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, wherein the sequence has 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).

[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 another aspect, the present disclosure refers to a recombinant AAV particle comprising the 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) or RHM15-6 (SEQ ID NO:10), wherein the particle encapsidates a vector genome.

[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 delivering or transferring a heterologous polynucleotide sequence into a mammal or a cell of a mammal, wherein the recombinant AAV particle is to be administered to said mammal or a cell of said mammal, thereby delivering or transferring the heterologous polynucleotide sequence into the mammal or cell of the mammal.

[0006e] 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 protein expression or function, wherein the recombinant AAV particle comprises ~~a~~ heterologous polynucleotide sequence encoding a polypeptide that can correct for the deficient protein expression or function, 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.

[0006f] In yet another aspect, the present disclosure refers to the recombinant AAV particle as described herein for use as a therapy.

[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.

[0009] AAV-Rh74 and related AAV vector mediated polynucleotide transfer produced protein expression levels that were significantly higher than several other serotypes currently studied in preclinical and clinical settings. In particular, AAV-Rh74 targets polynucleotides to the liver with efficiency at least comparable or superior to the gold standard for liver transduction, AAV8, both in mice and in hemophilia B dogs. (*see, e.g.*, Figures 1 and 2). AAV-Rh74 variants such as, for example, capsid variant RHM4-1 (SEQ ID NO:5) target polynucleotides to the liver with efficiency comparable to or superior to AAV8 and superior to Rh74 AAV in mice (*see, e.g.*, Figure 5). In addition, data in non-human primates show that AAV-Rh74 and AAV-Rh74 variants such as RHM4-1 are approximately two-fold more potent than AAV8 at mediating liver-derived expression of hFIX (*see, e.g.*, Figures 4 and 6).

[0010] Thus, AAV-Rh74 and AAV-Rh74 variants such as capsid variants (*e.g.*, RHM4-1) can be used to deliver polynucleotides, such as gene coding sequences, to express proteins that provide a desirable or therapeutic benefit, as well as for inhibitory nucleotides that reduce or inhibit expression of an undesirable or defective gene, thereby treating a variety of diseases. For example, AAV-Rh74 and AAV-Rh74capsid variants (*e.g.*, RHM4-1) can be used as vectors to deliver to cells, tissues and organs, such as liver therapeutic genes (*e.g.*, FIX, FVIII) to treat hemophilia A, B, etc. Such AAV-Rh74 and AAV-Rh74capsid variant (*e.g.*, RHM4-1) vectors can also be used to deliver genes for a wide range of other metabolic or plasma protein deficiencies, or for other therapeutic purposes, such as but not limited to genes encoding zinc finger nucleases to carry out genome editing in the liver, and for local (liver) delivery of immunomodulatory agents such as alpha-interferon for treatment of hepatitis virus infections, or to treat virtually any disease that requires either liver transduction or presence of the therapeutic transgene product in the bloodstream (which can be achieved by targeting the transgenes for liver expression).

[0011] In addition to efficient delivery of polynucleotides by AAV-Rh74 and related AAV vectors such as AAV-Rh74 variants such as capsid variants (*e.g.*, RHM4-1) into cells *in vitro*, *ex vivo* and *in vivo*, prevalence of anti-AAV-Rh74 antibodies in humans is lower than anti-AAV2 antibodies, and differs from that of anti-AAV8 antibodies (Table 1). Owing to low sero-prevalence, AAV-Rh74 and related AAV vectors such as AAV-Rh74 (capsid) variants (*e.g.*, RHM4-1) can be used in a greater percentage of humans which otherwise would not be eligible for gene transfer, for example, humans that may be sero-positive for other AAV serotypes (*e.g.*, AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, etc.). In addition, AAV-Rh74 and related vectors such as AAV-Rh74 variants including capsid variants (*e.g.*, RHM4-1) can be efficiently produced at high titers (Table 2). Thus, AAV-Rh74 and related AAV vectors can be produced in large amounts for more prevalent clinical diseases.

[0012] In accordance with the disclosure, there are provided recombinant AAV-Rh74 vectors and related AAV vectors such as AAV-Rh74 variants such as capsid variants (*e.g.*, RHM4-1) particles

that include (encapsidate, package) AAV vector genomes. In one example, a recombinant AAV vector includes a heterologous polynucleotide sequence. In another example, a recombinant AAV vector genome is encapsidated or packaged by an AAV-Rh74 capsid or a related AAV such as AAV-Rh74 variants such as a capsid variant (*e.g.*, RHM4-1).

[0013] In disclosure recombinant AAV vectors, such as AAV-Rh74 vectors and related AAV vectors such as AAV-Rh74 (capsid) variants (*e.g.*, RHM4-1) particles that include (encapsidate, package) recombinant AAV vector genomes, the heterologous polynucleotide sequence may or be transcribed and subsequently translated into a protein. Alternatively, the heterologous polynucleotide may be transcribed into a transcript that in itself has a function or activity (*e.g.*, as an inhibitory nucleic acid).

