AAV Vector Compositions and Methods for Gene Transfer to Cells, Organs and Tissues

Related Application Information

[0001] This application claims priority to application serial no. 61/600,415, filed February 17, 2012, and which application is expressly incorporated herein by reference in its entirety.

Introduction

[0002] Genetic disorders, caused by absence or a defect in a desirable gene (loss of function) or expression of an undesirable or defective gene or (gain of function) lead to a variety of diseases. One example of a loss of function genetic disorder is hemophilia, an inherited bleeding disorder caused by deficiency in either coagulation factor VIII (FVIII, hemophilia A) or factor IX (FIX, hemophilia B). One example of a gain of function genetic disorder is Huntington's disease, a disease caused by a pathologic "HTT" gene (encodes the huntingtin protein) that encodes a mutated protein that accumulates within and leads to gradual destruction of neurons, particularly in the basal ganglia and the cerebral cortex.

[0003] Current treatment for hemophilia consists in the intravenous administration of recombinant clotting factor either on demand, in case a bleeding occurs, or prophylactically. However, this therapeutic approach has several drawbacks such as the need for repeated infusions, the cost of the treatment, the risk of developing anti-therapeutic factor immune responses, and the risk of potentially fatal bleedings. These limitations have prompted the development of gene-based therapies for hemophilia. To this end, hemophilia is ideal for gene transfer based therapy as 1) the therapeutic window is very wide, as levels just above 1% of normal already can result in a change in phenotype from severe to moderate, and levels of 100% are not associated to any side effects; 2) tissue specific expression of the therapeutic transgene is not strictly required; and 3) there is a considerable experience in measuring the endpoints of therapeutic efficacy. Furthermore, liver expression of clotting factor has been demonstrated to induce immunological tolerance to the clotting factor itself, reducing the likelihood of potentially harmful immune responses against clotting factor.

[0004] Currently, adeno-associated virus (AAV) vectors are recognized as the gene transfer vectors of choice since they have the best safety and efficacy profile for the delivery of genes *in vivo*. Of the AAV serotypes isolated so far, AAV2 and AAV8 have been used to target the liver of humans affected by severe hemophilia B. Both vectors worked efficiently and in the case of AAV8 long-term expression of the therapeutic transgene was documented. Recent data in humans showed that 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 invention provides adeno-associated virus (AAV) serotype AAV-Rh74 vector, and related AAV vectors. 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. Furthermore, 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 (*see, e.g.*, Figures 1 and 2). In particular, AAV-Rh74 could target 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. Thus, AAV-Rh74 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 can be used to deliver therapeutic genes (*e.g.*, FIX, FVIII) to treat hemophilia A, B, and to deliver genes for a wide range of other metabolic or plasma protein deficiencies, or to delever genes 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).

[0007] In addition to efficient delivery of polynucleotides by AAV-Rh74 and related vectors 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 seroprevealence, AAV-Rh74 and related vectors 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 can be efficiently produced at high

titers (Table 2). Thus, AAV-Rh74 and related vectors can be produced in large amounts for more prevalent clinical diseases.

[0007a] In one aspect, there is provided the use of an adeno-associated virus (AAV) vector comprising a heterologous polynucleotide sequence and a VP1 sequence having 100% identity to:

MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDNGRGLVLPGYKYLG
PFNGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTS
FGGNLGRAVFQAKKRVLEPLGLVESPVKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQ
PAKKRLNFGQTGDSESVPDPQPIGEPPAGPSGLGSGTMAAGGGAPMADNNEGADGV
GSSSGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGSTNDNTYFGY
STPWGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTQNEGTKTI
ANNLTSTIQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGR
SSFYCLEYFPSQMLRTGNNFEFSYNFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSR
TQSTGGTAGTQQLLFSQAGPNNMSAQAKNWLPGPCYRQQRVSTTLSQNNNSNFAW
TGATKYHLNGRDSLVNPGVAMATHKDDEERFFPSSGVLMFGKQGAGKDNVDYSSV
MLTSEEEIKTTNPVATEQYGVVADNLQQQNAAPIVGAVNSQGALPGMVWQNRDVY
LQGPIWAKIPHTDGNFHPSPLMGGFGLKHPPPQILIKNTPVPADPPTTFNQAKLASFIT
QYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYYKSTNVDFAVNTEGTYSEPRPIGT
RYLTRNL,

in the manufacture of a medicament for treating cystic fibrosis, Choroideremia, LCA-Lebercilin, Gyrate Atrophy, X-linked Retinoschisis, Usher's Syndrome 1C, retinitis pigmentosa, Connexin 26 deafness, Achromatopsia, Polycystic kidney disease, a lysosomal storage disease, a polynucleotide repeat disease, dentatorubropallidolusyan atropy, spinobulbar muscular atrophy, spinocerebellar ataxia (type 1, 2, 3, 6, 7, 8, 12 17), fragile X syndrome, fragile X-associated tremor/ataxia syndrome, myotonic dystrophy, Friedreich's ataxia, amyotrophic lateral sclerosis (ALS), Parkinson's disease, Alzheimer's disease, hypercoloesterolemia, HIV infection, Rous sarcoma virus (RSV) infection, hepatitis C virus infection, acute kidney injury, delayed graft function kidney transplant, kidney injury acute renal failure, advance recurrent or metastatic solid malignancies, metastatic melanoma, chronic myeloid leukemia, solid tumors, liver tumors, familial adenomatous polyposis, glaucoma, diabetic macular oedma (DME), age-related macular degeneration, choroidal neovascularization, non-arteritic ischaemic optic neuropathy, pachyonychia congenital, influenza infection, severe acute respiratory syndrome (SARS), respiratory syncytial virus

infection, Ebola infection, hepatitis B virus infection, herpes simplex virus infection, coxsackievirus B3 infection, primary dystonia, autosomal dominantly inherited retinitis pigmentosa, lung disease, bleeding disorder, hemophilia A, hemophilia B, thalassemia, blood disorder, anemia, Huntington's disease, epilepsy, lysosomal storage disease, copper or iron accumulation disorders, Wilson's disease, Menkes disease, lysosomal acid lipase deficiency, a neurological or neurodegenerative disorder, cancer, type 1 diabetes, type 2 diabetes, Gaucher's disease, Hurler's disease, adenosine deaminase deficiency, glycogen storage diseases, a retinal degenerative disease, RPE65 deficiency, brain disease, liver disease, kidney disease, heart disease, infectious viral disease, bacterial disease or fungal disease,

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

[0007b] In one aspect, there is provided the use of an adeno-associated virus (AAV) vector in the manufacture of a medicament for treating a mammal deficient in protein expression or function, said vector comprising a heterologous polynucleotide 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 said vector comprises a VP1 sequence having 100% identity to:

MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDNGRGLVLPGYKYLG
PFNGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTS
FGGNLGRAVFQAKKRVLEPLGLVESPVKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQ
PAKKRLNFGQTGDSESVPDPQPIGEPPAGPSGLGSGTMAAGGGAPMADNNEGADGV
GSSSGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGSTNDNTYFGY
STPWGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTQNEGTKTI
ANNLTSTIQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGR
SSFYCLEYFPSQMLRTGNNFEFSYNFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSR
TQSTGGTAGTQQLLFSQAGPNNMSAQAKNWLPGPCYRQQRVSTTLSQNNNSNFAW
TGATKYHLNGRDSLVNPGVAMATHKDDEERFFPSSGVLMFGKQGAGKDNVDYSSV
MLTSEEEIKTTNPVATEQYGVVADNLQQQNAAPIVGAVNSQGALPGMVWQNRDVY
LQGPIWAKIPHTDGNFHPSPLMGGFGLKHPPPQILIKNTPVPADPPTTFNQAKLASFIT
QYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYYKSTNVDFAVNTEGTYSEPRPIGT

RYLTRNL and wherein said vector is to be administered to a mammal wherein said polypeptide is expressed in the mammal.

[0007c] In one aspect, there is provided the use of an adeno-associated virus (AAV) vector comprising a VP1 sequence having 100% identity to:

MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDNGRGLVLPGYKYLG
PFNGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTS
FGGNLGRAVFQAKKRVLEPLGLVESPVKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQ
PAKKRLNFGQTGDSESVPDPQPIGEPPAGPSGLGSGTMAAGGGAPMADNNEGADGV
GSSSGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGSTNDNTYFGY
STPWGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTQNEGTKTI
ANNLTSTIQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGR
SSFYCLEYFPSQMLRTGNNFEFSYNFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSR
TQSTGGTAGTQQLLFSQAGPNNMSAQAKNWLPGPCYRQQRVSTTLSQNNNSNFAW
TGATKYHLNGRDSLVNPGVAMATHKDDEERFFPSSGVLMFGKQGAGKDNVDYSSV
MLTSEEEIKTTNPVATEQYGVVADNLQQQNAAPIVGAVNSQGALPGMVWQNRDVY
LQGPIWAKIPHTDGNFHPSPLMGGFGLKHPPPQILIKNTPVPADPPTTFNQAKLASFIT
QYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYYKSTNVDFAVNTEGTYSEPRPIGT
RYLTRNL,

in the manufacture of a medicament for treating cystic fibrosis, Choroideremia, LCA-Lebercilin, Gyrate Atrophy, X-linked Retinoschisis, Usher's Syndrome 1C, retinitis pigmentosa, Connexin 26 deafness, Achromatopsia, Polycystic kidney disease, a lysosomal storage disease, a polynucleotide repeat disease, dentatorubropallidolusyan atropy, spinobulbar muscular atrophy, spinocerebellar ataxia (type 1, 2, 3, 6, 7, 8, 12 17), fragile X syndrome, fragile X-associated tremor/ataxia syndrome, myotonic dystrophy, Friedreich's

ataxia, amyotrophic lateral sclerosis (ALS), Parkinson's disease, Alzheimer's disease, hypercoloesterolemia, HIV infection, Rous sarcoma virus (RSV) infection, hepatitis C virus infection, acute kidney injury, delayed graft function kidney transplant, kidney injury acute renal failure, advance recurrent or metastatic solid malignancies, metastatic melanoma, chronic myeloid leukemia, solid tumors, liver tumors, familial adenomatous polyposis, glaucoma, diabetic macular oedma (DME), age-related macular degeneration, choroidal neovascularization, non-arteritic ischaemic optic neuropathy, pachyonychia congenital, influenza infection, severe acute respiratory syndrome (SARS), respiratory syncytial virus infection, Ebola infection, hepatitis B virus infection, herpes simplex virus infection, coxsackievirus B3 infection, primary dystonia, autosomal dominantly inherited retinitis pigmentosa, lung disease, bleeding disorder, hemophilia A, hemophilia B, thalassemia, blood disorder, anemia, Huntington's disease, epilepsy, lysosomal storage disease, copper or iron accumulation disorders, Wilson's disease, Menkes disease, lysosomal acid lipase deficiency, a neurological or neurodegenerative disorder, cancer, type 1 diabetes, type 2 diabetes, Gaucher's disease, Hurler's disease, adenosine deaminase deficiency, glycogen storage diseases, a retinal degenerative disease, RPE65 deficiency, brain disease, liver disease, kidney disease, heart disease, infectious viral disease, bacterial disease or fungal disease,

wherein said medicament is to be administered to a mammal or a cell of said mammal, thereby delivering or transferring a heterologous polynucleotide sequence into the mammal or cell of the mammal, wherein the mammal is sero-negative for AAV-Rh74.

