

ISOLATED MODIFIED VP1 CAPSID PROTEIN OF AAV5

FIELD OF INVENTION

This application relates to the fields of gene therapy and molecular biology. More specifically, the present invention relates to an isolated altered VP1 protein of adeno-associated virus serotype 5 (AAV5) capsid, which comprises one or more amino acid substitutions as compared to the VP1 protein of a wild-type AAV5 capsid, which improve transduction efficiency, as well as to a capsid and a vector based thereon.

BACKGROUND OF THE INVENTION

Adeno-associated virus (AAV) is a small (20 nm), independent replication-defective, nonenveloped virus. Many different AAV serotypes have been described in human and primates. The adeno-associated virus genome is composed of (+ or -) single-stranded DNA (ssDNA) being about 4,700 nucleotide long. The genomic DNA has terminal inverted repeats (ITRs) at the ends. The genome comprises two open reading frames (ORFs), Rep and Cap comprising several alternative reading frames encoding various protein products. The rep products are essential for AAV replication, whereas three capsid proteins (the VP1, VP2, and VP3), along with other alternative products, are encoded by the Cap gene. The VP1, VP2 and VP3 proteins are at 1:1:10 ratio to form an icosahedral capsid (Xie Q. et al. The atomic structure of adeno-associated virus (AAV-2), a vector for human gene therapy. *Proc Natl Acad Sci USA*, 2002; 99:10405-10410). During recombinant AAV (rAAV) vector production, an expression cassette flanked by ITRs is packaged into an AAV capsid. The genes required for AAV replication are not included in the cassette. Recombinant AAV is considered to be one of the safest and most widely used viral vectors for in vivo gene transfer. Vectors can infect cells of multiple tissue types to provide strong and sustained transgene expression. They are also non-pathogenic, and have a low immunogenicity profile (High KA et al., "rAAV human trial experience" *Methods Mol Biol*. 2011; 807:429-57).

One of the essential goals of trials in the field of development of effective gene therapy is to optimize the vector to maximize tissue transduction while minimizing vector dosage.

It is known that, the various AAV serotypes are characterized by affinity for distinct host cell surface receptors, toward which they have tropism. Thus, the primary known receptor for AAV2 is heparan sulfate proteoglycan, the coreceptors are integrin

heterodimer $\alpha\text{V}\beta 5$, fibroblast growth factor receptor type 1, and hepatocyte growth factor receptor, c-Met. AAV12 binds to heparan sulfate proteoglycans and sialic acid. AAV4 and AAV5 bind to N- and O-linked sialic acids, respectively. AAV5 activates the platelet-derived growth factor receptor. At the same time, a binding has been established between the amino acid sequence of AAV capsid proteins and the process of assembly thereof, encapsidation of the genome, affinity for different types of receptors represented on the surface of host cells (Govindasamy L. et. al. Structural insights into adeno-associated virus serotype 5. J Virol. 2013 Oct;87(20):11187-99).

International application WO 2012145601 discloses adeno-associated virus (AAV) virions with variant capsid protein, wherein the AAV virions exhibit greater infectivity of a retinal cell, when administered via intravitreal injection, compared to a wild-type AAV.

International application WO 2013158879 discloses an adeno-associated virus (AAV) vector delivering to a subject a heterologous nucleic acid sequence, comprising a VP1 capsid protein, which comprises one or more lysine substitutions, wherein one lysine substitution is K137R, wherein said lysine substitution is effective to inhibit ubiquitination of said capsid protein, thereby increasing transduction of said AAV vector in a target cell.

There is currently a need for AAVs with improved transduction ability, which include in structure thereof various transgenes, including clinically important transgenes, for patients in need thereof. Improved tissue transduction enables to minimize the dose of vector being administered to a subject.

The inventors have surprisingly found that the presence of one or more amino acid substitutions in the VP1 protein of the wild-type AAV5 capsid, which are selected from the group comprising:

S651A,

S2A and T711S or

S2A, S651A, and T711S,

caused an increase in the efficiency of transduction of target cells using the AAV serotype 5 vector with this/these modification(s) and a significant increase in the efficiency of transgene delivery by the rAAV vectors with the above mutations.

Brief description of invention

In one aspect, the present invention relates to an isolated altered VP1 protein of adeno-associated virus serotype 5 (AAV5) capsid for highly-efficient transduction of target cells, comprising the amino acid sequence of the VP1 protein of the wild-type AAV5 capsid, which is encoded by the *Cap* gene, with one or more substitutions selected from the group comprising:

S651A,

S2A and T711S,

S2A, S651A, and T711S.

In some embodiments, the amino acid sequence of the VP1 protein of the wild-type AAV5 capsid has the amino acid sequence represented by SEQ ID NO: 1.

In some embodiments, the isolated altered VP1 protein of AAV5 capsid includes one substitution at S651A position.

In some embodiments, the isolated altered VP1 protein of AAV5 capsid has the amino acid sequence represented by SEQ ID NO: 2.

In some embodiments, the isolated altered VP1 protein of AAV5 capsid includes the S2A and T711S substitutions.

