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 of Streptococcus pyogenes (IdeS) 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 wherein the IdeS is to be administered within 72 hours before the recombinant AAV vector is administered, and wherein the humoral immune response includes neutralizing antibodies that bind to the recombinant AAV vector.

[0007b] In another aspect, the present disclosure refers to use of an immunoglobulin G-degrading enzyme of Streptococcus pyogenes (IdeS) polypeptide in the manufacture of a first medicament and use of a recombinant gene therapy adeno-associated virus (AAV) vector in the manufacture of a second medicament, wherein the first and second medicaments are to be used in combination therapy for the treatment of a disease treated by said recombinant gene therapy AAV vector in a patient in need thereof, wherein the IdeS is to be administered within 72 hours before the recombinant AAV vector is to be

administered, wherein said patient has neutralizing anti-AAV antibodies that inhibit cell transduction of said recombinant gene therapy AAV vector, and 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.

[0007c]In yet another example, the present disclosure refers to use of a nucleic acid sequence encoding an immunoglobulin G-degrading enzyme of Streptococcus pyogenes (IdeS) 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, wherein the nucleic acid sequence encoding IdeS is to be administered within 72 hours before the recombinant gene therapy AAV vector is to be administered, and wherein said patient has neutralizing anti-AAV antibodies that inhibit cell transduction of said recombinant gene therapy AAV vector.

[0007d] In yet another example, there is disclosed a 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 example, there is disclosed 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 example, there is disclosed 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

WHAT IS CLAIMED IS:

1. Use of an immunoglobulin G-degrading enzyme of Streptococcus pyogenes (IdeS) 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, wherein the IdeS is to be administered within 72 hours before the recombinant AAV vector is administered, and wherein the humoral immune response includes neutralizing antibodies that bind to 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 of Streptococcus pyogenes (IdeS) polypeptide in the manufacture of a first medicament and use of a recombinant gene therapy adeno-associated virus (AAV) vector in the manufacture of a second medicament, wherein the first and second medicaments are to be used in combination therapy for the treatment of a disease treated by said recombinant gene therapy AAV vector in a patient in need thereof, wherein the IdeS is to be administered within 72 hours before the recombinant AAV vector is to be administered, wherein said patient has neutralizing anti-AAV antibodies that inhibit cell transduction of said recombinant gene therapy AAV vector, and 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.

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 IdeS polypeptide is able to cleave all four human subclasses of IgG at glycine residue 237 in the lower hinge region of the IgG heavy chains without cleaving the IgA, IgM, IgD and IgE isotypes.

6. The use according to any one of claims 1-5, wherein the immunoglobulin G-degrading enzyme polypeptide comprises the sequence of any one of SEQ ID NO: 4, or SEQ ID NO: 48.

7. The use according to claim 6, wherein said immunoglobulin G-degrading enzyme polypeptide further comprises a non-native signal sequence of any one of SEQ ID NOs:49-51.

8. The use according to claim 2, 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.

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

10. The use according to any one of claims 3-9, wherein the patient is a subject to whom the recombinant gene therapy AAV vector had previously been administered, and who has developed antibodies that bind and/or neutralize the recombinant gene therapy AAV vector, and to whom the recombinant gene therapy AAV vector is to be re-dosed or re-administered.

11. The use according to any one of claims 3-10, wherein the patient is a human.

12. The use according to any one of claims 3-11, wherein the IdeS polypeptide is to be administered 24 to 48 hours before the recombinant gene therapy AAV vector is to be administered.

13. The use according to any one of claims 1-12, wherein the IdeS polypeptide and/or recombinant gene therapy AAV vector is to be administered two or more times.
14. The use according to any one of claims 1-13, wherein a biological sample from said subject is analyzed for the presence or amount of recombinant gene therapy AAV vector binding antibodies present in said sample before administering the IdeS polypeptide, after administering the IdeS polypeptide but before administering the recombinant gene therapy AAV vector and/or after administering the IdeS polypeptide and recombinant gene therapy AAV vector.
15. The use according to claim 14, wherein said biological sample is a blood product.