[0007d] In one aspect, there is provided the use of an adeno-associated virus vector (AAV) comprising a VP1 sequence having 100% identity to:

MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDNGRGLVLPGYKYLG
PFNGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTS
FGGNLGRAVFQAKKRVLEPLGLVESPVKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQ
PAKKRLNFGQTGDSESVPDPQPIGEPPAGPSGLGSGTMAAGGGAPMADNNEGADGV
GSSSGNWHCDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGSTNDNTYFGY
STPWGYFDFNRFHCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTQNEGTKTI
ANNLTSTIQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGR
SSFYCLEYFPSQMLRTGNNFEFSYNFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSR
TQSTGGTAGTQQLLFSQAGPNNMSAQAKNWLPGPCYRQQRVSTTLSQNNNSNFAW
TGATKYHLNGRDSLVNPGVAMATHKDDEERFFPSSGVLMFGKQGAGKDNVDYSSV
MLTSEEEIKTTNPVATEQYGVVADNLQQQNAAPIVGAVNSQGALPGMVWQNRDVY

LQGPIWAKIPHTDGNFHPSPLMGGFGLKHPPPQILIKNTPVPADPPTTFNQAKLASFIT QYSTGQVSVEIEWELQKENSKRWNPEIQYTSNYYKSTNVDFAVNTEGTYSEPRPIGT RYLTRNL, and empty capsid AAV-Rh74,

in the manufacture of a medicament for treating cystic fibrosis, Choroideremia, LCA-Lebercilin, Gyrate Atrophy, X-linked Retinoschisis, Usher's Syndrome 1C, retinitis pigmentosa, Connexin 26 deafness, Achromatopsia, Polycystic kidney disease, a lysosomal storage disease, a polynucleotide repeat disease, dentatorubropallidolusyan atropy, spinobulbar muscular atrophy, spinocerebellar ataxia (type 1, 2, 3, 6, 7, 8, 12 17), fragile X syndrome, fragile X-associated tremor/ataxia syndrome, myotonic dystrophy, Friedreich's ataxia, amyotrophic lateral sclerosis (ALS), Parkinson's disease, Alzheimer's disease, hypercoloesterolemia, HIV infection, Rous sarcoma virus (RSV) infection, hepatitis C virus infection, acute kidney injury, delayed graft function kidney transplant, kidney injury acute renal failure, advance recurrent or metastatic solid malignancies, metastatic melanoma, chronic myeloid leukemia, solid tumors, liver tumors, familial adenomatous polyposis, glaucoma, diabetic macular oedma (DME), age-related macular degeneration, choroidal neovascularization, non-arteritic ischaemic optic neuropathy, pachyonychia congenital, influenza infection, severe acute respiratory syndrome (SARS), respiratory syncytial virus infection, Ebola infection, hepatitis B virus infection, herpes simplex virus infection, coxsackievirus B3 infection, primary dystonia, autosomal dominantly inherited retinitis pigmentosa, lung disease, bleeding disorder, hemophilia A, hemophilia B, thalassemia, blood disorder, anemia, Huntington's disease, epilepsy, lysosomal storage disease, copper or iron accumulation disorders, Wilson's disease, Menkes disease, lysosomal acid lipase deficiency, a neurological or neurodegenerative disorder, cancer, type 1 diabetes, type 2 diabetes, Gaucher's disease, Hurler's disease, adenosine deaminase deficiency, glycogen storage diseases, a retinal degenerative disease, RPE65 deficiency, brain disease, liver disease, kidney disease, heart disease, infectious viral disease, bacterial disease or fungal disease,

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

[0007e] In one aspect, there is provided a method for delivering or transferring a heterologous polynucleotide sequence into a mammal or a cell of a mammal, comprising administering an adeno-associated virus (AAV) vector, said vector comprising a heterologous

polynucleotide sequence, 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.

[0007f] In another aspect, there is provided use of an adeno-associated virus (AAV) vector in the manufacture of a medicament for treating a mammal deficient in protein expression or function, said vector comprising a heterologous polynucleotide 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; and wherein said vector is to be administered to a mammal wherein said polypeptide is expressed in the mammal.

[0008] In accordance with the invention, there are provided methods for delivering or transferring a heterologous polynucleotide sequence into a mammal or a cell of a mammal. In one embodiment, a method includes administering an adeno-associated virus (AAV) vector that includes a heterologous polynucleotide sequence 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, thereby delivering or transferring the heterologous polynucleotide. In one aspect, the method allows transfer/delivery of the heterologous polynucleotide into the mammal and/or cell. In another aspect, 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 aspect, 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 aspects 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.

Description of Drawings

[0009] **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.

[0010] **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.

[0011] **Figure 3** shows AAV-Rh74 VP1, VP2, and VP3 amino acid sequences and, for VP1, polynucleotide (DNA) sequence (SEQ ID NOs:1-4).

[0012] **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.

Detailed Description

[0013] The invention is based, at least in part, on data indicating that adeno-associated virus (AAV) serotype AAV-Rh74 has a high tropism for hepatocytes, which are cells of the liver. As a vector for polynucleotide (*e.g.* genes, inhibitory nucleic acid, etc.) transfer/delivery into cells, AAV-Rh74 can drive therapeutic levels of expression in liver after intravenous administration. Furthermore, AAV-Rh74 mediated gene transfer/delivery produced protein expression levels that were significantly higher than several other serotypes (*see, e.g.*, Figures 1 and 2). In particular, AAV-Rh74 targets genes for delivery 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. Thus, AAV-Rh74 can be used to transfer/deliver polynucleotides, such as coding sequences (genes) for proteins that provide a desirable or therapeutic benefit, as well as inhibitory (*e.g.*, anti-sense) nucleic acid that reduce or inhibit expression of an undesirable or defective (e.g., pathologic) gene, thereby treating a variety of diseases. For example, AAV-Rh74 can be used to transfer/deliver therapeutic genes to treat hemophilia A, B, and to transfer/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, and 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).

[0014] As set forth herein, adeno-associated virus (AAV) serotype AAV-Rh74 and related AAV vectors provide delivery of polynucleotide sequences to cells *ex vivo*, *in vitro* and *in vivo*. Such polynucleotide sequences can encode proteins such that the cells into which the polynucleotides are delivered express the encoded proteins. For example, AAV-Rh74 and related AAV vectors can include polynucleotides encoding a desired protein or peptide, or a polynucleotide that when transcribed comprises an inhibitory sequence (*e.g.*, RNA), for example, a sequence that targets a gene for inhibition of expression. Vector delivery or administration to a subject (*e.g.*, mammal) therefore provides not only polynucleotides encoding proteins and peptides to the subject, but also inhibitory nucleic acids that target genes for inhibition of expression or function in the subject.

[0015] Thus, in accordance with the invention adeno-associated virus (AAV) serotype AAV-Rh74, and related AAV vectors, including polynucleotide sequences encoding peptides and proteins, as well as polynucleotide sequences which directly or when transcribed comprise inhibitory nucleic acids that target genes for inhibition of expression or function, are provided. Such AAV-Rh74 and related AAV vector serotypes (*e.g.*, VP1, VP2, and/or VP3 sequences) are distinct from other AAV

serotypes, including, for example, AAV1-AAV11 or Rh10 (*e.g.*, distinct from VP1, VP2, and/or VP3 sequences of any of AAV1-AAV11, or Rh10 serotypes).

[0016] As used herein, the term "serotype" is a distinction used to refer to an AAV having a capsid that is serologically distinct from other AAV serotypes. Serologic distinctiveness is determined on the basis of the lack of cross-reactivity between antibodies to one AAV as compared to another AAV. Such cross-reactivity differences are usually due to differences in capsid protein sequences/antigenic determinants (*e.g.*, due to VP1, VP2, and/or VP3 sequence differences of AAV serotypes).

[0017] AAV-Rh74 has gene/protein sequences identical to sequences characteristic for AAV-Rh74 (*see, e.g.*, VP1, VP2, VP3 of Figure 3). As used herein, an "AAV vector related to AAV-Rh74" and grammatical variations thereof refers to one or more AAV proteins (*e.g.*, VP1, VP2, and/or VP3 sequences) that has substantial sequence identity to one or more polynucleotides or polypeptide sequences that comprise AAV-Rh74. Such AAV vectors related to AAV-Rh74 can therefore have one or more distinct sequences from AAV-Rh74, but can exhibit substantial sequence identity to one or more genes and/or have one or more functional characteristics of AAV-Rh74 (*e.g.*, such as cell/tissue tropism). Exemplary AAV-Rh74 sequences include VP1, VP2, and/or VP3 set forth in Figure 3. In one non-limiting exemplary embodiment, an AAV vector related to AAV-Rh74 has a polynucleotide, polypeptide or subsequence thereof that includes or consists of a sequence at least 80% or more (*e.g.*, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, etc.) identical to one or more AAV-Rh74 VP1, VP2, and/or VP3 sequences set forth in Figure 3.

[0018] In accordance with the invention, methods and uses include AAV-Rh74 sequences (polypeptides and nucleotides) and subsequences thereof that exhibit less than 100% sequence identity to a reference AAV-Rh74 gene or protein sequence (*e.g.*, VP1, VP2, and/or VP3 sequences set forth in Figure 3), but are distinct from and not identical to known AAV genes or proteins, such as AAV1-AAV11, AAV-Rh10, genes or proteins, etc. In one embodiment, an AAV-Rh74 polypeptide or subsequence thereof includes or consists of a sequence at least 80% or more identical, *e.g.*, 85%, 85%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, 99.5%, etc., i.e. up to 100% identical to any reference AAV-Rh74 sequence or subsequence thereof (*e.g.*, VP1, VP2 and/or VP3 sequences set forth in Figure 3).

[0019] AAV vectors, including AAV-Rh74, and AAV-Rh74 related vectors, can be constructed using recombinant techniques that are known to the skilled artisan, to include one or more heterologous polynucleotide sequences flanked with functional AAV ITRs. Incorporation of a heterologous polynucleotide defines the AAV as a recombinant vector, or an "rAAV vector." Such vectors can have one or more of the wild type AAV genes deleted in whole or in part, for example, a rep and/or cap gene, but retain at least one functional flanking ITR sequence, as necessary for the rescue, replication, and packaging of the AAV particle. Thus, an AAV vector includes sequences required in cis for viral replication and packaging (*e.g.*, functional ITRs).

[0020] The terms "polynucleotide" and "nucleic acid" are used interchangeably herein to refer to all forms of nucleic acid, oligonucleotides, including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Polynucleotides include genomic DNA, cDNA and antisense DNA, and spliced or unspliced mRNA, rRNA tRNA and inhibitory DNA or RNA (RNAi, *e.g.*, small or short hairpin (sh)RNA, microRNA, small or short interfering (si)RNA, trans-splicing RNA, or antisense RNA). Polynucleotides include naturally occurring, synthetic, and intentionally altered or modified polynucleotides as well as analogues and derivatives. Polynucleotides can be single, double, or triplex, linear or circular, and can be of any length.

[0021] A "heterologous" polynucleotide merely refers to a polynucleotide inserted into AAV for purposes of AAV mediated transfer/delivery of the polynucleotide into a cell. Heterologous polynucleotides are typically distinct from AAV nucleic acid. Once transferred/delivered into the cell, a heterologous polynucleotide, contained within the rAAV virion, can be expressed (*e.g.*, transcribed, and translated if appropriate). Alternatively, a transferred/delivered heterologous polynucleotide in a cell, contained within the rAAV virion, need not be expressed. Although the term "heterologous" is not always used herein in reference to polynucleotides, reference to a polynucleotide even in the absence of the modifier "heterologous" includes heterologous polynucleotides in spite of the omission.

[0022] The "polypeptides," "proteins" and "peptides" encoded by the "polynucleotide sequences," include full-length native sequences, as with naturally occurring proteins, as well as functional subsequences, modified forms or sequence variants so long as the subsequence, modified form or variant retains some degree of functionality of the native full-length protein. In methods and uses of the invention, such polypeptides, proteins and peptides encoded by the polynucleotide sequences can be but are not required to be identical to the endogenous protein that is defective, or whose expression is insufficient, or deficient in the treated mammal.

[0023] Invention adeno-associated virus (AAV) serotype AAV-Rh74, and related AAV vectors can be used to introduce/deliver polynucleotides stably or transiently into cells and progeny thereof. The term "transgene" is used to conveniently refer to such a heterologous polynucleotide that has been introduced into a cell or organism. Transgenes include any polynucleotide, such as a gene that encodes a polypeptide or protein, a polynucleotide that is transcribed into an inhibitory polynucleotide, or a polynucleotide that is not transcribed (e.g., lacks a expression control element, such as a promoter that drives transcription). For example, in a cell having a transgene, the transgene has been introduced/transferred by AAV "transformation" of the cell. A cell or progeny thereof into which the transgene has been introduced is referred to as a "transformed cell" or "transformant." Typically, a transgene is included in progeny of the transformant or becomes a part of the organism that develops from the cell. Accordingly, a "transformed" or "transfected" cell (*e.g.*, in a mammal, such as a cell or tissue or organ cell), means a genetic change in a cell following

incorporation of an exogenous molecule, for example, a polynucleotide or protein (*e.g.*, a transgene) into the cell. Thus, a "transfected" or "transformed" cell is a cell into which, or a progeny thereof in which an exogenous molecule has been introduced, for example. The cell(s) can be propagated and the introduced protein expressed, or nucleic acid transcribed.