[0014] In various examples, the heterologous polynucleotide sequence encodes a therapeutic protein. In particular examples, the protein is a blood clotting factor (*e.g.*, Factor XIII, Factor IX, Factor X, Factor VIII, Factor VIIa, or protein C), CFTR (cystic fibrosis transmembrane regulator protein), 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 or 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 (*e.g.*, insulin-like growth factors 1 and 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, etc.), a cytokine (*e.g.*, α-interferon, β-interferon, interferon-γ, interleukin-2, interleukin-4, interleukin 12, granulocyte-macrophage colony stimulating factor, lymphotoxin, etc.), a suicide gene product (*e.g.*, herpes simplex virus thymidine kinase, cytosine deaminase, diphtheria toxin, cytochrome P450, deoxycytidine kinase, tumor necrosis factor, etc.), a drug resistance protein (*e.g*, that provides resistance to a drug used in cancer therapy), a tumor suppressor protein (*e.g.*, 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 Tregitopes, or hCDR1, insulin, glucokinase, guanylate cyclase 2D (LCA-GUCY2D), Rab escort protein 1 (Choroideremia), LCA 5 (LCA-Lebercilin), ornithine ketoacid aminotransferase (Gyrate Atrophy), Retinoschisin 1 (X-linked Retinoschisis), USH1C (Usher's Syndrome 1C), X-linked retinitis pigmentosa GTPase (XLRP), MERTK (AR forms of RP: retinitis pigmentosa), DFNB1 (Connexin 26 deafness), ACHM 2, 3 and 4 (Achromatopsia), PKD-1 or PKD-2 (Polycystic kidney disease), TPP1, CLN2, gene deficiencies causative of lysosomal storage diseases (e.g., sulfatases, N-acetylglucosamine-1-phosphate transferase, cathepsin A, GM2-AP, NPC1, VPC2, Sphingolipid activator proteins, etc.), one or more zinc finger nucleases for genome editing, or donor sequences used as repair templates for genome editing.

[0015] In additional examples, the heterologous polynucleotide sequence encodes a therapeutic protein that in turn inhibits expression or function of an undesirable or aberrant (dysfunctional) protein present (endogenous) in a subject. In further examples, the heterologous polynucleotide sequence is a polynucleotide which, when transcribed, is transcribed into an inhibitory nucleic acid (e.g., inhibitory RNA). In more particular examples, an inhibitory nucleic acid is a single-stranded sequence, or forms a double- or triple-stranded sequence. In additional more particular examples, an inhibitory nucleic acid is a micro-RNA (miRNA), siRNA, shRNA, trans-splicing RNA, antisense RNA or triplex forming RNA.

[0016] In further more particular examples, an inhibitory nucleic acid inhibits expression of: huntingtin (HTT) gene, a gene associated with dentatorubropallidolusyan atropy (e.g., atrophin 1, ATN1); androgen receptor on the X chromosome in spinobulbar muscular atrophy, human Ataxin-1, -2, -3, and -7, $Ca_v2.1$ P/Q voltage-dependent calcium channel is encoded by the (CACNA1A), TATA-binding protein, Ataxin 8 opposite strand, also known as ATXN8OS, Serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B beta isoform in spinocerebellar ataxia (type 1, 2, 3, 6, 7, 8, 12 17), *FMR1* (fragile X mental retardation 1) in fragile X syndrome, *FMR1* (fragile X mental retardation 1) in fragile X-associated tremor/ataxia syndrome, *FMR1* (fragile X mental retardation 2) or AF4/FMR2 family member 2 in fragile XE mental retardation; Myotonin-protein kinase (MT-PK) in myotonic dystrophy; Frataxin in Friedreich's ataxia; a mutant of superoxide dismutase 1 (SOD1) gene in amyotrophic lateral sclerosis; a gene involved in pathogenesis of Parkinson's disease and/or Alzheimer's disease; apolipoprotein B (APOB) and proprotein convertase subtilisin/kexin type 9 (PCSK9), hypercoloesterolemia; HIV Tat, human immunodeficiency virus transactivator of transcription gene, in HIV infection; HIV TAR, HIV TAR, human immunodeficiency virus transactivator response element gene, in HIV infection; C-C chemokine receptor (CCR5) in HIV infection; Rous sarcoma virus (RSV) nucleocapsid protein in RSV infection, liver-specific microRNA (miR-122) in hepatitis C virus infection; p53, acute kidney injury or delayed graft function kidney transplant or kidney injury acute renal failure; protein kinase N3 (PKN3) in advance recurrent or metastatic solid malignancies; LMP2, LMP2 also known as proteasome subunit beta-type 9 (PSMB 9), metastatic melanoma; LMP7,also known as proteasome subunit beta-type 8 (PSMB 8), metastatic melanoma; MECL1 also known as proteasome subunit beta-type 10 (PSMB 10), metastatic melanoma; vascular endothelial growth factor (VEGF) in solid tumors; kinesin spindle protein in solid tumors, apoptosis suppressor B-cell CLL/lymphoma (BCL-2) in chronic myeloid leukemia; ribonucleotide reductase M2 (RRM2) in solid tumors; Furin in solid tumors; polo-like kinase 1 (PLK1) in liver tumors, diacylglycerol acyltransferase 1 (DGAT1) in hepatitis C infection, beta-catenin in familial adenomatous polyposis; beta2 adrenergic receptor, glaucoma; RTP801/Redd1 also known as DAN damage-inducible transcript 4 protein, in diabetic macular oedma (DME) or age-related macular degeneration; vascular endothelial growth factor

