Streptococcus pyogenes that exhibits specificity for its target sequence found in human IgG, in addition to several other species. IdeS is capable of cleaving IgG below the hinge region (von Pawel-Rammingen *et al.* (2003) Curr Opin Microbiol. 6, 50-5), leading to the generation of F(ab')2 and Fc/2 fragments (Ryan *et al.* (2008) Mol Immunol. 45, 1837-46).

[0006] By way of further example, EndoS is a naturally occurring glycosidase, specifically an endoglycosidase, from *S. pyogenes*, that specifically hydrolyzes glycans from human IgG and alters antibody effector functions, including Fc receptor binding.

[0007] Described herein are, *inter alia*, methods for treating patients that may develop or already have pre-existing neutralizing antibodies to gene therapy vectors, by administering a protease that cleaves peptide bonds present in immunoglobulins or by administering a glycosidase that cleaves carbohydrate residues present on immunoglobulins, or other similar enzymatic cleavage of immunoglobulins *in vivo*. Also described herein are, *inter alia*, methods for treating patients that may develop or already have pre-existing antibodies that bind to a heterologous polynucleotide or a protein or peptide encoded by the heterologous polynucleotide encapsidated by a gene therapy vector by administering a protease that cleaves peptide bonds present in immunoglobulins or by administering a glycosidase that cleaves carbohydrate residues present on immunoglobulins, or other similar enzymatic cleavage of immunoglobulins *in vivo*.

Summary

[0007a] In one aspect, the present disclosure refers to use of an immunoglobulin G-degrading enzyme polypeptide in the manufacture of a medicament for the prevention or treatment of an humoral immune response caused by administration of a recombinant adeno-associated virus (AAV) vector, wherein the humoral immune response is against the recombinant AAV vector and/or a heterologous polynucleotide or a protein or peptide encoded by a heterologous polynucleotide encapsidated by the recombinant AAV vector.

[0007b] In another aspect, the present disclosure refers to use of an immunoglobulin G-degrading enzyme polypeptide in the manufacture of a medicament for the treatment, in combination with a recombinant gene therapy adeno-associated virus (AAV) vector, of a disease treated by said recombinant gene therapy AAV vector in a patient in need thereof.

[0007c] In yet another aspect, the present disclosure refers to use of a nucleic acid sequence encoding an immunoglobulin G-degrading enzyme polypeptide in the manufacture of a medicament for the treatment, in combination with a recombinant gene therapy adeno-associated virus (AAV) vector, of a disease treated by said recombinant gene therapy AAV vector in a patient in need thereof.

[0007d] In yet another aspect, the present disclosure refers to use of an expression vector comprising a nucleic acid sequence encoding an immunoglobulin G-degrading enzyme polypeptide in the manufacture of a medicament for the treatment, in combination with a recombinant gene therapy adeno-associated virus (AAV) vector, of a disease treated by said recombinant gene therapy AAV vector in a patient in need thereof.

[0007e] In yet another aspect, the present disclosure refers to a package or kit comprising (a) a recombinant gene therapy adeno-associated virus (AAV) vector comprising a heterologous polynucleotide that encodes a heterologous protein, peptide or polynucleotide, (b) an immunoglobulin G-degrading enzyme polypeptide, and (c) a label with instructions for performing gene therapy, wherein (a) and (b) are in separate or the same container.

[0007f]In yet another aspect, the present disclosure refers to use of a package or kit as disclosed herein, in the manufacture of a medicament for treating a disease by gene therapy

[0008] Disclosed herein are methods for utilizing a protease such as IdeS to reduce antibody (*e.g.*, IgG) levels in human plasma. Methods according to the instant disclosure may be used, *inter alia*, to treat patients with pre-existing neutralizing antibodies to gene therapy vectors and to re-dose patients previously treated with a gene therapy vector.