[0024] Particular non-limiting examples of polynucleotides encoding gene products (proteins) which are useful in accordance with the invention include, but are not limited to: genes that comprise or encode CFTR (cystic fibrosis transmembrane regulator protein), a blood coagulation (clotting) factor (Factor XIII, Factor IX, Factor X, Factor VIII, Factor VIIa, protein C etc.) including gain of function blood coagulation factors, 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 [de Groot et al., *Blood* 2008 Oct 15;112(8):3303], or hCDR1 [Sharabi et al., *Proc Natl Acad Sci U S A.* 2006 Jun 6;103(23):8810-5], 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.

[0025] All mammalian and non-mammalian forms of polynucleotides encoding gene products, including the non-limiting genes and proteins disclosed herein are expressly included, either known or unknown. Thus, the invention includes genes and proteins from non-mammals, mammals other

than humans, and humans, which genes and proteins function in a substantially similar manner to the human genes and proteins described herein. A non-limiting example of non-mammalian gene is a Fok nuclease domain, which is bacterial in origin. Non-limiting examples of mammalian non-human FIX sequences are described in Yoshitake et al., 1985, supra; Kurachi et al., 1995, supra; Jallat et al., 1990, supra; Kurachi et al., 1982, Proc. Natl. Acad. Sci. USA 79:6461-6464; Jaye et al., 1983, Nucl. Acids Res. 11:2325-2335; Anson et al., 1984, EMBO J. 3: 1053-1060; Wu et al., 1990, Gene 86:275-278; Evans et al., *Proc Natl Acad Sci USA* 86:10095 (1989), Blood 74:207-212; Pendurthi et al., 1992, Thromb. Res. 65:177-186; Sakar et al., 1990, Genomics 1990, 6:133-143; and, Katayama et al., 1979, Proc. Natl. Acad. Sci. USA 76:4990-4994.

[0026] Polynucleotides, polypeptides and subsequences thereof include modified and variant forms. As used herein, the terms "modify" or "variant" and grammatical variations thereof, mean that a polynucleotide, polypeptide or subsequence thereof deviates from a reference sequence. Modified and variant sequences may therefore have substantially the same, greater or less activity or function than a reference sequence, but at least retain partial activity or function of the reference sequence.

[0027] Accordingly, the invention also includes naturally and non-naturally occurring variants. Such variants include gain and loss of function variants. For example, wild type human FIX DNA sequences, which protein variants or mutants retain activity, or are therapeutically effective, or are comparably or even more therapeutically active than invariant human FIX in the methods and uses of the invention. In a particular example, collagen IV serves to trap FIX, meaning that when introduced into the muscle tissue of a mammal some of the FIX is not available for participation in blood coagulation because it is retained in the interstitial spaces in the muscle tissue. A mutation in the sequence of FIX that results in a protein with reduced binding to collagen IV (*e.g.*, loss of function) is a mutant useful in the methods of the invention, for example, for treatment of hemophilia. An example of such a mutant human FIX gene encodes a human FIX protein with the amino acid alanine in place of lysine in the fifth amino acid position from the beginning of the mature protein.

[0028] Non-limiting examples of modifications include one or more amino acid substitutions (*e.g.*, 1-3, 3-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30-40, 40-50, or more residues), additions (*e.g.*, insertions or 1-3, 3-5, 5-10, 10-15, 15-20, 20-25, 25-30, 30-40, 40-50, or more residues) and deletions (*e.g.*, subsequences or fragments) of a reference sequence. In particular embodiments, a modified or variant sequence retains at least part of a function or an activity of unmodified sequence. Such modified forms and variants can have less than, the same, or greater, but at least a part of, a function or activity of a reference sequence, for example, as described herein.

[0029] A variant can have one or more non-conservative or a conservative amino acid sequence differences or modifications, or both. A "conservative substitution" is the replacement of one amino acid by a biologically, chemically or structurally similar residue. Biologically similar means that the

substitution does not destroy a biological activity. Structurally similar means that the amino acids have side chains with similar length, such as alanine, glycine and serine, or a similar size. Chemical similarity means that the residues have the same charge or are both hydrophilic or hydrophobic. Particular examples include the substitution of one hydrophobic residue, such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acids, or glutamine for asparagine, serine for threonine, and the like. Particular examples of conservative substitutions include the substitution of a hydrophobic residue such as isoleucine, valine, leucine or methionine for another, the substitution of a polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acids, or glutamine for asparagine, and the like. For example, conservative amino acid substitutions typically include substitutions within the following groups: glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and phenylalanine, tyrosine. A "conservative substitution" also includes the use of a substituted amino acid in place of an unsubstituted parent amino acid.

[0030] Accordingly, the invention includes gene and protein variants (*e.g.*, of polynucleotides encoding proteins described herein) which retain one or more biological activities (*e.g.*, function in blood clotting, etc.). Such variants of proteins or polypeptides include proteins or polypeptides which have been or may be modified using recombinant DNA technology such that the protein or polypeptide possesses altered or additional properties, for example, variants conferring enhanced protein stability in plasma or enhanced activity of the protein. Variants can differ from a reference sequence, such as naturally occurring polynucleotides, proteins or peptides.

[0031] At the nucleotide sequence level, a naturally and non-naturally occurring variant gene will typically be at least about 50% identical, more typically about 70% identical, even more typically about 80% identical (90% or more identity) to the reference gene. At the amino acid sequence level, a naturally and non-naturally occurring variant protein will typically be at least about 70% identical, more typically about 80% identical, even more typically about 90% or more identity to the reference protein, although substantial regions of non-identity are permitted in non-conserved regions (*e.g.*, less, than 70% identical, such as less than 60%, 50% or even 40%). In other embodiments, the sequences have at least 60%, 70%, 75% or more identity (*e.g.*, 80%, 85% 90%, 95%, 96%, 97%, 98%, 99% or more identity) to a reference sequence. Procedures for the introduction of nucleotide and amino acid changes in a polynucleotide, protein or polypeptide are known to the skilled artisan (*see, e.g.*, Sambrook et al. (1989)).

[0032] The term "identity," "homology" and grammatical variations thereof, mean that two or more referenced entities are the same, when they are "aligned" sequences. Thus, by way of example, when two polypeptide sequences are identical, they have the same amino acid sequence, at least within the referenced region or portion. Where two polynucleotide sequences are identical, they

have the same polynucleotide sequence, at least within the referenced region or portion. The identity can be over a defined area (region or domain) of the sequence. An "area" or "region" of identity refers to a portion of two or more referenced entities that are the same. Thus, where two protein or nucleic acid sequences are identical over one or more sequence areas or regions they share identity within that region. An "aligned" sequence refers to multiple polynucleotide or protein (amino acid) sequences, often containing corrections for missing or additional bases or amino acids (gaps) as compared to a reference sequence

[0033] The identity can extend over the entire sequence length or a portion of the sequence. In particular aspects, the length of the sequence sharing the percent identity is 2, 3, 4, 5 or more contiguous polynucleotide or amino acids, *e.g.*, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, etc. contiguous amino acids. In additional particular aspects, the length of the sequence sharing identity is 20 or more contiguous polynucleotide or amino acids, *e.g.*, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, etc. contiguous amino acids. In further particular aspects, the length of the sequence sharing identity is 35 or more contiguous polynucleotide or amino acids, *e.g.*, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 45, 47, 48, 49, 50, etc., contiguous amino acids. In yet further particular aspects, the length of the sequence sharing identity is 50 or more contiguous polynucleotide or amino acids, *e.g.*, 50-55, 55-60, 60-65, 65-70, 70-75, 75-80, 80-85, 85-90, 90-95, 95-100, 100-110, etc. contiguous polynucleotide or amino acids.

[0034] The terms "homologous" or "homology" mean that two or more referenced entities share at least partial identity over a given region or portion. "Areas, regions or domains" of homology or identity mean that a portion of two or more referenced entities share homology or are the same. Thus, where two sequences are identical over one or more sequence regions they share identity in these regions. "Substantial homology" means that a molecule is structurally or functionally conserved such that it has or is predicted to have at least partial structure or function of one or more of the structures or functions (*e.g.*, a biological function or activity) of the reference molecule, or relevant/corresponding region or portion of the reference molecule to which it shares homology.

[0035] The extent of identity (homology) between two sequences can be ascertained using a computer program and mathematical algorithm. Such algorithms that calculate percent sequence identity (homology) generally account for sequence gaps and mismatches over the comparison region or area. For example, a BLAST (*e.g.*, BLAST 2.0) search algorithm (*see, e.g.*, Altschul et al., *J. Mol. Biol.* 215:403 (1990), publicly available through NCBI) has exemplary search parameters as follows: Mismatch -2; gap open 5; gap extension 2. For polypeptide sequence comparisons, a BLASTP algorithm is typically used in combination with a scoring matrix, such as PAM100, PAM 250, BLOSUM 62 or BLOSUM 50. FASTA (*e.g.*, FASTA2 and FASTA3) and SSEARCH sequence comparison programs are also used to quantitate extent of identity (Pearson et al., *Proc. Natl. Acad. Sci.* USA 85:2444 (1988); Pearson, *Methods Mol Biol.* 132:185 (2000); and Smith et al.,

J. Mol. Biol. 147:195 (1981)). Programs for quantitating protein structural similarity using Delaunay-based topological mapping have also been developed (Bostick et al., *Biochem Biophys Res Commun.* 304:320 (2003)).

[0036] Polynucleotides include additions and insertions, for example, heterologous domains. An addition (*e.g.*, heterologous domain) can be a covalent or non-covalent attachment of any type of molecule to a composition. Typically additions and insertions (*e.g.*, a heterologous domain) confer a complementary or a distinct function or activity.

[0037] Additions and insertions include chimeric and fusion sequences, which is a polynucleotide or protein sequence having one or more molecules not normally present in a reference native (wild type) sequence covalently attached to the sequence. The terms "fusion" or "chimeric" and grammatical variations thereof, when used in reference to a molecule means that a portions or part of the molecule contains a different entity distinct (heterologous) from the molecule as they do not typically exist together in nature. That is, for example, one portion of the fusion or chimera, includes or consists of a portion that does not exist together in nature, and is structurally distinct.

[0038] As set forth herein, polynucleotide sequences include inhibitory and antisense nucleic acid sequences. Inhibitory, antisense, miRNA, shRNA, and RNAi nucleic acids can modulate expression of a target gene. Antisense includes single, double or triple stranded polynucleotides and peptide nucleic acids (PNAs) that bind RNA transcript or DNA (*e.g.*, genomic DNA). Oligonucleotides derived from the transcription initiation site of a target gene, *e.g.*, between positions -10 and +10 from the start site, are another particular example. Triplex forming antisense can bind to double strand DNA thereby inhibiting transcription of the gene. "RNAi" is the use of single or double stranded RNA sequences for inhibiting gene expression (*see, e.g.*, Kennerdell et al., *Cell* 95:1017 (1998); and Fire et al., *Nature*, 391:806 (1998)). Double stranded RNA sequences from a target gene coding region may therefore be used to inhibit or prevent gene expression/transcription in accordance with the methods and uses of the invention. Antisense and RNAi can be produced based upon nucleic acids encoding target gene sequences (*e.g.*, HTT), such as nucleic acid encoding mammalian and human HTT. For example, a single or double stranded nucleic acid (*e.g.*, RNA) can target HTT transcript (*e.g.*, mRNA).