receptor I (VEGFR1) in age-related macular degeneration or choroidal neivascularization, caspase 2 in non-arteritic ischaemic optic neuropathy; Keratin 6A N17K mutant protein in pachyonychia congenital; influenza A virus genome/gene sequences in influenza infection; severe acute respiratory syndrome (SARS) coronavirus genome/gene sequences in SARS infection; respiratory syncytial virus genome/gene sequences in respiratory syncytial virus infection; Ebola filovirus genome/gene sequence in Ebola infection; hepatitis B and C virus genome/gene sequences in hepatitis B and C infection; herpes simplex virus (HSV) genome/gene sequences in HSV infection, coxsackievirus B3 genome/gene sequences in coxsackievirus B3 infection; silencing of a pathogenic allele of a gene (allele-specific silencing) like torsin A (TOR1A) in primary dystonia, pan-class I and HLA-allele specific in transplant; mutant rhodopsin gene (RHO) in autosomal dominantly inherited retinitis pigmentosa (adRP); or the inhibitory nucleic acid binds to a transcript of any of the foregoing genes or sequences.

[0017] Disclosure recombinant AAV vectors and AAV-Rh74 and related AAV vectors such as AAV-Rh74 vector variants such as capsid variants (e.g., RHM4-1) particles that include (encapsidate, package) recombinant AAV vector genome include additional elements that function in cis or in trans. In particular examples, a recombinant viral (*e.g.*, AAV) vector and/or AAV-Rh74 vector or related AAV vector such as a AAV-Rh74 (capsid) variants (e.g., RHM4-1) particle that includes (encapsidate, package) recombinant AAV vector genome also has: one or more inverted terminal repeat (ITR) sequences that flank the 5' or 3' terminus of the heterologous polynucleotide sequence; an expression control sequence that drives transcription of the heterologous polynucleotide sequence (*e.g.*, a promoter or enhancer that contributes to transcription of the heterologous polynucleotide sequence, such as a constitutive or regulatable control element, or tissue-specific expression control element); a poly-Adenine sequence located 3' of the heterologous polynucleotide sequence; a selectable marker (*e.g.*, a protein that provides antibiotic resistance, such as Kanamycin resistance); and/or an origin of replication.

[0018] Disclosure recombinant AAV vectors and AAV-Rh74 vectors and related AAV vectors such as AAV-Rh74 variants including capsid variant (e.g., RHM4-1) particles that include (encapsidate, package) recombinant AAV vector genome can also include additional elements. In one example, a recombinant vector genome includes a heterologous polynucleotide sequence and a filler or stuffer polynucleotide sequence. In particular examples, a heterologous polynucleotide sequence has a length less than about 4.7 Kb. In further particular examples, a heterologous polynucleotide sequence has a length less than 4.7 Kb and is positioned within two adeno-associated virus (AAV) ITR sequences. In additional particular examples, a filler or stuffer polynucleotide sequence has a length that when combined with the heterologous polynucleotide sequence the total combined length of the heterologous polynucleotide sequence and filler or stuffer polynucleotide sequence is between about 3.0-5.5Kb, or between about 4.0-5.0Kb, or between about 4.3-4.8Kb.

[0019] Filler or stuffer polynucleotide sequences can be located in the vector sequence at any desired position such that it does not prevent a function or activity of the vector. In one example, a filler or stuffer polynucleotide sequence is not positioned between a 5' and/or 3' ITR that flanks the respective 5' and/or 3' termini of a heterologous polynucleotide sequence. In another example, a filler or stuffer polynucleotide sequence is positioned within a 5' and/or 3' ITR that flanks the respective 5' and/or 3' termini of a heterologous polynucleotide sequence. In an additional example, a filler or stuffer polynucleotide sequence is positioned adjacent to 5' and/or 3' ITR that flanks the respective 5' and/or 3' termini of a heterologous polynucleotide sequence. In a further example, a filler or stuffer polynucleotide sequence is positioned within a heterologous polynucleotide sequence, *e.g.*, analogous to an intron within a genomic nucleic acid.

[0020] Accordingly, in various examples, a filler or stuffer polynucleotide sequence is positioned adjacent to an AAV ITR sequence; positioned within two adeno-associated virus (AAV) ITR sequences; positioned outside two adeno-associated virus (AAV) ITR sequences; or there are two filler or stuffer polynucleotide sequences, a first filler or stuffer polynucleotide sequence positioned within two adeno-associated virus (AAV) ITR sequences, and a second filler or stuffer polynucleotide sequence positioned outside two adeno-associated virus (AAV) ITR sequences.

[0021] In more particular examples, a filler or stuffer polynucleotide sequence has a length that when combined with said heterologous polynucleotide sequence the total combined length of the heterologous polynucleotide sequence and filler or stuffer polynucleotide sequence is between about 3.0-5.5Kb, between about 4.0-5.0Kb, or between about 4.3-4.8Kb, when positioned within two adeno-associated virus (AAV) ITR sequences. In other more particular examples, a filler or stuffer polynucleotide sequence has a length greater than 4.7Kb, between about 5.0-10.0Kb, or between about 6.0-8.0Kb, when positioned outside two adeno-associated virus (AAV) ITR sequences.