[0009] In certain examples, a method of treating a subject in need of treatment for a disease caused by a loss of function or activity of a protein includes: (a) administering to the subject a recombinant viral vector comprising a heterologous polynucleotide that encodes a protein or peptide that provides or supplements a function or activity of the protein; and (b) administering to the subject an amount of a protease or glycosidase effective to degrade or digest and/or inhibit or

reduce effector function of antibodies that bind to said recombinant viral vector and/or the protein or peptide encoded by the heterologous polynucleotide.

[0010] In certain examples,a method of treating a subject in need of treatment for a disease caused by a gain of function activity or expression, of a protein includes: (a) administering to the subject a recombinant viral vector comprising a heterologous polynucleotide that is transcribed into a nucleic acid that inhibits, decreases or reduces expression of the gain of function, activity or expression of said protein; and (b) administering to the subject a protease or glycosidase effective to degrade or digest and/or inhibit or reduce effector function of antibodies that bind to said recombinant viral vector.

[0011] In certain examples, a method of treating a subject in need of treatment for a disease caused by a loss of function or activity of a protein includes: (a) administering to the subject a protease or glycosidase effective to degrade or digest and/or inhibit or reduce effector function of viral vector binding antibodies; and (b) administering to the subject a recombinant viral vector comprising a heterologous polynucleotide that encodes a protein or peptide that provides or supplements a function or activity of said protein.

[0012] In certain examples, a method of treating a subject in need of treatment for a disease caused by a gain of function activity or expression, of a protein includes: (a) administering to the subject a protease or glycosidase effective to degrade or digest and/or inhibit or reduce effector function of viral vector binding antibodies; and (b) administering to the subject a recombinant viral vector comprising a heterologous polynucleotide that is transcribed into a nucleic acid that inhibits, decreases or reduces expression of the gain of function, activity or expression of said protein.

[0013] In certain examples, in methods of treating a subject step (b) is performed within about 90 days after step (a) is performed. In certain examples, step (b) is performed within about 60 days before or after step (a). In certain examples, step (b) is performed within about 45 days before or after step (a). In certain examples, step (b) is performed within about 30 days before or after step (a). In certain examples, step (b) is performed within about 21 days before or after step (a). In certain examples, step (b) is performed within about 14 days before or after step (a). In certain examples, step (b) is performed within about 7 days before or after step (a). In certain examples, step (b) is performed within about 72 hours before or after step (a). In

certain examples, step (b) is performed within about 48 hours before or after step (a). In certain examples, step (b) is performed within about 24 hours before or after step (a). In certain examples, step (b) is performed within about 12 hours before or after step (a). In certain examples, step (b) is performed within about 6 hours before or after step (a).

[0014] In certain examples, the protease comprises a cysteine protease or a thiol protease. In certain examples, the protease comprises a protease from *Streptococcus pyogenes*, *Streptococcus equi* or *Mycoplasma canis*. In certain examples, the protease comprises IdeS or a modified variant thereof set forth in any of SEQ ID NOs:3 – 18 23 or 48.

[0015] In certain examples, the glycosidase comprises an endoglycosidase. In certain examples, the endoglycosidase comprises a sequence set forth in any of SEQ ID NOs:44 – 47.

[0016] In certain examples, the protease or glycosidase degrades or digests and/or inhibits or reduces effector function of human antibodies.

[0017] In certain examples, the viral vector comprises a lentiviral vector, an adenoviral vector or an adeno-associated virus (AAV) vector.

[0018] In certain examples, the lentiviral vector includes envelope proteins to which the antibodies bind.

[0019] In certain examples, the AAV vector includes capsid proteins to which the antibodies bind.

[0020] In certain examples, the AAV vector comprises VP1, VP2 and/or VP3 capsid proteins to which the antibodies bind.

[0021] In certain examples, the AAV vector comprises VP1, VP2 and/or VP3 capsid protein having 60% or more sequence identity to VP1, VP2 and/or VP3 capsid protein selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10, AAV3B, AAV-2i8, Rh10, Rh74, SEQ ID NO:1 and SEQ ID NO:2 VP1, VP2 and/or VP3 capsid proteins.