[0039] Particular non-limiting examples of genes (e.g., genomic DNA) or transcript of a pathogenic gene (e.e.g, RNA or mRNA) that may be targeted with inhibitory nucleic acid sequences in accordance with the invention include, but are not limited to: pathogenic genes associated with polynucleotide repeat diseases such as 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; or mutant rhodopsin gene (RHO) in autosomal dominantly inherited retinitis pigmentosa (adRP).

[0040] As used herein, the term "recombinant," as a modifier of AAV, such as recombinant AAV-Rh74 and related AAV vectors, as well as a modifier of sequences such as recombinant polynucleotides and polypeptides, means that the compositions have been manipulated (*i.e.*,

engineered) in a fashion that generally does not occur in nature. A particular example of a recombinant AAV would be where a polynucleotide that is not normally present in the wild-type AAV is within the AAV particle and/or genome. For example, a particular example of a recombinant polynucleotide would be where a polynucleotide (*e.g.*, gene) encoding a protein is cloned into a vector, with or without 5', 3' and/or intron regions that the gene is normally associated within the AAV genome. Although the term "recombinant" is not always used herein in reference to AAV, such as AAV-Rh74 and related AAV vectors, as well as sequences such as polynucleotides and polypeptides, recombinant forms of AAV, AAV-Rh74 and related AAV vectors, and sequences including polynucleotides and polypeptides, are expressly included in spite of any such omission.

[0041] Polynucleotide sequences in accordance with the invention can be inserted into a vector. The term "vector" refers to a plasmid, virus (*e.g.*, AAV) or other vehicle that can be manipulated by insertion or incorporation of a polynucleotide. Such vectors can be used for genetic manipulation (*i.e.*, "cloning vectors"), to introduce/transfer polynucleotides into cells, and to transcribe or translate the inserted polynucleotide in cells. A vector generally contains at least an origin of replication for propagation in a cell and expression control element (*e.g.*, a promoter). Control elements, including expression control elements as set forth herein, present within a vector are included to facilitate proper transcription and if appropriate translation (*e.g.*, splicing signal for introns, maintenance of the correct reading frame of the gene to permit in-frame translation of mRNA and, stop codons etc.).

[0042] Vectors including AAV-Rh74 and related AAV vectors of the invention can include one or more "expression control elements." Typically, expression control elements are nucleic acid sequence(s), such as promoters and enhancers, that influence expression of an operably linked polynucleotide. Such elements typically act in cis but may also act in trans.

[0043] Expression control can be effected at the level of transcription, translation, splicing, message stability, etc. Typically, an expression control element that modulates transcription is juxtaposed near the 5' end of the transcribed polynucleotide (*i.e.*, "upstream"). Expression control elements can also be located at the 3' end of the transcribed sequence (*i.e.*, "downstream") or within the transcript (*e.g.*, in an intron). Expression control elements can be located at a distance away from the transcribed sequence (*e.g.*, 100 to 500, 500 to 1000, 2000 to 5000, 5000 to 10,000 or more nucleotides from the polynucleotide), even at considerable distances. Nevertheless, owing to the polynucleotide length limitations, for AAV-Rh74 and related AAV vectors, such expression control elements will typically be within 1 to 1000 nucleotides from the polynucleotide.

[0044] Functionally, expression of the operably linked polynucleotide is at least in part controllable by the element (*e.g.*, promoter) such that the element modulates transcription of the polynucleotide and, as appropriate, translation of the transcript. A specific example of an expression control element is a promoter, which is usually located 5' of the transcribed sequence. Another example of

an expression control element is an enhancer, which can be located 5', 3' of the transcribed sequence, or within the transcribed sequence.

[0045] Expression control elements and promoters include those active in a particular tissue or cell type, referred to herein as a "tissue-specific expression control elements/promoters." Tissue-specific expression control elements are typically active in specific cell or tissue (*e.g.*, liver, brain, central nervous system, spinal cord, eye, retina or lung). Expression control elements are typically active in these cells, tissues or organs because they are recognized by transcriptional activator proteins, or other regulators of transcription, that are unique to a specific cell, tissue or organ type.

[0046] Expression control elements also include ubiquitous or promiscuous promoters/enhancers which are capable of driving expression of a polynucleotide in many different cell types. Such elements include, but are not limited to the cytomegalovirus (CMV) immediate early promoter/enhancer sequences, the Rous sarcoma virus (RSV) promoter/enhancer sequences and the other viral promoters/enhancers active in a variety of mammalian cell types, or synthetic elements that are not present in nature.

[0047] Expression control elements also can confer expression in a manner that is regulatable, that is, a signal or stimuli increases or decreases expression of the operably linked polynucleotide. A regulatable element that increases expression of the operably linked polynucleotide in response to a signal or stimuli is also referred to as an "inducible element" (*i.e.*, is induced by a signal). Particular examples include, but are not limited to, a hormone (*e.g.*, steroid) inducible promoter. A regulatable element that decreases expression of the operably linked polynucleotide in response to a signal or stimuli is referred to as a "repressible element" (*i.e.*, the signal decreases expression such that when the signal, is removed or absent, expression is increased). Typically, the amount of increase or decrease conferred by such elements is proportional to the amount of signal or stimuli present; the greater the amount of signal or stimuli, the greater the increase or decrease in expression.

[0048] As used herein, the term "operable linkage" or "operably linked" refers to a physical or functional juxtaposition of the components so described as to permit them to function in their intended manner. In the example of an expression control element in operable linkage with a polynucleotide, the relationship is such that the control element modulates expression of the nucleic acid. More specifically, for example, two DNA sequences operably linked means that the two DNAs are arranged (cis or trans) in such a relationship that at least one of the DNA sequences is able to exert a physiological effect upon the other sequence.

[0049] Vectors including AAV-Rh74 and related AAV vectors of the invention can include still additional nucleic acid elements. These elements include, without limitation one or more copies of an AAV ITR sequence, a promoter/enhancer element, a transcription termination signal, 5' or 3' untranslated regions (*e.g.*, polyadenylation sequences) which flank a polynucloetide sequence, or all or a portion of intron I. Such elements also optionally include a transcription termination signal. A

particular non-limiting example of a transcription termination signal is the SV40 transcription termination signal.

[0050] Inclusion of an intron element may enhance expression compared with expression in the absence of the intron element (Kurachi et al., 1995, supra). AAV vectors typically accept inserts of DNA having a defined size range which is generally about 4 kb to about 5.2 kb, or slightly more. Thus, for shorter sequences, it may be necessary to include additional nucleic acid in the insert fragment in order to achieve the required length which is acceptable for the AAV vector. Introns and intron fragments (*e.g.* portion of intron I of FIX) fulfill this requirement while also enhancing expression. Thus, the invention is not limited to the inclusion of intron I sequences in the AAV vector, and include other introns or other DNA sequences in place of portions of intron I. Accordingly, other 5' and 3' untranslated regions of nucleic acid may be used in place of those recited for human FIX, particularly when polyncucleotides encoding proteins other than human FIX are used in the AAV-Rh74 and related AAV vectors of the invention.

[0051] A "portion of intron I" as used herein, is meant region of intron I having a nucleotide length of from about 0.1 kb to about 1.7 kb, which region enhances expression of FIX, typically by about 1.5-fold or more on a plasmid or viral vector template when compared with expression of FIX in the absence of a portion of intron I. A more specific portion is a 1.3 kb portion of intron 1.

[0052] Polynucleotides and polypeptides including modified forms can be made using various standard cloning, recombinant DNA technology, via cell expression or *in vitro* translation and chemical synthesis techniques. Purity of polynucleotides can be determined through sequencing, gel electrophoresis and the like. For example, nucleic acids can be isolated using hybridization or computer-based database screening techniques. Such techniques include, but are not limited to: (1) hybridization of genomic DNA or cDNA libraries with probes to detect homologous nucleotide sequences; (2) antibody screening to detect polypeptides having shared structural features, for example, using an expression library; (3) polymerase chain reaction (PCR) on genomic DNA or cDNA using primers capable of annealing to a nucleic acid sequence of interest; (4) computer searches of sequence databases for related sequences; and (5) differential screening of a subtracted nucleic acid library.

[0053] Polynucleotides and polypeptides including modified forms can also be produced by chemical synthesis using methods known in the art, for example, an automated synthesis apparatus (*see, e.g.*, Applied Biosystems, Foster City, CA). Peptides can be synthesized, whole or in part, using chemical methods (*see, e.g.*, Caruthers (1980). *Nucleic Acids Res. Symp. Ser.* 215; Horn (1980); and Banga, A.K., Therapeutic Peptides and Proteins, Formulation, Processing and Delivery Systems (1995) Technomic Publishing Co., Lancaster, PA). Peptide synthesis can be performed using various solid phase techniques (*see, e.g.*, Roberge *Science* 269:202 (1995); Merrifield, *Methods Enzymol.* 289:3(1997)) and automated synthesis may be achieved, *e.g.*, using

the ABI 431A Peptide Synthesizer (Perkin Elmer) in accordance with the manufacturer's instructions.

[0054] The term "isolated," when used as a modifier of a composition, means that the compositions are made by the hand of man or are separated, completely or at least in part, from their naturally occurring *in vivo* environment. Generally, isolated compositions are substantially free of one or more materials with which they normally associate with in nature, for example, one or more protein, nucleic acid, lipid, carbohydrate, cell membrane. The term "isolated" does not exclude alternative physical forms of the composition, such as fusions/chimeras, multimers/oligomers, modifications (*e.g.*, phosphorylation, glycosylation, lipidation) or derivatized forms, or forms expressed in host cells produced by the hand of man.

[0055] In accordance with the invention, treatment methods and uses are provided that include therapeutic methods and uses. Methods and uses of the invention are broadly applicable to diseases amenable to treatment by introducing a gene encoding a protein, or increasing or stimulating gene expression or function, *e.g.*, gene addition or replacement. Methods and uses of the invention are also broadly applicable to diseases amenable to treatment by reducing or decreasing gene expression or function, *e.g.*, gene knockout or reduction of gene expression.

[0056] Non-limiting particular examples of diseases treatable in accordance with the invention include those set forth herein as well as a lung disease (*e.g.*, cystic fibrosis), a blood coagulation or bleeding disorder (*e.g.*, hemophilia A or hemophilia B with or without inhibitors), thalassemia, a blood disorder (*e.g.*, anemia), Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis (ALS), epilepsy, lysosomal storage diseases, a copper or iron accumulation disorders (*e.g.*, 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 (*e.g.*, glycogen storage diseases), a retinal degenerative disease (such as RPE65 deficiency, choroideremia, and other diseases of the eye), and a disease of a solid organ (*e.g.*, brain, liver, kidney, heart).

[0057] Thus, in one embodiment, a method of the invention includes: (a) providing adeno-associated virus (AAV) vector, said vector comprising a heterologous polynucleotide encoding a protein, wherein the heterologous polynucleotide sequence is operably linked to an expression control element conferring transcription of said polynucleotide sequence; and (b) administering an *amount of the AAV vector to the mammal wherein said protein is expressed in the mammal.* In particular aspects, expression of the protein provides a therapeutic benefit to the mammal.

[0058] Methods of the invention include treatment methods, which result in any therapeutic or beneficial effect. In various methods embodiments, an methods of the invention further include inhibiting, decreasing or reducing one or more adverse (*e.g.*, physical) symptoms, disorders, illnesses,

diseases or complications caused by or associated with the disease, such as reduced blood clotting time, reduced administration dosage of supplemental clotting factor protein.

[0059] A therapeutic or beneficial effect of treatment is therefore any objective or subjective measurable or detectable improvement or benefit provided to a particular subject. A therapeutic or beneficial effect can but need not be complete ablation of all or any particular adverse symptom, disorder, illness, or complication of a disease. Thus, a satisfactory clinical endpoint is achieved when there is an incremental improvement or a partial reduction in an adverse symptom, disorder, illness, or complication caused by or associated with a disease, or an inhibition, decrease, reduction, suppression, prevention, limit or control of worsening or progression of one or more adverse symptoms, disorders, illnesses, or complications caused by or associated with the disease, over a short or long duration (hours, days, weeks, months, etc.).