[0022] In various additional examples, a filler or stuffer polynucleotide sequence is a sequence between about 1-10, 10-20, 20-30, 30-40, 40-50, 50-60, 60-75, 75-100, 100-150, 150-200, 200-250, 250-300, 300-400, 400-500, 500-750, 750-1,000, 1,000-1,500, 1,500-2,000, 2,000-2,500, 2,500-3,000, 3,000-3,500, 3,500-4,000, 4,000-4,500, 4,500-5,000, 5,500-6,000, 6,000-7,000, 7,000-8,000, or 8,000-9,000 nucleotides in length.

[0023] Typically, a filler or stuffer polynucleotide sequence is inert or innocuous and has no function or activity. In various particular examples, a filler or stuffer polynucleotide sequence is not a bacterial polynucleotide sequence, a filler or stuffer polynucleotide sequence is not a sequence that encodes a protein or peptide, a filler or stuffer polynucleotide sequence is a sequence distinct from any of: the heterologous polynucleotide sequence, an AAV inverted terminal repeat (ITR) sequence, an expression control element, an origin of replication, a selectable marker or a poly-Adenine (poly-A) sequence.

[0024] In various additional particular examples, a filler or stuffer polynucleotide sequence is an intron sequence that is related to or unrelated to the heterologous polynucleotide sequence. In particular examples, the intron sequence is positioned within the heterologous polynucleotide sequence. In other particular examples, the intron sequence is related to the heterologous polynucleotide sequence as the intron is in genomic DNA, such as the genomic DNA that encodes a protein which protein is also encoded by the heterologous polynucleotide sequence.

[0025] Disclosure recombinant AAV vectors and AAV-Rh74 and related AAV vectors such as AAV-Rh74 variants such as capsid variant (e.g., RHM4-1) particles that include (encapsidate, package) recombinant AAV vector genome can be included within cells. In such examples, cells can comprise helper cells lysed to produce virus (AAV) particles (*e.g.*, AAV-Rh74 vectors or related AAV vectors such as AAV-Rh74 capsid variants (e.g., RHM4-1)), or target cells in which it is desired to express the heterologous polynucleotide sequence.

[0026] Disclosure recombinant AAV vectors and AAV-Rh74 vectors and related AAV vectors such as AAV-Rh74 variants (e.g., capsid variants such as RHM4-1) particles that include (encapsidate, package) recombinant AAV vector genome can be included within pharmaceutical compositions. Such compositions are useful for administration of recombinant vector (*e.g.*, AAV) and virus particles such as AAV-Rh74 vectors and related AAV vectors such as AAV-Rh74 variants (e.g., capsid variants such as RHM4-1) that include (encapsidate, package) recombinant vector (*e.g.*, AAV) genomes to a subject.

[0027] Disclosure recombinant AAV vectors and AAV-Rh74 vectors and related AAV vectors such as AAV-Rh74 variants (e.g., capsid variants such as RHM4-1) particles that include (encapsidate, package) recombinant AAV vector genome may be employed in various methods and uses. Accordingly, there are provided methods and uses for delivering or transferring a heterologous polynucleotide sequence into an organism or cell, such as a mammal or a cell of a mammal.

[0028] In one example, a method or use includes administering an adeno-associated virus (AAV) vector that includes a heterologous polynucleotide sequence (*e.g.*, via an AAV-Rh74 vector or related AAV such as AAV-Rh74 variants (e.g., capsid variants such as RHM4-1)) particle that includes (encapsidate, package) the vector genome to a mammal or a cell of a mammal under suitable conditions to deliver or transfer the heterologous polynucleotide sequence into the mammal or the cell of a mammal. In one example, the method or use allows transfer/delivery of the heterologous polynucleotide into the mammal and/or cell. In another example, the method allows transfer/delivery of the heterologous polynucleotide into the mammal and/or cell, and subsequent transcription of the heterologous polynucleotide thereby forming a transcript. In a further example, the method allows transfer/delivery of the heterologous polynucleotide into the cell, subsequent transcription to form a transcript and subsequent translation to form a gene product (protein). In particular, for example, in the latter two examples a heterologous polynucleotide sequence is operably

linked to an expression control element conferring transcription of the heterologous polynucleotide sequence, and optionally subsequent translation of the transcript.

[0029] In additional examples, a method or use is for delivering or transferring a heterologous polynucleotide sequence into a subject (*e.g.*, mammal) or a cell of a subject (*e.g.*, mammal), and includes administering a viral (*e.g.,* AAV-Rh74 or related AAV such as AAV-Rh74 capsid variants (e.g., RHM4-1)) particle, a plurality of such viral (*e.g.*, AAV) particles, or a pharmaceutical composition of such a viral (*e.g.*, AAV-Rh74 or related AAV such as AAV-Rh74 capsid variants (e.g., RHM4-1)) particle or plurality of such viral (*e.g.*, AAV) particles to a subject (*e.g.*, mammal) or a cell of the subject (*e.g.*, mammal), thereby delivering or transferring a heterologous polynucleotide sequence into the subject (*e.g.*, mammal) or cell of the subject (*e.g.*, mammal). In another example, a method or use is for treating a subject (*e.g.*, mammal) deficient or in need of protein expression or function, or in need of reduced expression or function of an endogenous protein (e.g., an undesirable, aberrant or dysfunctional protein), that includes providing a viral (*e.g.*, AAV-Rh74 and related AAV such as AAV-Rh74 variants (e.g., capsid variants such as RHM4-1)) particle, a plurality of such viral (*e.g.*, AAV) particles, or a pharmaceutical composition of a viral (*e.g.*, AAV-Rh74 and related AAV such as AAV-Rh74 variants (e.g., capsid variants such as RHM4-1)) particle or plurality of such viral (*e.g.*, AAV) particles; and administering the viral (*e.g.*, AAV-Rh74 and related AAV such as AAV-Rh74 variants (e.g., capsid variants such as RHM4-1)) particle, plurality of such viral (*e.g.*, AAV) particles, or pharmaceutical composition of viral (*e.g.*, AAV-Rh74 and related AAV such as AAV-Rh74 variants (e.g., capsid variants such as RHM4-1)) particle or plurality of such viral (*e.g.*, AAV) particles to the subject (*e.g.*, mammal), where the heterologous polynucleotide sequence is expressed in the mammal, or wherein the heterologous polynucleotide sequence encodes an inhibitory sequence or protein that reduces expression or function of an endogenous protein (*e.g.*, an undesirable, aberrant or dysfunctional protein) in the subject (*e.g.*, mammal).