[0022] In certain examples, the AAV vector comprises VP1, VP2 and/or VP3 capsid protein having 100% sequence identity to VP1, VP2 and/or VP3 capsid protein selected from the group consisting of AAV1, AAV2, AAV3, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV10,

AAV3B, AAV-2i8, Rh10, Rh74, SEQ ID NO:1 and SEQ ID NO:2 VP1, VP2 and/or VP3 capsid proteins.

[0023] In certain examples, the subject has antibodies that bind to said viral vector.

[0024] In certain examples, antibodies that bind to the viral vector are absent from the subject.

[0025] In certain examples, the subject has antibodies that bind to the protein or peptide encoded by the heterologous polynucleotide.

[0026] In certain examples, the antibodies comprise IgG, IgM, IgA, IgD and/or IgE.

[0027] In certain examples, a method includes determining the presence of, quantifying the amount of or an effector function of viral vector binding antibodies present in said subject before performing step (a), after performing step (a) but before performing step (b) and/or after performing steps (a) and (b).

[0028] In certain examples, a method includes analyzing a biological sample from said subject for the presence, amount or an effector function of viral vector binding antibodies present in said sample before performing step (a), after performing step (a) but before performing step (b) and/or after performing steps (a) and (b).

[0029] In certain examples, a biological sample from the subject is a blood product. In certain examples, a blood product comprises plasma or serum.

[0030] In certain examples, a method leads to a reduction of 20-50%, 50-75%, 75-90%, 90-95% or 95% or more of said viral vector binding antibodies.

[0031] In certain examples, the viral vector binding antibodies present in the biological sample or blood product from the subject is less than about 1:100,000 where 1 part of said biological sample or blood product diluted in 100,000 parts of buffer results in 50% viral vector neutralization.

[0032] In certain examples, the viral vector binding antibodies present in said biological sample or blood product from said subject is less than about 1:50,000, where 1 part of the biological sample or blood product diluted in 50,000 parts of buffer results in 50% viral vector neutralization.

[0033] In certain examples, the viral vector binding antibodies present in the biological sample or blood product from the subject is less than about 1:10,000, where 1 part of the biological sample or blood product diluted in 10,000 parts of buffer results in 50% viral vector neutralization.

[0034] In certain examples, the viral vector binding antibodies present in the biological sample or blood product from the subject is less than about 1:1,000, where 1 part of the biological sample or blood product diluted in 1,000 parts of buffer results in 50% viral vector neutralization.

[0035] In certain examples, the viral vector binding antibodies present in the biological sample or blood product from the subject is less than about 1:100, where 1 part of the biological sample or blood product diluted in 100 parts of buffer results in 50% viral vector neutralization.

[0036] In certain examples, the viral vector binding antibodies present in the biological sample or blood product from the subject is less than about 1:10, where 1 part of the biological sample or blood product diluted in 10 parts of buffer results in 50% viral vector neutralization.

[0037] In certain examples, the viral vector binding antibodies present in the biological sample or blood product is less than about 1:5, where 1 part of the biological sample or blood product diluted in 5 parts of buffer results in 50% viral vector neutralization.

[0038] In certain examples, the ratio of viral vector binding antibodies present in the biological sample or blood product is less than about 1:4, where 1 part of the biological sample or blood product diluted in 4 parts of buffer results in 50% viral vector neutralization.

[0039] In certain examples, the ratio of viral vector binding antibodies present in the biological sample or blood product is less than about 1:3, where 1 part of the biological sample or blood product diluted in 3 parts of buffer results in 50% viral vector neutralization.

[0040] In certain examples, the ratio of viral vector binding antibodies present in the subject, biological sample or blood product is less than about 1:2, where 1 part of the biological sample or blood product diluted in 2 parts of buffer results in 50% viral vector neutralization.