[0060] Compositions, methods and uses of the invention, can be administered in a sufficient or effective amount to a subject in need thereof. An "effective amount" or "sufficient amount" refers to an amount that provides, in single or multiple doses, alone or in combination, with one or more other compositions (therapeutic agents such as a drug), treatments, protocols, or therapeutic regimens agents, a detectable response of any duration of time (long or short term), an expected or desired outcome in or a benefit to a subject of any measurable or detectable degree or for any duration of time (*e.g.*, for minutes, hours, days, months, years, or cured).

[0061] The AAV vector dose to achieve a therapeutic effect, *e.g.*, the dose in vector genomes/per kilogram of body weight (vg/kg), will vary based on several factors including, but not limited to: route of administration, the level of heterologous polynucleotide expression required to achieve a therapeutic effect, the specific disease treated, any host immune response to the AAV vector, a host immune response to the heterologous polynucleotide or expression product (protein), and the stability of the protein expressed. One skilled in the art can readily determine a AAV virion dose range to treat a patient having a particular disease or disorder based on the aforementioned factors, as well as other factors. Generally, doses will range from at least $1X10^8$, or more, for example, $1X10^9$, $1X10^{10}$, $1X10^{11}$, $1X10^{12}$, $1X10^{13}$ or $1X10^{14}$, or more, vector genomes per kilogram (vg/kg) of the weight of the subject, to achieve a therapeutic effect.

[0062] Using hemophilia as an example, generally speaking, it is believed that, in order to achieve a therapeutic effect, a blood coagulation factor concentration that is greater than 1% of factor concentration found in a normal individual is needed to change a severe disease phenotype to a moderate one. A severe phenotype is characterized by joint damage and life-threatening bleeds. To convert a moderate disease phenotype into a mild one, it is believed that a blood coagulation factor concentration greater than 5% of normal is needed. With respect to treating such a hemophilic subject, a typical dose is at least $1X10^{10}$ vector genomes (vg) per kilogram (vg/kg) of the weight of the subject, or between about $1X10^{10}$ to $1X10^{11}$ vg/kg of the weight of the subject, or between about

$1X10^{11}$ to $1X10^{12}$ vg/kg of the weight of the subject, or between about $1X10^{12}$ to $1X10^{13}$ vg/kg of the weight of the subject, to achieve a desired therapeutic effect.

[0063] The doses of an "effective amount" or "sufficient amount" for treatment (*e.g.*, to ameliorate or to provide a therapeutic benefit or improvement) typically are effective to provide a response to one, multiple or all adverse symptoms, consequences or complications of the disease, one or more adverse symptoms, disorders, illnesses, pathologies, or complications, for example, caused by or associated with the disease, to a measurable extent, although decreasing, reducing, inhibiting, suppressing, limiting or controlling progression or worsening of the disease is a satisfactory outcome.

[0064] An effective amount or a sufficient amount can but need not be provided in a single administration, may require multiple administrations, and, can but need not be, administered alone or in combination with another composition (*e.g.*, agent), treatment, protocol or therapeutic regimen. For example, the amount may be proportionally increased as indicated by the need of the subject, type, status and severity of the disease treated or side effects (if any) of treatment. In addition, an effective amount or a sufficient amount need not be effective or sufficient if given in single or multiple doses without a second composition (*e.g.*, another drug or agent), treatment, protocol or therapeutic regimen, since additional doses, amounts or duration above and beyond such doses, or additional compositions (*e.g.*, drugs or agents), treatments, protocols or therapeutic regimens may be included in order to be considered effective or sufficient in a given subject. Amounts considered effective also include amounts that result in a reduction of the use of another treatment, therapeutic regimen or protocol, such as administration of recombinant clotting factor protein for treatment of a clotting disorder (*e.g.*, hemophilia A or B).

[0065] An effective amount or a sufficient amount need not be effective in each and every subject treated, nor a majority of treated subjects in a given group or population. An effective amount or a sufficient amount means effectiveness or sufficiency in a particular subject, not a group or the general population. As is typical for such methods, some subjects will exhibit a greater response, or less or no response to a given treatment method or use. Thus, appropriate amounts will depend upon the condition treated, the therapeutic effect desired, as well as the individual subject (*e.g.*, the bioavailability within the subject, gender, age, etc.).

[0066] The term "ameliorate" means a detectable or measurable improvement in a subject's disease or symptom thereof, or an underlying cellular response. A detectable or measurable improvement includes a subjective or objective decrease, reduction, inhibition, suppression, limit or control in the occurrence, frequency, severity, progression, or duration of the disease, or complication caused by or associated with the disease, or an improvement in a symptom or an underlying cause or a consequence of the disease, or a reversal of the disease.

[0067] Thus, a successful treatment outcome can lead to a "therapeutic effect," or "benefit" of decreasing, reducing, inhibiting, suppressing, limiting, controlling or preventing the occurrence,

frequency, severity, progression, or duration of a disease, or one or more adverse symptoms or underlying causes or consequences of the disease in a subject. Treatment methods and uses affecting one or more underlying causes of the disease or adverse symptoms are therefore considered to be beneficial. A decrease or reduction in worsening, such as stabilizing the disease, or an adverse symptom thereof, is also a successful treatment outcome.

[0068] A therapeutic benefit or improvement therefore need not be complete ablation of the disease, or any one, most or all adverse symptoms, complications, consequences or underlying causes associated with the disease. Thus, a satisfactory endpoint is achieved when there is an incremental improvement in a subject's disease, or a partial decrease, reduction, inhibition, suppression, limit, control or prevention in the occurrence, frequency, severity, progression, or duration, or inhibition or reversal, of the disease (*e.g.*, stabilizing one or more symptoms or complications), over a short or long duration of time (hours, days, weeks, months, etc.). Effectiveness of a method or use, such as a treatment that provides a potential therapeutic benefit or improvement of a disease, can be ascertained by various methods.

[0069] Invention methods and uses can be combined with any compound, agent, drug, treatment or other therapeutic regimen or protocol having a desired therapeutic, beneficial, additive, synergistic or complementary activity or effect. Exemplary combination compositions and treatments include second actives, such as, biologics (proteins), agents and drugs. Such biologics (proteins), agents, drugs, treatments and therapies can be administered or performed prior to, substantially contemporaneously with or following any other method or use of the invention, for example, a therapeutic method of treating a subject for a blood clotting disease.

[0070] The compound, agent, drug, treatment or other therapeutic regimen or protocol can be administered as a combination composition, or administered separately, such as concurrently or in series or sequentially (prior to or following) an AAV vector of the invention. The invention therefore provides combinations in which a method or use of the invention is in a combination with any compound, agent, drug, therapeutic regimen, treatment protocol, process, remedy or composition, set forth herein or known to one of skill in the art. The compound, agent, drug, therapeutic regimen, treatment protocol, process, remedy or composition can be administered or performed prior to, substantially contemporaneously with or following administration of an AAV vector of the invention, to a subject. Specific non-limiting examples of combination embodiments therefore include the foregoing or other compound, agent, drug, therapeutic regimen, treatment protocol, process, remedy or composition.

[0071] Methods and uses of the invention also include, among other things, methods and uses that result in a reduced need or use of another compound, agent, drug, therapeutic regimen, treatment protocol, process, or remedy. For example, for a blood clotting disease, a method or use of the invention has a therapeutic benefit if in a given subject a less frequent or reduced dose or elimination

of administration of a recombinant clotting factor protein to supplement for the deficient or defective (abnormal or mutant) endogenous clotting factor in the subject. Thus, in accordance with the invention, methods and uses of reducing need or use of another treatment or therapy are provided.

[0072] The term "subject" refers to an animal, typically a mammal, such as humans, non-human primates (apes, gibbons, gorillas, chimpanzees, orangutans, macaques), a domestic animal (dogs and cats), a farm animal (poultry such as chickens and ducks, horses, cows, goats, sheep, pigs), and experimental animals (mouse, rat, rabbit, guinea pig). Subjects include animal disease models, for example, mouse and other animal models of blood clotting diseases and others known to those of skill in the art.

[0073] Subjects appropriate for treatment in accordance with the invention include those having or at risk of producing an insufficient amount or having a deficiency in a functional gene product (protein), or produce an aberrant, partially functional or non-functional gene product (protein), which can lead to disease. Subjects appropriate for treatment in accordance with the invention also include those having or at risk of producing an aberrant, or defective (mutant) gene product (protein) that leads to a disease such that reducing amounts, expression or function of the aberrant, or defective (mutant) gene product (protein) would lead to treatment of the disease, or reduce one or more symptoms or ameliorate the disease. Target subjects therefore include subjects that have such defects regardless of the disease type, timing or degree of onset, progression, severity, frequency, or type or duration of the symptoms.

[0074] Subjects appropriate for treatment in accordance with the invention also include those having or at risk of producing antibodies against AAV. AAV vectors can be administered or delivered to such subjects using several techniques. For example, empty capsid AAV (i.e., AAV lacking a heterologous polynucleotide) can be delivered to bind to the AAV antibodies thereby allowing the AAV vector bearing the heterologous polynucleotide to transform cells of the subject. Amounts of empty capsid AAV to administer can be calibrated based upon the amount of AAV antibodies produced in a particular subject. Alternatively or in addition to, AAV vector can be delivered by direct intramuscular injection (*e.g.*, one or more slow-twitch fibers of a muscle). In another alternative, a catheter introduced into the femoral artery can be used to delivery AAV vectors to liver via the hepatic artery. Non-surgical means can also be employed, such as endoscopic retrograde cholangiopancreatography (ERCP), to deliver AAV vectors directly to the liver, thereby bypassing the bloodstream and AAV antibodies. Other ductal systems, such as the ducts of the submandibular gland, can also be used as portals for delivering AAV vectors into a subject with preexisting anti-AAV antibodies.

[0075] "Prophylaxis" and grammatical variations thereof mean a method in which contact, administration or *in vivo* delivery to a subject is prior to disease. Administration or *in vivo* delivery to a subject can be performed prior to development of an adverse symptom, condition, complication,

etc. caused by or associated with the disease. For example, a screen (*e.g.*, genetic) can be used to identify such subjects as candidates for invention methods and uses, but the subject may not manifest the disease. Such subjects therefore include those screened positive for an insufficient amount or a deficiency in a functional gene product (protein), or produce an aberrant, partially functional or non-functional gene product (protein), which can lead to disease; and subjects that screen positive for an aberrant, or defective (mutant) gene product (protein) that leads to disease, even though such subjects do not manifest symptoms of the disease.

[0076] Methods and uses of the invention include delivery and administration systemically, regionally or locally, or by any route, for example, by injection, infusion, orally (*e.g.*, ingestion or inhalation), or topically (*e.g.*, transdermally). Such delivery and administration include intravenously, intramuscularly, intraperitoneally, intradermally, subcutaneously, intracavity, intracranially, transdermally (topical), parenterally, *e.g.* transmucosally or rectally. Exemplary administration and delivery routes include intravenous (i.v.), intraperitoneal (i.p.), intrarterial, intramuscular, parenteral, subcutaneous, intra-pleural, topical, dermal, intradermal, transdermal, parenterally, *e.g.* transmucosal, intra-cranial, intra-spinal, oral (alimentary), mucosal, respiration, intranasal, intubation, intrapulmonary, intrapulmonary instillation, buccal, sublingual, intravascular, intrathecal, intracavity, iontophoretic, intraocular, ophthalmic, optical, intraglandular, intraorgan, intralymphatic.

[0077] Doses for delivery and administration can be based upon current existing protocols, empirically determined, using animal disease models or optionally in human clinical trials. Initial study doses can be based upon animal studies set forth herein, for a mouse or dog, for example.

[0078] Doses can vary and depend upon whether the treatment is prophylactic or therapeutic, the type, onset, progression, severity, frequency, duration, or probability of the disease to which treatment is directed, the clinical endpoint desired, previous or simultaneous treatments, the general health, age, gender, race or immunological competency of the subject and other factors that will be appreciated by the skilled artisan. The dose amount, number, frequency or duration may be proportionally increased or reduced, as indicated by any adverse side effects, complications or other risk factors of the treatment or therapy and the status of the subject. The skilled artisan will appreciate the factors that may influence the dosage and timing required to provide an amount sufficient for providing a therapeutic or prophylactic benefit.