[0030] Methods and uses for administration or delivery include any mode compatible with a subject. In particular examples, a viral (*e.g.*, AAV-Rh74 or related AAV such as AAV-Rh74 capsid variant (e.g., RHM4-1)) particle or plurality of such viral (*e.g.*, AAV) particles (*e.g.*, AAV-Rh74 vectors or related AAV vectors such as AAV-Rh74 capsid variants (e.g., RHM4-1)) is administered or delivered intravenously, intraarterially, intramuscularly, subcutaneously, orally, by intubation, via catheter, dermally, intra-cranially, via inhalation, intra-cavity, or mucosally.

[0031] Subjects include mammals, such as humans and non-humans (e.g., primates). In particular examples, a subject would benefit from or is in need of expression of a heterologous polynucleotide sequence.

[0032] In accordance with the disclosure, methods of producing recombinant vector (*e.g.*, AAV) plasmids and virus (*e.g.*, AAV-Rh74 and related AAV such as AAV-Rh74 variants (e.g., capsid

variants such as RHM4-1)) particles that include (encapsidate, package) recombinant vector (*e.g.*, AAV) are provided. In one example, a method of producing recombinant viral or AAV particles includes introducing into a packaging helper cell a recombinant vector (*e.g.*, AAV) plasmid to produce a productive viral (*e.g.*, AAV-Rh74 and related AAV such as AAV-Rh74 variants (e.g., capsid variants such as RHM4-1)) infection; and culturing the helper cells under conditions to produce recombinant viral (*e.g.*, AAV-Rh74 or related AAV such as AAV-Rh74 capsid variants (e.g., RHM4-1)) particles. In another example, a method of producing recombinant viral or AAV particles with reduced amounts of recombinant viral (*e.g.*, AAV-Rh74 and related AAV such as AAV-Rh74 variants (e.g., capsid variants such as RHM4-1)) particles in which the recombinant viral vector includes contaminating nucleic acid, includes introducing into a packaging helper cell a recombinant vector (*e.g.*, AAV) plasmid; and culturing the helper cells under conditions to produce recombinant viral (*e.g.*, AAV-Rh74 and related AAV such as AAV-Rh74 variants (e.g., capsid variants such as RHM4-1)) particles, wherein the recombinant viral (*e.g.*, AAV-Rh74 and related AAV such as AAV-Rh74 variants (e.g., capsid variants such as RHM4-1)) particles produced have reduced numbers of viral (*e.g.*, AAV-Rh74 and related AAV such as AAV-Rh74 variants (e.g., capsid variants such as RHM4-1)) particles with recombinant vector genomes that contain contaminating nucleic acid compared to the numbers of viral (*e.g.*, AAV-Rh74 and related AAV such as AAV-Rh74 variants (e.g., capsid variants such as RHM4-1)) particles that contain contaminating nucleic acid produced under conditions in which the filler or stuffer polynucleotide sequence is absent from the recombinant viral vector. In particular examples, the contaminating nucleic acid is bacterial nucleic acid; or a sequences other than the heterologous polynucleotide sequence, or ITR, promoter, enhancer, origin of replication, poly-Adenine sequence, or selectable marker.

[0033] Helper cells include mammalian cells. In particular examples, a helper cell provides helper (*e.g.*, AAV) functions to package the heterologous polynucleotide sequence into a viral particle (*e.g.*, AAV particle such as AAV-Rh74 and related AAV vectors such as AAV-Rh74 variants (e.g., capsid variants such as RHM4-1)). In particular examples, a helper cell provides AAV Rep and/or Cap proteins (*e.g.*, Rep78 or/and Rep68 proteins); a helper cell is stably or transiently transfected with polynucleotide(s) encoding Rep and/or Cap protein sequence(s); a helper cell is stably or transiently transfected with Rep78 and/or Rep68 protein polynucleotide encoding sequence(s).