[0041] In certain examples, the ratio of viral vector binding antibodies present in the subject, biological sample or blood product is less than about 1:1, where 1 part of the biological sample or blood product diluted in 1 part of buffer results in 50% viral vector neutralization.

[0042] In certain examples, a method includes determining the presence of or quantifying the amount of antibodies that bind to the polypeptide or peptide encoded by the heterologous

polynucleotide after performing step (a) but before performing step (b) and/or after performing steps (a) and (b).

[0043] In certain examples, a method includes determining the presence of or quantifying the amount of antibodies that bind to the nucleic acid after performing step (a) but before performing step (b) and/or after performing steps (a) and (b).

[0044] In certain examples, a subject 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, a lysosomal storage disease (*e.g.*, aspartylglucosaminuria, Batten disease, late infantile neuronal ceroid lipofuscinosis type 2 (CLN2), cystinosis, Fabry disease, Gaucher disease types I, II, and III, glycogen storage disease II (Pompe disease), GM2-gangliosidosis type I (Tay Sachs disease), GM2-gangliosidosis type II (Sandhoff disease), mucolipidosis types I (sialidosis type I and II), II (I-cell disease), III (pseudo-Hurler disease) and IV, mucopolysaccharide storage diseases (Hurler disease and variants, Hunter, Sanfilippo Types A,B,C,D, Morquio Types A and B, Maroteaux-Lamy and Sly diseases), Niemann-Pick disease types A/B, C1 and C2, and Schindler disease types I and II), hereditary angioedema (HAE), a copper or iron accumulation disorder (*e.g.*, Wilson’s or Menkes disease), lysosomal acid lipase deficiency, a neurological or neurodegenerative disorder, cancer, type 1 or type 2 diabetes, adenosine deaminase deficiency, a metabolic defect (*e.g.*, glycogen storage diseases), 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. In certain examples, a subject has a blood clotting disorder. In certain examples, a subject has hemophilia A, hemophilia A with inhibitory antibodies, hemophilia B, hemophilia B with inhibitory antibodies, a deficiency in any coagulation Factor: VII, VIII, IX, X, XI, V, XII, II, von Willebrand factor, or a combined FV/FVIII deficiency, thalassemia, vitamin K epoxide reductase C1 deficiency or gamma-carboxylase deficiency.

[0045] In certain examples, a subject has anemia, bleeding associated with trauma, injury, thrombosis, thrombocytopenia, stroke, coagulopathy, disseminated intravascular coagulation (DIC); over-anticoagulation associated with heparin, low molecular weight heparin,

pentasaccharide, warfarin, small molecule antithrombotics (*i.e.*, FXa inhibitors), or a platelet disorder such as, Bernard Soulier syndrome, Glanzmann thrombasthenia, or storage pool deficiency.

[0046] In certain examples, a subject has a disease that affects or originates in the central nervous system (CNS). In certain examples, the disease is a neurodegenerative disease. In certain examples, the CNS or neurodegenerative disease is Alzheimer's disease, Huntington's disease, ALS, hereditary spastic hemiplegia, primary lateral sclerosis, spinal muscular atrophy, Kennedy's disease, a polyglutamine repeat disease, or Parkinson's disease. In certain examples, the CNS or neurodegenerative disease is a polyglutamine repeat disease. In certain examples, the polyglutamine repeat disease is a spinocerebellar ataxia (SCA1, SCA2, SCA3, SCA6, SCA7, or SCA17).