[0079] Methods and uses of the invention as disclosed herein can be practiced within 1-2, 2-4, 4-12, 12-24 or 24-72 hours after a subject has been identified as having the disease targeted for treatment, has one or more symptoms of the disease, or has been screened and is identified as positive as set forth herein even though the subject does not have one or more symptoms of the disease. Of course, methods and uses of the invention can be practiced 1-7, 7-14, 14-21, 21-48 or more days, months or

years after a subject has been identified as having the disease targeted for treatment, has one or more symptoms of the disease, or has been screened and is identified as positive as set forth herein.

[0080] AAV vectors and other compositions, agents, drugs, biologics (proteins) can be incorporated into pharmaceutical compositions, *e.g.*, a pharmaceutically acceptable carrier or excipient. Such pharmaceutical compositions are useful for, among other things, administration and delivery to a subject *in vivo* or *ex vivo*.

[0081] As used herein the term "pharmaceutically acceptable" and "physiologically acceptable" mean a biologically acceptable formulation, gaseous, liquid or solid, or mixture thereof, which is suitable for one or more routes of administration, *in vivo* delivery or contact. Such formulations include solvents (aqueous or non-aqueous), solutions (aqueous or non-aqueous), emulsions (*e.g.*, oil-in-water or water-in-oil), suspensions, syrups, elixirs, dispersion and suspension media, coatings, isotonic and absorption promoting or delaying agents, compatible with pharmaceutical administration or *in vivo* contact or delivery. Aqueous and non-aqueous solvents, solutions and suspensions may include suspending agents and thickening agents. Such pharmaceutically acceptable carriers include tablets (coated or uncoated), capsules (hard or soft), microbeads, powder, granules and crystals. Supplementary active compounds (*e.g.*, preservatives, antibacterial, antiviral and antifungal agents) can also be incorporated into the compositions.

[0082] Pharmaceutical compositions can be formulated to be compatible with a particular route of administration or delivery, as set forth herein or known to one of skill in the art. Thus, pharmaceutical compositions include carriers, diluents, or excipients suitable for administration by various routes.

[0083] Formulations suitable for parenteral administration comprise aqueous and non-aqueous solutions, suspensions or emulsions of the active compound, which preparations are typically sterile and can be isotonic with the blood of the intended recipient. Non-limiting illustrative examples include water, saline, dextrose, fructose, ethanol, animal, vegetable or synthetic oils.

[0084] For transmucosal or transdermal administration (*e.g.*, topical contact), penetrants can be included in the pharmaceutical composition. Penetrants are known in the art, and include, for example, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. For transdermal administration, the active ingredient can be formulated into aerosols, sprays, ointments, salves, gels, or creams as generally known in the art. For contact with skin, pharmaceutical compositions typically include ointments, creams, lotions, pastes, gels, sprays, aerosols, or oils. Carriers which may be used include Vaseline, lanolin, polyethylene glycols, alcohols, transdermal enhancers, and combinations thereof.

[0085] Cosolvents and adjuvants may be added to the formulation. Non-limiting examples of cosolvents contain hydroxyl groups or other polar groups, for example, alcohols, such as isopropyl alcohol; glycols, such as propylene glycol, polyethyleneglycol, polypropylene glycol, glycol ether;

glycerol; polyoxyethylene alcohols and polyoxyethylene fatty acid esters. Adjuvants include, for example, surfactants such as, soya lecithin and oleic acid; sorbitan esters such as sorbitan trioleate; and polyvinylpyrrolidone.

[0086] Pharmaceutical formulations and delivery systems appropriate for the compositions and methods of the invention are known in the art (*see, e.g.*, Remington: The Science and Practice of Pharmacy (2003) 20th ed., Mack Publishing Co., Easton, PA; Remington's Pharmaceutical Sciences (1990) 18th ed., Mack Publishing Co., Easton, PA; The Merck Index (1996) 12th ed., Merck Publishing Group, Whitehouse, NJ; Pharmaceutical Principles of Solid Dosage Forms (1993), Technonic Publishing Co., Inc., Lancaster, Pa.; Ansel and Stoklosa, Pharmaceutical Calculations (2001) 11th ed., Lippincott Williams & Wilkins, Baltimore, MD; and Poznansky et al., Drug Delivery Systems (1980), R. L. Juliano, ed., Oxford, N.Y., pp. 253-315).

[0087] A "unit dosage form" as used herein refers to physically discrete units suited as unitary dosages for the subject to be treated; each unit containing a predetermined quantity optionally in association with a pharmaceutical carrier (excipient, diluent, vehicle or filling agent) which, when administered in one or more doses, is calculated to produce a desired effect (*e.g.*, prophylactic or therapeutic effect). Unit dosage forms may be within, for example, ampules and vials, which may include a liquid composition, or a composition in a freeze-dried or lyophilized state; a sterile liquid carrier, for example, can be added prior to administration or delivery *in vivo*. Individual unit dosage forms can be included in multi-dose kits or containers. Pharmaceutical formulations can be packaged in single or multiple unit dosage form for ease of administration and uniformity of dosage.

[0088] The invention provides kits with packaging material and one or more components therein. A kit typically includes a label or packaging insert including a description of the components or instructions for use *in vitro*, *in vivo*, or *ex vivo*, of the components therein. A kit can contain a collection of such components, *e.g.*, an AAV vector and optionally a second active, such as another compound, agent, drug or composition.

[0089] The term "packaging material" refers to a physical structure housing the components of the kit. The packaging material can maintain the components sterilely, and can be made of material commonly used for such purposes (*e.g.*, paper, corrugated fiber, glass, plastic, foil, ampules, vials, tubes, etc.).

[0090] Labels or inserts can include identifying information of one or more components therein, dose amounts, clinical pharmacology of the active ingredient(s) including mechanism of action, pharmacokinetics and pharmacodynamics. Labels or inserts can include information identifying manufacturer, lot numbers, manufacture location and date, expiration dates. Labels or inserts can include information identifying manufacturer information, lot numbers, manufacturer location and date. Labels or inserts can include information on a disease for which a kit component may be used. Labels or inserts can include instructions for the clinician or subject for using one or more of the kit

components in a method, use, or treatment protocol or therapeutic regimen. Instructions can include dosage amounts, frequency or duration, and instructions for practicing any of the methods, uses, treatment protocols or prophylactic or therapeutic regimes described herein.

[0091] Labels or inserts can include information on any benefit that a component may provide, such as a prophylactic or therapeutic benefit. Labels or inserts can include information on potential adverse side effects, complications or reactions, such as warnings to the subject or clinician regarding situations where it would not be appropriate to use a particular composition. Adverse side effects or complications could also occur when the subject has, will be or is currently taking one or more other medications that may be incompatible with the composition, or the subject has, will be or is currently undergoing another treatment protocol or therapeutic regimen which would be incompatible with the composition and, therefore, instructions could include information regarding such incompatibilities.

[0092] Labels or inserts include "printed matter," *e.g.*, paper or cardboard, or separate or affixed to a component, a kit or packing material (*e.g.*, a box), or attached to an ampule, tube or vial containing a kit component. Labels or inserts can additionally include a computer readable medium, such as a bar-coded printed label, a disk, optical disk such as CD- or DVD-ROM/RAM, DVD, MP3, magnetic tape, or an electrical storage media such as RAM and ROM or hybrids of these such as magnetic/optical storage media, FLASH media or memory type cards.

[0093] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described herein.

[0094] All applications, publications, patents and other references, GenBank citations and ATCC citations cited herein are incorporated by reference in their entirety. In case of conflict, the specification, including definitions, will control.

[0095] All of the features disclosed herein may be combined in any combination. Each feature disclosed in the specification may be replaced by an alternative feature serving a same, equivalent, or similar purpose. Thus, unless expressly stated otherwise, disclosed features (*e.g.*, compound structures) are an example of a genus of equivalent or similar features.

[0096] As used herein, the singular forms "a", "and," and "the" include plural referents unless the context clearly indicates otherwise. Thus, for example, reference to "a polynucleotide" includes a plurality of such polynucleotides, such as a plurality of genes.

[0097] As used herein, all numerical values or numerical ranges include integers within such ranges and fractions of the values or the integers within ranges unless the context clearly indicates otherwise. Thus, to illustrate, reference to at least 90% identity, includes 91%, 92%, 93%, 94%,

95%, 95%, 97%, 98%, 99%, etc., as well as 91.1%, 91.2%, 91.3%, 91.4%, 91.5%, etc., 92.1%, 92.2%, 92.3%, 92.4%, 92.5%, etc., and so forth.

[0098] Reference to a number with more (greater) or less than includes any number greater or less than the reference number, respectively. Thus, for example, a reference to less than 1,000, includes 999, 998, 997, etc. all the way down to the number one (1); and less than 100, includes 99, 98, 97, etc. all the way down to the number one (1).

[0099] As used herein, all numerical values or ranges include fractions of the values and integers within such ranges and fractions of the integers within such ranges unless the context clearly indicates otherwise. Thus, to illustrate, reference to a numerical range, such as a percentage range, 90-100%, includes 91%, 92%, 93%, 94%, 95%, 95%, 97%, etc., as well as 91.1%, 91.2%, 91.3%, 91.4%, 91.5%, etc., 92.1%, 92.2%, 92.3%, 92.4%, 92.5%, etc., and so forth. Reference to a range of 1-50 therefore includes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, etc., as well as 1.1, 1.2, 1.3, 1.4, 1.5, etc., 2.1, 2.2, 2.3, 2.4, 2.5, etc., and so forth.

[0100] Reference to a series of ranges includes ranges which combine the values of the boundaries of different ranges within the series. Thus, to illustrate reference to a series of ranges of 2-72 hours, 2-48 hours, 4-24 hours, 4-18 hours and 6-12 hours, includes ranges of 2-6 hours, 2, 12 hours, 2-18 hours, 2-24 hours, etc., and 4-27 hours, 4-48 hours, 4-6 hours, etc.

[0101] The invention is generally disclosed herein using affirmative language to describe the numerous embodiments and aspects. The invention also specifically includes embodiments in which particular subject matter is excluded, in full or in part, such as substances or materials, method steps and conditions, protocols, or procedures. For example, in certain embodiments or aspects of the invention, materials and/or method steps are excluded. Thus, even though the invention is generally not expressed herein in terms of what the invention does not include aspects that are not expressly excluded in the invention are nevertheless disclosed herein.

[0102] A number of embodiments of the invention have been described. Nevertheless, one skilled in the art, without departing from the spirit and scope of the invention, can make various changes and modifications of the invention to adapt it to various usages and conditions. Accordingly, the following examples are intended to illustrate but not limit the scope of the invention claimed.

Examples

Example 1

This example includes a description of various materials and methods.

[0103] **Mice:** Male C57BL/6J (WT) mice 8-10 weeks of age, n=5 per experimental group. The dog is a HB dog from the University of North Carolina Chapel Hill colony carrying a missense mutation in the FIX gene (Evans et al., *Proc Natl Acad Sci USA* 86:10095 (1989)).

[0104] **AAV Vector Constructs:** The in vivo studies in mice were performed using a construct expressing human FIX under the control of the ApoE-hAAT liver specific promoter. The study in dogs used a nearly identical promoter and the canine FIX transgene.

[0105] **Gene Transfer Methodology:** All vectors were delivered intravenously. In mice via the tail vein (a volume of 200 microliters per mouse was administered, vector was diluted in PBS). In dogs the vector was delivered via the saphenous vein.

[0106] **FIX Expression Determination:** ELISA was used to measure FIX levels. In mice, the human FIX ELISA antibody pair (capture and secondary) is from Affinity Biologicals. In dogs, an antibody pair also from Affinity Biologicals was used as described in Haurigot et al. (*Mol Ther* 18:1318 (2010)).

[0107] **Statistical analysis:** Statistical analysis was performed with unpaired, two tailed t test. p values <0.05 were considered statistically significant.