[0034] Disclosure recombinant vector (*e.g.*, AAV) plasmids can be based upon any strain or serotype, including hybrids or chimeras of different serotypes. Disclosure recombinant viral (*e.g.*, AAV) particles are typically based upon AAV-Rh74 or related AAV such as AAV-Rh74 and related AAV such as AAV-Rh74 variants (e.g., capsid variants such as RHM4-1)), but also include hybrids or chimeras with different serotypes. Representative AAV serotypes include, without limitation,

AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, and Rh10 serotypes. Accordingly, disclosure recombinant viral (*e.g.*, AAV) particles comprising vector genomes can include a capsid protein from a different serotype, a mixture of serotypes, or hybrids or chimeras of different serotypes, such as a VP1, VP2 or VP3 capsid protein of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, Rh10 serotype. Furthermore, disclosure recombinant vectors (*e.g.*, AAV), sequences, plasmids, vector genomes, can include elements from any one serotype, a mixture of serotypes, or hybrids or chimeras of different serotypes. In various examples, a recombinant AAV vector includes a Cap, Rep, and/or ITR sequence derived from AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV11, Rh74, or Rh10 serotype, or a mixture, hybrid or chimera of any of the foregoing AAV serotypes.

Description of Drawings

[0035] Figure 1 shows human factor IX (FIX) plasma levels in C57BL/6 mice (n=5 per group) injected via the tail vein with AAV vectors expressing the FIX transgene under the control of a liver-specific promoter. Vector dose 2.5^{10} vector genomes per mouse. FIX transgene product (FIX protein) plasma levels were measured by ELISA at weeks 1, 2, and 4 post gene transfer. AAV-Rh74 conferred the highest levels of FIX transgene expression.

[0036] Figure 2 shows canine FIX plasma levels in hemophilia B dogs after the delivery of 3^{12} vector genomes per kilogram (kg) of weight. AAV vectors were infused intravenously (IV) though the saphenous vein and FIX levels were monitored by ELISA. Expression of the therapeutic FIX transgene was driven by a liver specific promoter. AAV8 and AAV-Rh74 vectors performed roughly equally in hemophilia B dogs and were both superior to AAV6.

[0037] Figure 3 shows AAV-Rh74 VP1, VP2, and VP3 amino acid sequences and, for VP1, polynucleotide (DNA) sequence (SEQ ID NOs:1-4).

[0038] Figure 4 shows administration of AAV8 and AAVrh74 vector expressing human Factor IX (FIX) (under the control of a liver-specific promoter) to rhesus macaques, a non-human primate, and expression of FIX in the animals. Animals receiving the AAVrh74-FIX vectors (last two bars towards the right margin) expressed the FIX transgene at higher levels compared to the other groups of animals injected at the same dose.

[0039] Figure 5 shows plasma human factor IX expression levels in animals administered AAV human factor IX expression vector encapsidated by the indicated AAV (Rh74 variants, such as RHM4-1 variant), compared to Rh74 andAAV8 encapsidated AAV human factor IX expression vector.

What is claimed:

1. An AAV capsid sequence, wherein the sequence has 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).

2. The AAV capsid sequence of claim 1, 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 RH74 VP1 capsid sequence (SEQ ID NO:1).

3. The AAV capsid sequence of claim 1, wherein the 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 of RH74 VP1 capsid sequence (SEQ ID NO:1).

4. The AAV capsid sequence of claim 1, wherein the 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 of RH74 VP1 capsid sequence (SEQ ID NO:1).

5. The AAV capsid sequence of claim 1, wherein the 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 of RH74 VP1 capsid sequence (SEQ ID NO:1).

6. The AAV capsid sequence of claim 1, wherein the 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 of RH74 VP1 capsid sequence (SEQ ID NO:1).

7. A recombinant AAV particle, comprising the AAV capsid sequence of any one of claims 1 to 6 , wherein the particle encapsidates a vector genome.

8. A recombinant AAV particle comprising the 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) or RHM15-6 (SEQ ID NO:10), wherein the particle encapsidates a vector genome.

9. The recombinant AAV particle comprising claim 7 or 8, wherein the particle encapsidates an AAV 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 delivering or transferring a heterologous polynucleotide sequence into a mammal or a cell of a mammal, wherein the recombinant AAV particle is to be administered to said mammal or a cell of said mammal, thereby delivering or transferring the heterologous polynucleotide sequence into the mammal or cell of the mammal.

12. The use of claim 11, wherein the recombinant AAV particle comprises an AAV capsid sequence of any one 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).

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 protein expression or function, wherein the recombinant AAV particle comprises a heterologous polynucleotide sequence encoding a polypeptide that can correct for the deficient protein expression or function, 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 recombinant AAV particle of claim 10 or 13, wherein said recombinant AAV particle comprises a VP1 sequence having 90% or more sequence identity to:
MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDNGRGLVLPGYKYLGPFNGL
DKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGNLGRAV
FQAKKRVLEPLGLVESPVKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPAKKRLNFGQTGDS
ESVPDPQPIGEPPAGPSGLGSGTMAAGGGAPMADNNEGADGVGSSSGNWHCDSTWLGDRV
ITTSTRTWALPTYNNHLYKQISNGTSGGSTNDNTYFGYSTPWGYFDFNRFHCHFSPRDWQR
LINNNWGFRPKRLNFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFTDSEYQLPYVLGSAHQG
CLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFEFSYNFEDVPFHS
SYAHSQSLDRLMNPLIDQYLYYLSRTQSTGGTAGTQQLLFSQAGPNNMSAQAKNWLPGPC
YRQQRVSTTLSQNNNSNFAWTGATKYHLNGRDSLVNPGVAMATHKDDEERFFPSSGVLMF
GKQGAGKDNVDYSSVMLTSEEEIKTTNPVATEQYGVVADNLQQQNAAPIVGAVNSQGALP
GMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPPPQILIKNTPVPADPPTTFNQ
AKLASFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYYKSTNVDFAVNTEGTYSEPRP
IGTRYLTRNL, or 1-50 amino acid substitutions, deletions or additions thererto;

