targeting the liver with an AAV vector achieves long-term expression of the FIX transgene at therapeutic levels.

[0005] While these data are promising, the identification of AAV serotypes with high tropism for liver and low seroprevalence in humans (a natural host for wild type AAV) is fundamental for 1) achieving therapeutic levels of transgene expression in liver at the lowest vector dose possible to decrease risk of triggering anti-AAV capsid immune responses; and 2) alternate AAV serotypes with unique seroprevalence will allow to treat those patients populations otherwise not eligible for AAV gene transfer due to pre-existing humoral immunity to AAVs. The invention addresses these needs and provides additional benefits.

Summary

[0006] The disclosure provides adeno-associated virus (AAV) serotype AAV-Rh74 vector, and related AAV vectors and viral particles. Such vectors include AAV-Rh74 which target hepatocyte cells of the liver, among other cell types. As a vector for polynucleotide sequence delivery, AAV-Rh74 and related AAV vectors drive expression of the polynucleotide in cells. Polynucleotides that encode proteins, such as proteins for therapeutic applications are able to be expressed at therapeutic levels after administration.

[0006a] In one aspect, the present disclosure refers to an AAV capsid sequence of any one of RHM4-1 (SEQ ID NO:5), RHM15-1 (SEQ ID NO:6), RHM15-2 (SEQ ID NO:7), RHM15-3/RHM15-5 (SEQ ID NO:8), RHM15-4 (SEQ ID NO:9) and RHM15-6 (SEQ ID NO:10).

[0006b] In another aspect, the present disclosure refers to a recombinant AAV particle, comprising the AAV capsid sequence as describe herein, wherein the particle encapsidates a vector genome.

[0006c] In yet another aspect, the present disclosure refers to a use of the recombinant AAV particle as described herein in the manufacture of a medicament for treating a lysosomal storage disease of a mammal, wherein the recombinant AAV particle is to be administered to said mammal or a cell of said mammal, thereby treating the mammal.

[0006d] In yet another aspect, the present disclosure refers to a use of the recombinant AAV particle as described herein in the manufacture of a medicament for treating a mammal deficient in an enzyme involved in a lysosomal storage disease, wherein the recombinant AAV particle comprises ~~a~~ heterologous polynucleotide sequence encoding a polypeptide that can correct for the enzyme involved in the lysosomal storage disease, wherein the heterologous polynucleotide sequence is operably linked to an expression control element conferring transcription of said heterologous polynucleotide sequence, wherein the recombinant AAV particle is to be administered to the mammal wherein said polypeptide is expressed in the mammal.

[0007] Exemplary AAV-Rh74 and related AAV vectors include AAV-Rh74 variants. Particular capsid variants include an Rh74 capsid sequence with an amino acid substitution at any one of amino acid positions 195, 199, 201 or 202, of RH74 VP1 capsid sequence (SEQ ID NO:1). In particular

examples, the residues correspond to an A, V, P or N amino acid at any one of amino acid positions 195, 199, 201 or 202 of RH74 VP1 capsid sequence (SEQ ID NO:1). In more particular examples, the capsid sequence has an A residue at amino acid position 195; a V residue at amino acid positions 199, a P residue at amino acid position 201, or an N residue at amino acid position 202 of RH74 VP1 capsid sequence (SEQ ID NO:1). In further more particular examples, the capsid sequence has any two, three or all four of the following: an A residue at amino acid position 195; a V residue at amino acid positions 199, a P residue at amino acid position 201, or an N residue at amino acid position 202 of RH74 VP1 capsid sequence (SEQ ID NO:1).

[0008] Recombinant AAV particles of the disclosure include an AAV capsid sequence of any capsid variant such as RHM4-1 (SEQ ID NO:5), RHM15-1 (SEQ ID NO:6), RHM15-2 (SEQ ID NO:7), RHM15-3/RHM15-5 (SEQ ID NO:8), RHM15-4 (SEQ ID NO:9) or RHM15-6 (SEQ ID NO:10). In particular examples, a recombinant AAV particle encapsidates or packages a vector genome (*e.g.*, viral vector such as an AAV vector genome). Such disclosure recombinant AAV particles include a viral (*e.g.*, AAV) vector genome which also includes a heterologous polynucleotide sequence.

What is claimed:

1. An AAV capsid sequence of any one of RHM4-1 (SEQ ID NO:5), RHM15-1 (SEQ ID NO:6), RHM15-2 (SEQ ID NO:7), RHM15-3/RHM15-5 (SEQ ID NO:8), RHM15-4 (SEQ ID NO:9) and RHM15-6 (SEQ ID NO:10).