[0047] In certain examples, the heterologous polynucleotide encodes a protein selected from the group consisting of insulin, glucagon, growth hormone (GH), parathyroid hormone (PTH), growth hormone releasing factor (GRF), follicle stimulating hormone (FSH), luteinizing hormone (LH), human chorionic gonadotropin (hCG), vascular endothelial growth factor (VEGF), angiopoietins, angiostatin, granulocyte colony stimulating factor (GCSF), erythropoietin (EPO), connective tissue growth factor (CTGF), basic fibroblast growth factor (bFGF), acidic fibroblast growth factor (aFGF), epidermal growth factor (EGF), transforming growth factor α (TGFα), platelet-derived growth factor (PDGF), insulin growth factors I and II (IGF-I and IGF-II), TGFβ, activins, inhibins, bone morphogenic protein (BMP), nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophins NT-3 and NT4/5, ciliary neurotrophic factor (CNTF), glial cell line derived neurotrophic factor (GDNF), neurturin, agrin, netrin-1 and netrin-2, hepatocyte growth factor (HGF), ephrins, noggin, sonic hedgehog and tyrosine hydroxylase.

[0048] In certain examples, the heterologous polynucleotide encodes a protein selected from the group consisting of thrombopoietin (TPO), interleukins (IL1 through IL-36), monocyte chemoattractant protein, leukemia inhibitory factor, granulocyte-macrophage colony stimulating factor, Fas ligand, tumor necrosis factors α and β, interferons α, β, and γ, stem cell factor, flk-2/flt3 ligand, IgG, IgM, IgA, IgD and IgE, chimeric immunoglobulins, humanized antibodies, single

chain antibodies, T cell receptors, chimeric T cell receptors, single chain T cell receptors, class I and class II MHC molecules.

[0049] In certain examples, the heterologous polynucleotide encodes CFTR (cystic fibrosis transmembrane regulator protein), a blood coagulation (clotting) factor (Factor XIII, Factor IX, Factor VIII, 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 sulfatase, N-acetylglucosamine-1-phosphate transferase, cathepsin A, GM2-AP, NPC1, VPC2, a sphingolipid activator protein, one or more zinc finger nuclease for genome editing, and one or more donor sequence used as repair templates for genome editing.

[0050] In certain examples, the heterologous polynucleotide encodes an inhibitory nucleic acid. In certain examples, the inhibitory nucleic acid is selected from the group consisting of

a siRNA, an antisense molecule, miRNA, RNAi, a ribozyme and a shRNA. In certain examples, the inhibitory nucleic acid binds to a gene, a transcript of a gene, or a transcript of a gene associated with a polynucleotide repeat disease selected from the group consisting of a huntingtin (HTT) gene, a gene associated with dentatorubropallidoluysian atrophy (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 (CACNA1A), TATA-binding protein, Ataxin 8 opposite strand (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), hypercholesterolemia; 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 DNA damage-inducible transcript 4 protein, in diabetic

macular edema (DME) or age-related macular degeneration; vascular endothelial growth factor receptor I (VEGFR1) in age-related macular degeneration or choroidal neovascularization, 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; and mutant rhodopsin gene (RHO) in autosomal dominantly inherited retinitis pigmentosa (adRP).

[0051] In certain examples, the protein encoded by the heterologous polynucleotide comprises a gene editing nuclease. In certain examples, the gene editing nuclease comprises a zinc finger nuclease (ZFN) or a transcription activator-like effector nuclease (TALEN). In certain examples, the gene editing nuclease comprises a functional Type II CRISPR-Cas9.

[0052] In certain examples, step (a) and/or step (b) of a method according to the instant disclosure are performed two or more times.

[0053] In certain examples, the subject is a mammal. In certain examples, the subject is a human.

[0054] Also disclosed herein are compositions, for example and without limitation, packages and kits, having components that may be used to practice methods according to the instant disclosure.

[0055] In certain examples, a package or kit has disposed therein: (a) a recombinant viral vector comprising a heterologous polynucleotide that encodes a protein or peptide; (b) a protease or glycosidase that degrades or digests antibodies; and (c) a label with instructions for performing a method as disclosed herein. In certain examples, (a) and (b) are in separate or the same container.

[0056] In certain examples, a package or kit has disposed therein: (a) a recombinant viral vector comprising a heterologous polynucleotide that is transcribed into a nucleic acid that inhibits, decreases or reduces expression of a protein; (b) a protease or glycosidase that degrades or digests antibodies; and (c) a label with instructions for performing a method as disclosed herein. In certain examples, (a) and (b) are in separate or the same container.