a VP2 sequence having 90% or more sequence identity to:
TAPGKKRPVEPSPQRSPDSSTGIGKKGQQPAKKRLNFGQTGDSESVPDPQPIGEPPAGPSGLG
SGTMAAGGGAPMADNNEGADGVGSSSGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYK
QISNGTSGGSTNDNTYFGYSTPWGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLNFKLFNI
QVKEVTQNEGTKTIANNLTSTIQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTL
NNGSQAVGRSSFYCLEYFPSQMLRTGNNFEFSYNFEDVPFHSSYAHSQSLDRLMNPLIDQYL
YYLSRTQSTGGTAGTQQLLFSQAGPNNMSAQAKNWLPGPCYRQQRVSTTLSQNNNSNFAW
TGATKYHLNGRDSLVNPGVAMATHKDDEERFFPSSGVLMFGKQGAGKDNVDYSSVMLTS
EEEIKTTNPVATEQYGVVADNLQQQNAAPIVGAVNSQGALPGMVWQNRDVYLQGPIWAKI
PHTDGNFHPSPLMGGFGLKHPPPQILIKNTPVPADPPTTFNQAKLASFITQYSTGQVSVEIEW
ELQKENSKRWNPEIQYTSNYYKSTNVDFAVNTEGTYSEPRPIGTRYLTRNL, or 1-50 amino acid substitutions, deletions or additions thererto; and/or
a VP3 sequence having 90% or more sequence identity to:
MAAGGGAPMADNNEGADGVGSSSGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQIS
NGTSGGSTNDNTYFGYSTPWGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQV
KEVTQNEGTKTIANNLTSTIQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNN
GSQAVGRSSFYCLEYFPSQMLRTGNNFEFSYNFEDVPFHSSYAHSQSLDRLMNPLIDQYLYY
LSRTQSTGGTAGTQQLLFSQAGPNNMSAQAKNWLPGPCYRQQRVSTTLSQNNNSNFAWTG
ATKYHLNGRDSLVNPGVAMATHKDDEERFFPSSGVLMFGKQGAGKDNVDYSSVMLTSEE
EIKTTNPVATEQYGVVADNLQQQNAAPIVGAVNSQGALPGMVWQNRDVYLQGPIWAKIPH
TDGNFHPSPLMGGFGLKHPPPQILIKNTPVPADPPTTFNQAKLASFITQYSTGQVSVEIEWEL
QKENSKRWNPEIQYTSNYYKSTNVDFAVNTEGTYSEPRPIGTRYLTRNL, or 1-50 amino acid substitutions, deletions or additions thererto.

16. 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).

17. The use of claim 16, 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.

18. The use of claim 16, 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.

19. The use of claim 16, 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.

20. The use of claim 16, 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.

21. The use of claim 16, 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.

22. The recombinant AAV particle of claim 10 or 13, wherein said recombinant AAV particle comprises an AAV with a VP1, VP2, or VP3 sequence having 95% 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 96% or more identity to one or more VP1, VP2, or VP3 sequences set forth in Figure 3.

24. The recombinant AAV particle of claim 10 or 13, wherein said recombinant AAV particle comprises a VP1, VP2, or VP3 sequence having 97% or more identity to one or more VP1, VP2, or VP3 sequences set forth in Figure 3.

25. 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.

26. 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.

27. 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.

28. 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).

29. 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.

30. 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.

31. 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.

32. The use of claim 31, wherein the cell comprises a secretory cell.

33. The use of claim 31, wherein the cell comprises an endocrine cell.

34. The use of claim 31, 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.

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

36. The use of any one of claims 11, 12 or 14, wherein the mammal produces an insufficient amount of a protein, or a defective or aberrant protein.

37. 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.

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

39. The recombinant AAV particle of claim 38, 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.

40. The recombinant AAV particle of claim 10 or 13, wherein the heterologous polynucleotide sequence comprises an inhibitory nucleic acid.

41. The recombinant AAV particle of claim 40, wherein the inhibitory nucleic acid comprises micro-RNA (miRNA), siRNA, shRNA, trans-splicing RNA, antisense RNA or triplex forming RNA.