[0108] **AAV Antibody Measurements:** An in vitro neutralization assay described in Manno et al., (*Nat Med* 12:342 (2006)) and Mingozzi et al. (*Nat Med* 13:419 (2007)) was used for antibody measurement. In brief, two AAV vector constructs were used in the assay, a single-stranded vector expressing β-galactosidase under the control of the CMV promoter (ssAAV-LacZ), or a self-complementary vector expressing the Renilla reporter gene, AAV-Rh74-CBA-Renilla, under the control of the chicken β-actin promoter (CBA). To increase the efficiency of transduction of AAV vectors *in vitro*, 2V6.11 cells (ATCC) were used, which expressed the adenoviral gene E4 under the control of an inducible promoter. Cells were seeded in a 96-well plate at a density of $1.25x10^4$ cells/well and a 1:1000 dilution of ponasterone A (Invitrogen) was added to the medium to induce E4 expression. At the day of assay, serial half-log dilutions of heat-inactivated test serum were mixed with medium containing virus. For the ssAAV-LacZ vector, virus concentration used in the assay was ~$1x10^{10}$vg/ml for AAV2 and ~$5.5x10^{10}$vg/ml for AAV5, 6, or 8. For the scAAV-Luc vector, virus concentration in the assay was between ~50 and 150-fold lower. Residual activity of the reporter transgene was measured using either a colorimetric assay (ssAAV-LacZ) or a luminometer (scAAV-Luc).

[0109] Anti-AAV capsid total IgG or Ig subclasses were measured with a capture assay; ELISA plates were coated with $5x10^{10}$ capsid particles/ml of AAV empty capsids. Plates were blocked with 2% BSA, 0.05% Tween 20 in PBS for 2 hours at room temperature and serial dilutions of samples were loaded onto the wells and incubated overnight at 4°C. Biotin-conjugated anti-human IgG1, IgG2, IgG3, IgG4, or IgM antibody (Sigma) were used as detecting antibodies; streptavidin-HRP was the added for substrate detection. Ig concentration was determined against standard curves made with serial dilution of human purified IgG1, IgG2, IgG3, IgG4, or IgM (Sigma).

[0110] AAV Production: The process for vector production is described in detail in Ayuso et al. (*Gene Ther* 17:503 (2010)).

Example 2

This example includes a description of human FIX gene transfer animal(Mice) studies and FIX expression after gene transfer.

[0111] C57BL/6 mice (n=5 per group) were injected via the tail vein with AAV vectors bearing the Factor IX (FIX) gene (2.5^{10} vector genomes per mouse) under the control of a liver-specific promoter. Human FIX transgene product (protein) plasma levels in the mice were determined by ELISA at week 1, 2, and 4 post gene transfer, and are illustrated in **Figure 1**. AAV-Rh74 showed the highest level of transgene expression in the animals.

Example 3

This example includes a description of animal studies and data demonstrating effective AAV-Rh74 mediated delivery of FIX at therapeutic levels in hemophilia dogs.

[0112] In brief, hemophilia B dogs were infused intravenously (IV) though the saphenous vein with $3X10^{12}$ vector genomes per kg of weight. Expression of the therapeutic FIX transgene was driven by a liver specific promoter. Vectors and FIX levels were monitored by ELISA. Canine FIX plasma levels are shown in **Figure 2**. AAV-Rh74 and AAV8 performed roughly equally in hemophilia B dogs, and both were superior to AAV6.

Example 4

This example includes a description of studies showing the presence of anti-AAV neutralizing antibodies (NAb) in humans.

[0113] The data in Table 1 show anti-AAV neutralizing antibodies (NAb) measured in humans with an in vitro assay. Subjects with a NAb titer of 1:1 are defined as naïve or low-titer for anti-AAV antibodies, and are eligible for gene transfer for that AAV serotype (highlighted in grey). AAV-Rh74 exhibited the lowest prevalence of anti-AAV Nab compared to AAV-2 and AAV-8.

Table 1

Subject ID	AAV2 NAb	AAV8 NAb	AAV-Rh74 NAb
GenImm 005	<1:3.16	1:1	1:1
GenImm 006	1:31.6-1:100	1:10-1:31.6	1:31.6-1:100
GenImm 015	1:3.1-1:10	1:1	1:1
GenImm 016	1:10-1:31.6	1:31.6-1:100	1:31.6-1:100
GenImm 017	>1:3160	>3160	>1:3160
GenImm 028	1:3.1-1:10	1:10-1:31.6	1:1
GenImm 035	>1:3160	1:100-1:316	1:316-1:1000
GenImm 040	1:1	1:1	1:1
GenImm 049	1:1	1:3.1-1:10	1:3.1-1:10
GenImm 053	1:3160	1:31.6-1:100	1:316-1:1000
GenImm 054	1:1000-1:3160	1:10-1:31.6	1:31.6-1:100
GenImm 055	1:1000-1:3160	1:31.6-1:100	1:100-1:316
GenImm 056	1:10-1:31.6	1:10-1:31.6	1:3.1-1:10
GenImm 058	1:3.1-1:10	1:1	1:1
GenImm 060	>1:3160	1:1000-1:3160	1:1000-1:3160
GenImm 068	1:3160	1:316-1:1000	1:316-1:1000
GenImm 069	>1:3160	>1:3160	>1:3160
GenImm 070	1:10-1:31.6	1:1	1:1
GenImm 072	1:3.1-1:10	1:100-1:316	1:1
GenImm 074	1:3160	1:100-1:316	1:316-1:1000
GenImm 075	1:316-1:1000	1:31.6-1:100	1:31.6-1:100
GenImm 080	1:10-1:31.6	1:1	1:1
GenImm 082	1:3.1-1:10	1:1	1:1
GenImm 083	1:1	1:1	1:1
GenImm 084	1:3.1-1:10	1:3.1-1:10	1:1
GenImm 087	1:3.1-1:10	1:1	1:1
GenImm 088			>1:3160
GenImm 095			1:1
GenImm 100	1:3.1-1:10	1:1	1:1

Example 5

This example includes a description of data showing production amounts of different AAV serotypes including AAV-Rh74.

[0114] The data in Table 2 show production yield of different AAV serotypes. Reported are the virus batch size in roller bottles, the total vector yield, and the yield per bottle. All serotypes were packaged with the same expression cassette. AAV-Rh74 has a yield comparable to or greater than the other serotypes evaluated, namely AAV-8, AAV-dj, and AAV-2.

Table 2

Serotype	Batch size (number of roller bottles)	Total vector yield (vector genomes)	Yield per roller bottle (vector genomes)
AAV-Rh74	80	1.21E+15	1.51E+13
AAV-Rh74	10	1.23E+14	1.29E+13
AAV-8	30	2.54E+14	8.47E+12
AAV-dj	20	1.79E+14	8.95E+12
AAV-2	30	1.38E+14	4.60E+12

Example 6

This example includes a description of data showing that AAVrh74 vector expressing human Factor IX (FIX) under the control of a liver-specific promoter administered to rhesus macaques led to production amounts of FIX in animals, and at higher levels than AAV8 vector administered at the same amount .

[0115] In brief, animals were administered either AAV8 or AAVrh74 at a dose of $2x10^{12}$ vector genomes (vg)/kg of weight. Vectors were either formulated in saline or in a mixture of vector and empty AAV capsid (denoted EC).

[0116] **Figure 4** is a histogram plot of the average (weeks 2 to 8) and standard error or the mean of human FIX measured by an ELISA that detects specifically human FIX in rhesus macaque plasma. Animals receiving the AAVrh74-FIX vector are shown in the last two bars towards the right margin. The data shows that animals receiving the AAVrh74 vectors (last two bars towards right margin) expressed the FIX transgene at higher levels compared to the other groups of animals injected at the same dose (black and grey bars). Average levels were compared using unpaired, two-tailed student t test.

What is claimed:

1. Use of an adeno-associated virus (AAV) vector comprising a heterologous polynucleotide sequence and a VP1 sequence having 100% identity to:
MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDNGRGLVLPGYKYLGPF
NGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGN
LGRAVFQAKKRVLEPLGLVESPVKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPAKKRL
NFGQTGDSESVPDPQPIGEPPAGPSGLGSGTMAAGGGAPMADNNEGADGVGSSSGNWH
CDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGSTNDNTYFGYSTPWGYFDFN
RFHCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFTD
SEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLR
TGNNFEFSYNFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQSTGGTAGTQQLLFSQ
AGPNNMSAQAKNWLPGPCYRQQRVSTTLSQNNNSNFAWTGATKYHLNGRDSLVNPG
VAMATHKDDEERFFPSSGVLMFGKQGAGKDNVDYSSVMLTSEEEIKTTNPVATEQYGV
VADNLQQQNAAPIVGAVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMG
GFGLKHPPPQILIKNTPVPADPPTTFNQAKLASFITQYSTGQVSVEIEWELQKENSKRWNP
EIQYTSNYYKSTNVDFAVNTEGTYSEPRPIGTRYLTRNL,

in the manufacture of a medicament for treating cystic fibrosis, Choroideremia, LCA-Lebercilin, Gyrate Atrophy, X-linked Retinoschisis, Usher's Syndrome 1C, retinitis pigmentosa, Connexin 26 deafness, Achromatopsia, Polycystic kidney disease, a lysosomal storage disease, a polynucleotide repeat disease, dentatorubropallidolusyan atropy, spinobulbar muscular atrophy, spinocerebellar ataxia (type 1, 2, 3, 6, 7, 8, 12 17), fragile X syndrome, fragile X-associated tremor/ataxia syndrome, myotonic dystrophy, Friedreich's ataxia, amyotrophic lateral sclerosis (ALS), Parkinson's disease, Alzheimer's disease, hypercoloesterolemia, HIV infection, Rous sarcoma virus (RSV) infection, hepatitis C virus infection, acute kidney injury, delayed graft function kidney transplant, kidney injury acute renal failure, advance recurrent or metastatic solid malignancies, metastatic melanoma, chronic myeloid leukemia, solid tumors, liver tumors, familial adenomatous polyposis, glaucoma, diabetic macular oedma (DME), age-related macular degeneration, choroidal neovascularization, non-arteritic ischaemic optic neuropathy, pachyonychia congenital, influenza infection, severe acute respiratory syndrome (SARS), respiratory syncytial virus infection, Ebola infection, hepatitis B virus infection, herpes simplex

virus infection, coxsackievirus B3 infection, primary dystonia, autosomal dominantly inherited retinitis pigmentosa, lung disease, bleeding disorder, hemophilia A, hemophilia B, thalassemia, blood disorder, anemia, Huntington's disease, epilepsy, lysosomal storage disease, copper or iron accumulation disorders, Wilson's disease, Menkes disease, lysosomal acid lipase deficiency, a neurological or neurodegenerative disorder, cancer, type 1 diabetes, type 2 diabetes, Gaucher's disease, Hurler's disease, adenosine deaminase deficiency, glycogen storage diseases, a retinal degenerative disease, RPE65 deficiency, brain disease, liver disease, kidney disease, heart disease, infectious viral disease, bacterial disease or fungal disease,

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

2. The use of claim 1, wherein the heterologous polynucleotide sequence is operably linked to an expression control element conferring transcription of said heterologous polynucleotide sequence.

3. Use of an adeno-associated virus (AAV) vector in the manufacture of a medicament for treating a mammal deficient in protein expression or function, said vector comprising a heterologous polynucleotide 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 said vector comprises a VP1 sequence having 100% identity to:
MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDNGRGLVLPGYKYLGPF
NGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGN
LGRAVFQAKKRVLEPLGLVESPVKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPAKKRL
NFGQTGDSESVPDPQPIGEPPAGPSGLGSGTMAAGGGAPMADNNEGADGVGSSSGNWH
CDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGSTNDNTYFGYSTPWGYFDFN
RFHCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFTD
SEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLR
TGNNFEFSYNFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQSTGGTAGTQQLLFSQ
AGPNNMSAQAKNWLPGPCYRQQRVSTTLSQNNNSNFAWTGATKYHLNGRDSLVNPG

VAMATHKDDEERFFPSSGVLMFGKQGAGKDNVDYSSVMLTSEEEIKTTNPVATEQYGV
VADNLQQQNAAPIVGAVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMG
GFGLKHPPPQILIKNTPVPADPPTTFNQAKLASFITQYSTGQVSVEIEWELQKENSKRWNP
EIQYTSNYYKSTNVDFAVNTEGTYSEPRPIGTRYLTRNL and wherein said vector is to be administered to a mammal wherein said polypeptide is expressed in the mammal.