2. The AAV capsid sequence of claim 1, wherein the sequence comprises RHM4-1 (SEQ ID NO:5).

3. The AAV capsid sequence of claim 1, wherein the sequence comprises RHM15-1 (SEQ ID NO:6).

4. The AAV capsid sequence of claim 1, wherein the sequence comprises RHM15-2 (SEQ ID NO:7).

5. The AAV capsid sequence of claim 1, wherein the sequence comprises RHM15-3/RHM15-5 (SEQ ID NO:8).

6. The AAV capsid sequence of claim 1, wherein the sequence comprises RHM15-4 (SEQ ID NO:9).

7. The AAV capsid sequence of claim 1, wherein the sequence comprises RHM15-4 (SEQ ID NO:9).

8. The AAV capsid sequence of claim 1, wherein the sequence comprises RHM15-6 (SEQ ID NO:10).

9. A recombinant AAV particle, comprising the AAV capsid sequence of any one of claims 1 to 8, wherein the particle encapsidates a vector genome.

10. The recombinant AAV particle of claim 9, wherein the AAV vector genome comprises a heterologous polynucleotide sequence.

11. Use of the recombinant AAV particle of claim 10 in the manufacture of a medicament for treating a lysosomal storage disease of a mammal, wherein the recombinant AAV particle is to be administered to said mammal or a cell of said mammal, thereby treating the mammal.

12. The use of claim 11, wherein the recombinant AAV particle comprises an AAV capsid sequence of RHM4-1 (SEQ ID NO:5).

13. The recombinant AAV particle of claim 10, wherein the heterologous polynucleotide sequence is operably linked to an expression control element conferring transcription of said heterologous polynucleotide sequence.

14. Use of the recombinant AAV particle of claim 10 or 13 in the manufacture of a medicament for treating a mammal deficient in an enzyme involved in a lysosomal storage disease, wherein the recombinant AAV particle comprises a heterologous polynucleotide sequence encoding a polypeptide that can correct for the enzyme involved in the lysosomal storage disease, wherein the heterologous polynucleotide sequence is operably linked to an expression control element conferring transcription of said heterologous polynucleotide sequence, wherein the recombinant AAV particle is to be administered to the mammal wherein said polypeptide is expressed in the mammal.

15. The use of claim 14, wherein said recombinant AAV particle comprises an amino acid substitution at any one of amino acid positions 195, 199, 201 or 202, of VP1 capsid sequence (SEQ ID NO:1).

16. The use of claim 15, wherein the residues correspond to an A, V, P or N amino acid at any one of amino acid positions 195, 199, 201 or 202 of VP1 capsid sequence.

17. The use of claim 15, wherein the VP1 capsid sequence has an A residue at amino acid position 195; a V residue at amino acid position 199, a P residue at amino acid position 201, or an N residue at amino acid position 202.

18. The use of claim 15, wherein the VP1 capsid sequence has any two of an A residue at amino acid position 195; a V residue at amino acid position 199, a P residue at amino acid position 201, or an N residue at amino acid position 202.

19. The use of claim 15, wherein the VP1 capsid sequence has any three of an A residue at amino acid position 195; a V residue at amino acid position 199, a P residue at amino acid position 201, or an N residue at amino acid position 202.

20. The use of claim 15, wherein the VP1 capsid sequence has an A residue at amino acid position 195; a V residue at amino acid position 199, a P residue at amino acid position 201, and an N residue at amino acid position 202.

21. The recombinant AAV particle of claim 10 or 13, wherein said recombinant AAV particle comprises a VP1, VP2, or VP3 sequence having 98% or more identity to one or more VP1, VP2, or VP3 sequences set forth in Figure 3.

22. The recombinant AAV particle of claim 10 or 13, wherein said recombinant AAV particle comprises a VP1, VP2, or VP3 sequence having 99% or more identity to one or more VP1, VP2, or VP3 sequences set forth in Figure 3.

23. The recombinant AAV particle of claim 10 or 13, wherein said recombinant AAV particle comprises a VP1, VP2, or VP3 sequence having 99.5% or more identity to one or more VP1, VP2, or VP3 sequences set forth in Figure 3.

24. The use of any one of claims 11, 12 or 14, wherein said recombinant AAV particle comprises a VP1, VP2, or VP3 sequence of AAV-Rh74 set forth in Figure 3 or AAV-Rh74, RHM4-1 (SEQ ID NO:5), RHM15-1 (SEQ ID NO:6), RHM15-2 (SEQ ID NO:7), RHM15-3/RHM15-5 (SEQ ID NO:8), RHM15-4 (SEQ ID NO:9) or RHM15-6 (SEQ ID NO:10).