[0057] In certain examples, a method for treating a disease treated by gene therapy using a vector comprising administering to a subject in need thereof a therapeutically effective amount of an immunoglobulin G-degrading enzyme polypetide.

Description of Drawings

[0058] **Figure 1** is a graph showing hydrolysis of human IgG in mice reconstituted with IVIg. Wild-type C57BL/6 mice were reconstituted with human IgG (IVIg, 9 mg/mouse) and injected 30 min later with IdeS (250 IU/mouse, 3 mice) or PBS (2 mice). Blood was collected before the injection of IdeS and 1, 6 and 10 hours later. Intact human IgG was measured in mouse serum by ELISA and is expressed as µg/mL using IVIg as a standard.

[0059] **Figure 2** is a scheme of the experimental protocol for the in vivo neutralization of anti-AAV IgG. Wild-type C57BL/6 mice (6 mice per group) or Hemophilia B (HB) mice (4 mice per group) were reconstituted with human IgG (IVIg, 9 mg/mouse, day (D) minus 1 (D-1)) and injected 30 minutes (min, mn) later with IdeS (250, 500 or 1250 IU/mouse, D-1 + 30 min). Thirty hours (hr, h) later (D0), the mice were injected with AAV8 vector (2x1010 vg/mouse). Blood was collected 15 min after IVIg injection, 24 hours after IdeS injection, as well as at D-7, D7, D14 and D28. At euthanasia (D28), livers were collected for a vector genome copy number (VGCN) assessment. Control mice received IVIg alone, IdeS alone or PBS only prior to AAV vectors injection.

[0060] **Figures 3A, 3B, 3C, 3D, 3E, and 3F** are graphs showing the effect of IdeS on human anti-AAV8 IgG, anti-AAV8 neutralizing antibody (NAb), anti-AAV2 IgG, anti-AAV2 NAb, anti-AAV9 IgG, and anti-AAV9 NAb, respectively, in passively immunized mice. Blood was collected from mice (6 mice/group) injected with IVIg and IdeS (IVIg/IdeS) or with IVIg alone (IVIg/PBS), at D-1+15min and D-1+24 (see scheme in Figure 2). The dose of IdeS was 250 IU/mouse in Figs.

WHAT IS CLAIMED IS:

1. Use of an immunoglobulin G-degrading enzyme polypeptide in the manufacture of a medicament for the prevention or treatment of an humoral immune response caused by administration of a recombinant adeno-associated virus (AAV) vector, wherein the humoral immune response is against the recombinant AAV vector and/or a heterologous polynucleotide or a protein or peptide encoded by a heterologous polynucleotide encapsidated by the recombinant AAV vector.
2. The use according to claim 1, wherein the recombinant AAV vector is a gene therapy vector.
3. Use of an immunoglobulin G-degrading enzyme polypeptide in the manufacture of a medicament for the treatment, in combination with a recombinant gene therapy adeno-associated virus (AAV) vector, of a disease treated by said recombinant gene therapy AAV vector in a patient in need thereof.
4. The use according to any one of claims 1-3, wherein said recombinant AAV vector comprises capsid proteins selected from the group consisting of AAV1, AAV2, an AAV2 variant, AAV3, an AAV3 variant, AAV3B, an AAV3B variant, AAV4, AAV5, AAV6, an AAV6 variant, AAV7, AAV8, AAV9, AAV10, AAVcy10, AAVrh10, AAVrh74, AAVdj, AAV-Anc80, SEQ ID NO:1, SEQ ID NO:2 or AAV2i8.
5. The use according to any one of claims 1-4, wherein the immunoglobulin G-degrading enzyme polypeptide comprises the sequence of any one of SEQ ID NOs:3-18, 23, or 48.
6. The use according to claim 5, wherein said immunoglobulin G-degrading enzyme polypeptide further comprises a non-native signal sequence of any one of SEQ ID NOs:49-51.
7. The use according to any one of claims 2-5, wherein the disease treated by the recombinant gene therapy AAV vector is selected from the group consisting of proliferative diseases (cancers, tumors, dysplasias, etc.), Crigler-Najjar and metabolic diseases like metabolic diseases of the liver, Friedreich ataxia, infectious diseases, addiction (*e.g.*, to tobacco, alcohol, or drugs), epilepsy, Canavan's disease, adrenoleukodystrophy, viral diseases (induced, *e.g.*, by hepatitis B or C viruses, HIV, herpes, retroviruses, etc.), genetic diseases