4. Use of an adeno-associated virus (AAV) vector comprising a VP1 sequence having 100% identity to:

MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDNGRGLVLPGYKYLGPF
NGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGN
LGRAVFQAKKRVLEPLGLVESPVKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPAKKRL
NFGQTGDSESVPDPQPIGEPPAGPSGLGSGTMAAGGGAPMADNNEGADGVGSSSGNWH
CDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGSTNDNTYFGYSTPWGYFDFN
RFHCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFTD
SEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLR
TGNNFEFSYNFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQSTGGTAGTQQLLFSQ
AGPNNMSAQAKNWLPGPCYRQQRVSTTLSQNNNSNFAWTGATKYHLNGRDSLVNPG
VAMATHKDDEERFFPSSGVLMFGKQGAGKDNVDYSSVMLTSEEEIKTTNPVATEQYGV
VADNLQQQNAAPIVGAVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMG
GFGLKHPPPQILIKNTPVPADPPTTFNQAKLASFITQYSTGQVSVEIEWELQKENSKRWNP
EIQYTSNYYKSTNVDFAVNTEGTYSEPRPIGTRYLTRNL,

in the manufacture of a medicament for treating cystic fibrosis, Choroideremia, LCA-Lebercilin, Gyrate Atrophy, X-linked Retinoschisis, Usher's Syndrome 1C, retinitis pigmentosa, Connexin 26 deafness, Achromatopsia, Polycystic kidney disease, a lysosomal storage disease, a polynucleotide repeat disease, dentatorubropallidolusyan atropy, spinobulbar muscular atrophy, spinocerebellar ataxia (type 1, 2, 3, 6, 7, 8, 12 17), fragile X syndrome, fragile X-associated tremor/ataxia syndrome, myotonic dystrophy, Friedreich's ataxia, amyotrophic lateral sclerosis (ALS), Parkinson's disease, Alzheimer's disease, hypercoloesterolemia, HIV infection, Rous sarcoma virus (RSV) infection, hepatitis C virus infection, acute kidney injury, delayed graft function kidney transplant, kidney injury acute renal failure, advance recurrent or metastatic solid malignancies, metastatic melanoma, chronic myeloid leukemia, solid tumors, liver tumors,

familial adenomatous polyposis, glaucoma, diabetic macular oedma (DME), age-related macular degeneration, choroidal neovascularization, non-arteritic ischaemic optic neuropathy, pachyonychia congenital, influenza infection, severe acute respiratory syndrome (SARS), respiratory syncytial virus infection, Ebola infection, hepatitis B virus infection, herpes simplex virus infection, coxsackievirus B3 infection, primary dystonia, autosomal dominantly inherited retinitis pigmentosa, lung disease, bleeding disorder, hemophilia A, hemophilia B, thalassemia, blood disorder, anemia, Huntington's disease, epilepsy, lysosomal storage disease, copper or iron accumulation disorders, Wilson's disease, Menkes disease, lysosomal acid lipase deficiency, a neurological or neurodegenerative disorder, cancer, type 1 diabetes, type 2 diabetes, Gaucher's disease, Hurler's disease, adenosine deaminase deficiency, glycogen storage diseases, a retinal degenerative disease, RPE65 deficiency, brain disease, liver disease, kidney disease, heart disease, infectious viral disease, bacterial disease or fungal disease,

wherein said medicament is to be administered to a mammal or a cell of said mammal, thereby delivering or transferring a heterologous polynucleotide sequence into the mammal or cell of the mammal, wherein the mammal is sero-negative for AAV-Rh74.

5. Use of an adeno-associated virus vector (AAV) comprising a VP1 sequence having 100% identity to:

MAADGYLPDWLEDNLSEGIREWWDLKPGAPKPKANQQKQDNGRGLVLPGYKYLGPF
NGLDKGEPVNAADAAALEHDKAYDQQLQAGDNPYLRYNHADAEFQERLQEDTSFGGN
LGRAVFQAKKRVLEPLGLVESPVKTAPGKKRPVEPSPQRSPDSSTGIGKKGQQPAKKRL
NFGQTGDSESVPDPQPIGEPPAGPSGLGSGTMAAGGGAPMADNNEGADGVGSSSGNWH
CDSTWLGDRVITTSTRTWALPTYNNHLYKQISNGTSGGSTNDNTYFGYSTPWGYFDFN
RFHCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTQNEGTKTIANNLTSTIQVFTD
SEYQLPYVLGSAHQGCLPPFPADVFMIPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLR
TGNNFEFSYNFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTQSTGGTAGTQQLLFSQ
AGPNNMSAQAKNWLPGPCYRQQRVSTTLSQNNNSNFAWTGATKYHLNGRDSLVNPG
VAMATHKDDEERFFPSSGVLMFGKQGAGKDNVDYSSVMLTSEEEIKTTNPVATEQYGV
VADNLQQQNAAPIVGAVNSQGALPGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMG
GFGLKHPPPQILIKNTPVPADPPTTFNQAKLASFITQYSTGQVSVEIEWELQKENSKRWNP
EIQYTSNYYKSTNVDFAVNTEGTYSEPRPIGTRYLTRNL, and

empty capsid AAV-Rh74,

in the manufacture of a medicament for treating cystic fibrosis, Choroideremia, LCA-Lebercilin, Gyrate Atrophy, X-linked Retinoschisis, Usher's Syndrome 1C, retinitis pigmentosa, Connexin 26 deafness, Achromatopsia, Polycystic kidney disease, a lysosomal storage disease, a polynucleotide repeat disease, dentatorubropallidolusyan atropy, spinobulbar muscular atrophy, spinocerebellar ataxia (type 1, 2, 3, 6, 7, 8, 12 17), fragile X syndrome, fragile X-associated tremor/ataxia syndrome, myotonic dystrophy, Friedreich's ataxia, amyotrophic lateral sclerosis (ALS), Parkinson's disease, Alzheimer's disease, hypercoloesterolemia, HIV infection, Rous sarcoma virus (RSV) infection, hepatitis C virus infection, acute kidney injury, delayed graft function kidney transplant, kidney injury acute renal failure, advance recurrent or metastatic solid malignancies, metastatic melanoma, chronic myeloid leukemia, solid tumors, liver tumors, familial adenomatous polyposis, glaucoma, diabetic macular oedma (DME), age-related macular degeneration, choroidal neovascularization, non-arteritic ischaemic optic neuropathy, pachyonychia congenital, influenza infection, severe acute respiratory syndrome (SARS), respiratory syncytial virus infection, Ebola infection, hepatitis B virus infection, herpes simplex virus infection, coxsackievirus B3 infection, primary dystonia, autosomal dominantly inherited retinitis pigmentosa, lung disease, bleeding disorder, hemophilia A, hemophilia B, thalassemia, blood disorder, anemia, Huntington's disease, epilepsy, lysosomal storage disease, copper or iron accumulation disorders, Wilson's disease, Menkes disease, lysosomal acid lipase deficiency, a neurological or neurodegenerative disorder, cancer, type 1 diabetes, type 2 diabetes, Gaucher's disease, Hurler's disease, adenosine deaminase deficiency, glycogen storage diseases, a retinal degenerative disease, RPE65 deficiency, brain disease, liver disease, kidney disease, heart disease, infectious viral disease, bacterial disease or fungal disease,

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

6. The use of any of claims 1 to 5, wherein said adeno-associated virus (AAV) vector comprises a VP2 (SEQ ID NO: 3), or VP3 (SEQ ID NO: 4) sequence of AAV-Rh74 set forth in Figure 3.

7. The use of any of claims 1 to 5, wherein said heterologous polynucleotide sequence, or said protein, is expressed at levels having a therapeutic effect on the mammal.

8. The use of any of claims 1 to 5, wherein said adeno-associated virus (AAV) vector has increased tropism for hepatocytes compared to AAV2 or AAV8.

9. The use of any of claims 1 to 5, wherein said heterologous polynucleotide sequence or said protein is expressed in a cell, tissue or organ of said mammal.

10. The use of claim 9, wherein the cell comprises a secretory cell.

11. The use of claim 9, wherein the cell comprises an endocrine cell.

12. The use of claim 9, wherein the cell comprises hepatocyte, a retinal cell, an epithelial cell, or a totipotent, pluripotent or multipotent stem cell.

13. The use of claim 9, wherein the tissue or organ of said mammal comprises liver, eye or retina.

14. The use of any of claims 1 to 5, wherein the mammal produces an insufficient amount of a protein, or a defective or aberrant protein.

15. The use of claim 14, wherein the protein comprises CFTR (cystic fibrosis transmembrane regulator protein), a blood coagulation (clotting) factor (Factor XIII, Factor IX, Factor X, Factor VIII, Factor VIIa, protein C, etc.) 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 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, 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 (*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 Tregitope 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, a gene product implicated in lysosomal storage diseases (e.g., 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.

16. The use of any of claims 1 to 5, wherein the heterologous polynucleotide sequence comprises a gene encoding a polypeptide, peptide or a protein.

17. The use of claim 16, wherein the gene encodes a therapeutic peptide or protein.

18. The use of claim 16, wherein the gene comprises or encodes CFTR (cystic fibrosis transmembrane regulator protein), a blood coagulation (clotting) factor (Factor XIII, Factor IX, Factor X, Factor VII, Factor VIIa, protein C, etc.) 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 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, 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 (*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 Tregitope 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, a gene product implicated in lysosomal storage diseases (e.g., 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.

19. The use of claim 1, wherein the heterologous polynucleotide sequence comprises an inhibitory nucleic acid.

20. The use of claim 19, wherein the inhibitory nucleic acid comprises micro-RNA (miRNA), siRNA, shRNA, trans-splicing RNA, antisense RNA or triplex forming RNA.

21. The use of claim 19, 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, 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; or mutant rhodopsin gene (RHO) in autosomal dominantly inherited retinitis pigmentosa (adRP).

22. The use of any of claims 1 to 5, wherein the mammal has a lung disease (*e.g.*, cystic fibrosis), a bleeding disorder (*e.g.*, hemophilia A or hemophilia B with or without inhibitors), thalassemia, a blood disorder (*e.g.*, anemia), Alzheimer's disease, Parkinson's disease, Huntington's disease, amyotrophic lateral sclerosis (ALS), epilepsy, lysosomal storage diseases, a copper or iron accumulation disorders (*e.g.*, 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 (*e.g.*, glycogen storage diseases), a retinal degenerative disease (such as RPE65 deficiency, choroideremia, and other diseases of the eye), a disease of solid organs (*e.g.*, brain, liver, kidney, heart), or an infectious viral (*e.g.*, hepatitis B and C, HIV, etc.), bacterial or fungal disease.

23. The use of any of claims 1 to 5, wherein the expression control element comprises a constitutive or regulatable control element.

24. The use of any of claims 1 to 5, wherein the expression control element comprises a tissue-specific expression control element or promoter.

25. The use of any of claims 1 to 5, wherein the AAV vector is prepared to be delivered intravenously, intraarterially, intramuscularly, subcutaneously, orally, by intubation, via catheter, dermally, intra-cranially, via inhalation, intra-cavity, or mucosally.

26. The use of any of claims 1 to 5, wherein the mammal is human.

27. The use of any of claims 1 to 5, wherein the mammal is sero-positive for an AAV serotype other than AAV-Rh74.

28. The use of any of claims 1 to 5, wherein the mammal is sero-positive for an AAV serotype AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10 or AAV11.

29. The use of any of claims 1 to 3 or 5, wherein the mammal is sero-negative for AAV-Rh74.

30. The use of any of claims 1 to 4, further comprising use of empty capsid AAV in the preparation of a pharmaceutical.

31. The use of any of claims 1 to 4, further comprising use of empty capsid AAV-Rh74.

32. The use of any of claims 1 to 5, wherein the AAV comprises a pharmaceutical composition.

33. The use of any of claims 1 to 4, wherein the pharmaceutical composition comprises empty capsid AAV.

34. The use of any of claims 1 to 4, wherein the pharmaceutical composition comprises empty capsid AAV-Rh74.