25. The use of any one of claims 11, 12 or 14, wherein said heterologous polynucleotide sequence, or said protein, is expressed at levels having a therapeutic effect on the mammal.

26. The recombinant AAV particle of claim 10 or 13, wherein said recombinant AAV particle has increased tropism for hepatocytes compared to Rh74, AAV2 or AAV8 serotypes.

27. The use of any one of claims 11, 12 or 14, wherein said heterologous polynucleotide sequence or said protein is expressed in a cell, tissue or organ of said mammal.

28. The use of claim 27, wherein the cell comprises a secretory cell.

29. The use of claim 27, wherein the cell comprises an endocrine cell.

30. The use of claim 27, wherein the cell comprises hepatocyte, a neural cell, a glial cell, a retinal cell, an epithelial cell, a lung cell or a pluripotent or multipotent stem cell.

31. The use of claim 27, wherein the tissue or organ of said mammal comprises liver, brain, central nervous system, spinal cord, eye, retina or lung.

32. The use of any one of claims 11, 12 or 14, wherein the mammal produces an insufficient amount of the enzyme involved in the lysosomal storage disease.

33. The recombinant AAV particle of claim 10 or 13, wherein the heterologous polynucleotide sequence encodes CFTR (cystic fibrosis transmembrane regulator protein), a blood coagulation or clotting factor, Factor XIII, Factor IX, Factor X, Factor VIII, Factor VIIa, protein C, a gain of function blood coagulation factor, an antibody, retinal pigment epithelium-specific 65 kDa protein (RPE65), erythropoietin, LDL receptor, lipoprotein lipase, ornithine transcarbamylase, β-globin, α-globin, spectrin, α-antitrypsin, adenosine deaminase (ADA), a metal transporter, ATP7A, ATP7,

sulfamidase, an enzyme involved in lysosomal storage disease, ARSA, hypoxanthine guanine phosphoribosyl transferase, β-25 glucocerebrosidase, sphingomyelinase, lysosomal hexosaminidase, branched-chain keto acid dehydrogenase, a hormone, a growth factor, insulin-like growth factor 1 or 2, platelet derived growth factor, epidermal growth factor, nerve growth factor, neurotrophic factor -3 and -4, brain-derived neurotrophic factor, glial derived growth factor, transforming growth factor α and β, a cytokine, α-interferon, β-interferon, interferon-γ, interleukin-2, interleukin-4, interleukin 12, granulocyte-macrophage colony stimulating factor, lymphotoxin, a suicide gene product, herpes simplex virus thymidine kinase, cytosine deaminase, diphtheria toxin, cytochrome P450, deoxycytidine kinase, tumor necrosis factor, a drug resistance protein, a tumor suppressor protein, p53, Rb, Wt-1, NF1, Von Hippel–Lindau (VHL), adenomatous polyposis coli (APC), a peptide with immunomodulatory properties, a tolerogenic or immunogenic peptide or protein Tregitope or hCDR1, insulin, glucokinase, guanylate cyclase 2D (LCA-GUCY2D), Rab escort protein 1, LCA 5, ornithine ketoacid aminotransferase, Retinoschisin 1, USH1C, X-linked retinitis pigmentosa GTPase (XLRP), MERTK, DFNB1, ACHM 2, 3 and 4, PKD-1, PKD-2, TPP1, CLN2, a gene product implicated in lysosomal storage diseases, sulfatase, N-acetylglucosamine-1-phosphate transferase, cathepsin A, GM2-AP, NPC1, VPC2, a sphingolipid activator protein, or one or more zinc finger nucleases for genome editing, or donor sequences used as repair templates for genome editing.

34. The recombinant AAV particle of claim 10 or 13, wherein the heterologous polynucleotide sequence comprises a gene encoding a polypeptide, peptide or a protein.