(cystic fibrosis, dystroglycanopathies, myopathies such as Duchenne muscular myopathy or dystrophy, myotubular myopathy, hemophilia A, hemophilia B, hemophilia A with inhibitors, hemophilia B with inhibitors, sickle-cell anemia, sickle cell disease, Fanconi's anemia, diabetes, amyotrophic lateral sclerosis (ALS), myotubularin myopathy, motor neuron diseases such as spinal muscular atrophy (SMA), spinobulbar muscular atrophy, or Charcot-Marie-Tooth disease, arthritis, severe combined immunodeficiencies (such as RS-SCID, ADA-SCID or X-SCID), Wiskott-Aldrich syndrome, X-linked thrombocytopenia, X-linked congenital neutropenia, chronic granulomatous disease, etc.), clotting factor deficiencies, cardiovascular disease (restenosis, ischemia, dyslipidemia, homozygous familial hypercholesterolemia, etc.), eye diseases such as retinitis pigmentosa, Leber congenital amaurosis, Leber hereditary optic neuropathy, and Stargardt disease; hereditary angioedema (HAE); lysosomal storage diseases such as San Filippo syndrome; hyperbilirubinemia such as CN type I or II or Gilbert's syndrome; Fabry disease, glycogen storage disease such as GSDI, GSDII (Pompe disease), GSDIII, GSDIV, GSDV, GSDVI, GSDVII, GSDVIII and lethal congenital glycogen storage disease of the heart.

8. The use according to any one of claims 2-6, wherein the recombinant gene therapy AAV vector comprises a therapeutic polynucleotide appropriate for treating the disease of claim 7.

9. The use according to any one of claims 3-8, wherein the recombinant gene therapy AAV vector and the immunoglobulin G-degrading enzyme polypeptide are to be administered simultaneously, separately, or sequentially.

10. The use according to any one of claims 3-9, wherein the immunoglobulin G-degrading enzyme polypeptide is to be administered before or simultaneously with said recombinant gene therapy AAV vector.

11. Use of a nucleic acid sequence encoding an immunoglobulin G-degrading enzyme polypeptide in the manufacture of a medicament for the treatment, in combination with a recombinant gene therapy adeno-associated virus (AAV) vector, of a disease treated by said recombinant gene therapy AAV vector in a patient in need thereof.

12. Use of an expression vector comprising a nucleic acid sequence encoding an immunoglobulin G-degrading enzyme polypeptide in the manufacture of a medicament for the treatment, in combination with a recombinant gene therapy adeno-associated virus (AAV) vector, of a disease treated by said recombinant gene therapy AAV vector in a patient in need thereof.

13. A package or kit comprising (a) a recombinant gene therapy adeno-associated virus (AAV) vector comprising a heterologous polynucleotide that encodes a heterologous protein, peptide or polynucleotide, (b) an immunoglobulin G-degrading enzyme polypeptide, and (c) a label with instructions for performing gene therapy, wherein (a) and (b) are in separate or the same container.

14. Use of a package or kit according to claim 13, in the manufacture of a medicament for treating a disease by gene therapy.

15. The use according to any one of claims 11-12 and 14, or the package or kit according to claim 13, wherein the immunoglobulin G-degrading enzyme polypeptide comprises the sequence of any of SEQ ID NOs:3-18, 23, or 48.