim 11, wherein

(i) the nucleic acid sequence of (a) comprises at least SEQ ID NO: 1, or a sequence at least 70% to at least 99% identical to SEQ ID NO: 1 which encodes the amino acid sequence of SEQ ID NO:2; and/or

(ii) the system further comprises a nucleic acid molecule comprising a nucleic acid sequence of nucleotide 607 to nucleotide 2211 of SEQ ID NO:1 encoding the AAVhu68 vp3 of amino acid 203 to amino acid 736 of SEQ ID NO: 2.

13. The system according to claim 11 or 12, wherein the cell culture comprises human embryonic kidney 293 cells.

14. The system according to any one of claims 11 to 13, wherein the AAV rep is

(i) is from AAV2;

(ii) AAVhu68rep characterized by the amino acid sequence of SEQ ID NO: 4,; or

(iii) the AAV rep is encoded by the nucleic acid sequence of SEQ ID NO:3.

15. The system according to any one of claims 11 to 14, wherein the AAV rep coding sequence and cap genes are on the same nucleic acid molecule, wherein there is optionally a spacer between the rep sequence and cap gene; optionally wherein the spacer is atgacttaaaccaggt SEQ ID NO: 9.

16. A nucleic acid molecule comprising a nucleic acid sequence encoding an AAVhu68 rep protein under the control of exogenous regulatory control sequences which direct expression thereof in a host cell, wherein the rep protein has the amino acid sequence of SEQ ID NO: 4.

17. A packaging host cell comprising a nucleic acid sequence encoding SEQ ID NO:2.

18. A plasmid comprising a nucleic acid sequence encoding SEQ ID NO: 2.

19. The host cell of claim 17 or the plasmid of claim 18, wherein the nucleic acid sequence is SEQ ID NO:1 or a sequence at least 99% identical thereto encoding SEQ ID NO: 2.