35. The recombinant AAV particle of claim 34, wherein the gene comprises or encodes CFTR (cystic fibrosis transmembrane regulator protein), a blood coagulation or clotting factor, Factor XIII, Factor IX, Factor X, Factor VII, Factor VIIa, protein C, a gain of function blood coagulation factor, an antibody, retinal pigment epithelium-specific 65 kDa protein (RPE65), erythropoietin, LDL receptor, lipoprotein lipase, ornithine transcarbamylase, β-globin, α-globin, spectrin, α-antitrypsin, adenosine deaminase (ADA), a metal transporter, ATP7A, ATP7, sulfamidase, an enzyme involved in lysosomal storage disease, ARSA, hypoxanthine guanine phosphoribosyl transferase, β-25 glucocerebrosidase, sphingomyelinase, lysosomal hexosaminidase, branched-chain keto acid dehydrogenase, a hormone, a growth factor, insulin-like growth factor 1 or 2, platelet derived growth factor, epidermal growth factor, nerve growth factor, neurotrophic factor -3 and -4, brain-derived neurotrophic factor, glial derived growth factor, transforming growth factor α and β, a cytokine, α-interferon, β-interferon, interferon-γ, interleukin-2, interleukin-4, interleukin 12, granulocyte-macrophage colony stimulating factor, lymphotoxin, a suicide gene product, herpes simplex virus thymidine kinase, cytosine deaminase, diphtheria toxin, cytochrome P450, deoxycytidine kinase, tumor necrosis factor, a drug resistance protein, a tumor suppressor protein, p53, Rb, Wt-1, NF1, Von Hippel–Lindau (VHL), adenomatous polyposis coli (APC), a peptide with immunomodulatory

properties, a tolerogenic or immunogenic peptide or protein Tregitope or hCDR1, insulin, glucokinase, guanylate cyclase 2D (LCA-GUCY2D), Rab escort protein 1, LCA 5, ornithine ketoacid aminotransferase, Retinoschisin 1, USH1C, X-linked retinitis pigmentosa GTPase, MERTK, DFNB1, ACHM 2, 3 and 4, PKD-1, or PKD-2, TPP1, CLN2, a gene product implicated in lysosomal storage diseases, sulfatases, N-acetylglucosamine-1-phosphate transferase, cathepsin A, GM2-AP, NPC1, VPC2, a sphingolipid activator protein or one or more zinc finger nucleases for genome editing, or donor sequences used as repair templates for genome editing.

36. The use of any one of claims 11, 12 or 14, wherein the mammal has lysosomal acid lipase deficiency, Gaucher's disease, Hurler's disease, or a metabolic defect.

37. The recombinant AAV particle of claim 13, wherein the expression control element comprises a constitutive or regulatable control element.

38. The recombinant AAV particle of claim 13, wherein the expression control element comprises a tissue-specific expression control element or promoter.

39. The use of any one of claims 11, 12 or 14, wherein the AAV vector is delivered intravenously, intraarterially, intramuscularly, subcutaneously, orally, by intubation, via catheter, dermally, intra-cranially, via inhalation, intra-cavity, or mucosally.

40. The use of any one of claims 11, 12 or 14, wherein the mammal is human.

41. The use of any one of claims 11, 12 or 14, wherein the mammal is sero-positive for an AAV serotype other than AAV-Rh74.

42. The use of any one of claims 11, 12 or 14, wherein the mammal is sero-positive for an AAV serotype AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10 or AAV11.

43. The use of any one of claims 11, 12 or 14, wherein the mammal is sero-negative or sero-positive for AAV-Rh74.

44. The use of any one of claims 11, 12 or 14, wherein an empty capsid AAV is to be further administered.

45. The use of any one of claims 11, 12 or 14, wherein an empty capsid AAV-Rh74 or an empty capsid comprising an AAV capsid sequence of any of claims 1 to 6 or RHM4-1 (SEQ ID NO:5), RHM15-1 (SEQ ID NO:6), RHM15-2 (SEQ ID NO:7), RHM15-3/RHM15-5 (SEQ ID NO:8), RHM15-4 (SEQ ID NO:9) or RHM15-6 (SEQ ID NO:10) is to be further administered.

46. The recombinant AAV particle of any one of claims 10, 13 and 15, wherein the AAV particle comprises a pharmaceutical composition.

47. The recombinant AAV particle of claim 46, wherein the pharmaceutical composition comprises empty capsid AAV.

48. The recombinant AAV particle of claim 46, wherein the pharmaceutical composition comprises empty capsid AAV-Rh74 or empty capsid comprising an AAV capsid sequence of any of claims 1 to 6 or RHM4-1 (SEQ ID NO:5), RHM15-1 (SEQ ID NO:6), RHM15-2 (SEQ ID NO:7), RHM15-3/RHM15-5 (SEQ ID NO:8), RHM15-4 (SEQ ID NO:9) or RHM15-6 (SEQ ID NO:10).

49. The use of any one of claims 11, 12 or 14, wherein the mammal has Pompe's disease.