42. The recombinant AAV particle of claim 40, wherein the inhibitory nucleic acid binds to a pathogenic gene, a transcript of a pathogenic gene, or a gene transcript associated with a polynucleotide repeat disease, a huntingtin (HTT) gene, a gene associated with dentatorubropallidolusyan atropy, atrophin 1, androgen receptor on the X chromosome in spinobulbar muscular atrophy, human Ataxin-1, -2, -3, and -7, $Ca_v2.1$ P/Q voltage-dependent calcium channel encoded by CACNA1A, TATA-binding protein, Ataxin 8 opposite strand, also known as ATXN8OS, Serine/threonine-protein phosphatase 2A 55 kDa regulatory subunit B beta isoform, *FMR1* (fragile X mental retardation 1), *FMR1* (fragile X mental retardation 1) in fragile X-associated tremor/ataxia syndrome, *FMR2* (fragile X mental retardation 2), AF4/FMR2 family member 2 in fragile XE mental retardation; Myotonin-protein kinase (MT-PK) in myotonic dystrophy; Frataxin in Friedreich's ataxia; a mutant of superoxide dismutase 1 (SOD1) gene in amyotrophic lateral sclerosis; a gene involved in pathogenesis of Parkinson's disease and/or Alzheimer's disease; apolipoprotein B (APOB), proprotein convertase subtilisin/kexin type 9 (PCSK9), hypercoloesterolemia; HIV Tat, human immunodeficiency virus transactivator of transcription gene, in HIV infection; HIV TAR, HIV TAR, human immunodeficiency virus transactivator response element gene; C-C chemokine receptor (CCR5); Rous sarcoma virus (RSV) nucleocapsid protein in RSV infection, liver-specific microRNA (miR-122) in hepatitis C virus infection; p53, acute kidney injury or delayed graft function kidney transplant or kidney injury acute renal failure; protein kinase N3 (PKN3) in advance recurrent or metastatic solid malignancies; LMP2, LMP2 also known as proteasome subunit beta-type 9 (PSMB 9), metastatic melanoma; LMP7, also known as proteasome subunit beta-type 8 (PSMB 8), metastatic melanoma; MECL1 also known as proteasome subunit beta-type 10 (PSMB 10), metastatic melanoma; vascular endothelial growth factor (VEGF) in solid tumors; kinesin spindle protein in solid tumors, apoptosis suppressor B-cell CLL/lymphoma (BCL-2) in chronic myeloid leukemia; ribonucleotide reductase M2 (RRM2) in solid tumors; Furin in solid tumors; polo-like kinase 1 (PLK1) in liver tumors, diacylglycerol acyltransferase 1 (DGAT1) in hepatitis C infection, beta-catenin in familial adenomatous polyposis; beta2 adrenergic receptor, glaucoma; RTP801/Redd1 also known as DAN damage-inducible transcript 4 protein, in diabetic macular oedma (DME) or age-related macular degeneration; vascular endothelial growth factor receptor I (VEGFR1) in age-related macular degeneration or choroidal neivascularization, caspase 2 in non-arteritic ischaemic optic neuropathy; Keratin 6A N17K mutant protein in pachyonychia congenital; influenza A virus genome/gene sequences in influenza infection; severe acute respiratory syndrome (SARS) coronavirus genome/gene sequences in SARS infection; respiratory syncytial virus genome/gene sequences in respiratory syncytial virus infection; Ebola filovirus genome/gene sequence in Ebola infection; hepatitis B and C virus genome/gene sequences in hepatitis B and C infection; herpes simplex virus (HSV) genome/gene sequences in HSV infection, coxsackievirus B3 genome/gene sequences in coxsackievirus B3 infection; silencing

of a pathogenic allele of a gene, torsin A (TOR1A) in primary dystonia, pan-class I and HLA-allele specific in transplant; or mutant rhodopsin gene (RHO) in autosomal dominantly inherited retinitis pigmentosa (adRP).

43. The use of any one of claims 11, 12 or 14, wherein the mammal has Choroideremia, LCA-Lebercilin, Gyrate Atrophy, X-linked Retinoschisis, Usher's Syndrome 1C, X-linked retinitis pigmentosa (XLRP), retinitis pigmentosa, Connexin 26 deafness, Achromatopsia, Polycystic kidney disease, spinocerebellar ataxia type 1, 2, 3, 6, 7, 8, 12 or 17, fragile X-associated tremor/ataxia syndrome, myotonic dystrophy, Friedreich's ataxia, amyotrophic lateral sclerosis, an HIV infection, a lung disease , cystic fibrosis, a bleeding disorder, hemophilia A or hemophilia B with or without inhibitors, thalassemia, a blood disorder, anemia, Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis (ALS), epilepsy, lysosomal storage diseases, a copper or iron accumulation disorders, Wilson's or Menkes disease, lysosomal acid lipase deficiency, a neurological or neurodegenerative disorder, cancer, type 1 or type 2 diabetes, Gaucher's disease, Hurler's disease, adenosine deaminase deficiency, a metabolic defect, glycogen storage diseases, a retinal degenerative disease, RPE65 deficiency, choroideremia, disease of the eye, a disease of a solid organ, brain disease, liver disease, kidney disease, or heart disease, an infectious viral disease, hepatitis B and C, HIV, bacterial disease or fungal disease.

44. The recombinant AAV particle of claim 10 or 13, wherein the expression control element comprises a constitutive or regulatable control element.

45. The recombinant AAV particle of claim 10 or 13, wherein the expression control element comprises a tissue-specific expression control element or promoter.

46. 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.

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

48. 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.

49. 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.

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

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

52. 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.

53. The recombinant AAV particle of claim 15, wherein the VP1, VP2 or VP3 sequence has 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10, conservative, non-conservative, or conservative and non-conservative amino acid substitutions.

54. The recombinant AAV particle of any one of claims 10, 13, 15 and 53, wherein the AAV particle comprises a pharmaceutical composition.

55. The recombinant AAV particle of claim 54, wherein the pharmaceutical composition comprises empty capsid AAV.

56. The recombinant AAV particle of claim 54, 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).

57. The use of any one of claims 11, 12 or 14, wherein the mammal has a lysosomal storage disease.

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

59. The recombinant AAV particle of claim 10 or 13 for use as a therapy.