In some embodiments, the isolated altered VP1 protein of AAV5 capsid has the amino acid sequence represented by SEQ ID NO: 3.

In some embodiments, the isolated altered VP1 protein of AAV5 capsid includes the S2A, S651A, and T711S substitutions.

In some embodiments, the isolated altered VP1 protein of AAV5 capsid has the amino acid sequence represented by SEQ ID NO: 4.

In one aspect, the present invention relates to an isolated nucleic acid encoding the above altered VP1 protein of adeno-associated virus serotype 5 (AAV5) capsid, which is used for highly-efficient transduction of target cells.

In some embodiments, the isolated nucleic acid encoding the altered VP1 protein of adeno-associated virus serotype 5 (AAV5) capsid with the amino acid S651A substitution is represented by the nucleic acid sequence with SEQ ID NO: 5 or any other sequence encoding the corresponding amino acid sequence of the altered protein of adeno-associated virus serotype 5 (AAV5) capsid with the amino acid S651A substitution.

In some embodiments, the isolated nucleic acid encoding the altered VP1 protein of adeno-associated virus serotype 5 (AAV5) capsid with the amino acid S2A and T711S substitutions is represented by the nucleic acid sequence with SEQ ID NO: 6 or any other sequence encoding the corresponding amino acid sequence of the altered protein of adeno-associated virus serotype 5 (AAV5) capsid with the amino acid S2A and T711S substitutions.

In some embodiments, the isolated nucleic acid encoding the altered VP1 protein of adeno-associated virus serotype 5 (AAV5) capsid with the amino acid S2A, S651A, and T711S substitutions is represented by the nucleic acid sequence with SEQ ID NO: 7 or any other sequence encoding the corresponding amino acid sequence of the altered protein of adeno-associated virus serotype 5 (AAV5) capsid with the amino acid S2A, S651A, and T711S substitutions.

In one aspect, the present invention relates to an isolated capsid for highly-efficient transduction of target cells, which includes the above altered VP1 protein of adeno-associated virus serotype 5 (AAV5) capsid.

In some embodiments, the isolated capsid includes the above altered VP1 protein of adeno-associated virus serotype 5 (AAV5) capsid, a VP2 protein of AAV5 capsid or an altered variant thereof, and a VP3 protein of AAV5 capsid or an altered variant thereof.

In some embodiments, the isolated capsid includes a VP2 protein of the wild-type AAV5 capsid.

In some embodiments, the isolated capsid includes a VP1 protein of the wild-type AAV5 capsid, which has the amino acid sequence represented by SEQ ID NO: 8.

In some embodiments, the isolated capsid includes an altered VP2 protein of adeno-associated virus serotype 5 (AAV5) capsid.

In some embodiments, the isolated capsid includes an altered VP2 protein of AAV5 capsid, which comprises the T575S substitution.

In some embodiments, the isolated capsid includes an altered VP2 protein of AAV5 capsid, which comprises the T575S substitution, and has the amino acid sequence represented by SEQ ID NO: 9.

In some embodiments, the isolated capsid includes an altered VP2 protein of AAV5 capsid, which comprises the S515A and T575S substitutions.

In some embodiments, the isolated capsid includes an altered VP2 protein of AAV5 capsid, which comprises the S515A and T575S substitutions, and has the amino acid sequence represented by SEQ ID NO: 10.

In some embodiments, the isolated capsid includes a VP3 protein of the wild-type AAV5 capsid.

In some embodiments, the isolated capsid includes the VP3 protein of the wild-type AAV5 capsid, which has the amino acid sequence represented by SEQ ID NO: 11.

In some embodiments, the isolated capsid includes an altered VP3 protein of adeno-associated virus serotype 5 (AAV5) capsid.

In some embodiments, the isolated capsid includes the altered VP3 protein of AAV5 capsid, which comprises the T519S substitution.

In some embodiments, the isolated capsid includes the altered VP3 protein of AAV5 capsid, which comprises the T519S substitution, and has the amino acid sequence represented by SEQ ID NO: 12.

In some embodiments, the isolated capsid includes the altered VP3 protein of AAV5 capsid, which comprises the S459A and T519S substitutions.

In some embodiments, the isolated capsid includes the altered VP3 protein of AAV5 capsid, which comprises the S459A and T519S substitutions, and has the amino acid sequence represented by SEQ ID NO: 13.

In one aspect, the present invention relates to an isolated nucleic acid encoding the above capsid, which is used for highly-efficient transduction of target cells.

In one aspect, the present invention relates to a vector based on recombinant adeno-associated virus serotype 5 (rAAV5) for delivery to a subject of a heterologous nucleic acid sequence, which includes:

- 1) the above capsid, and
- 2) a heterologous nucleic acid sequence comprising regulatory sequences that promote the expression of a product encoded by the heterologous nucleic acid sequence, in target cells.

In some embodiments, the vector based on rAAV5 comprises a heterologous nucleic acid sequence encoding a product that is a therapeutic polypeptide or a reporter polypeptide.

In some embodiments, the vector based on rAAV5 comprises a heterologous nucleic acid sequence encoding a product that is a therapeutic polypeptide, wherein the therapeutic polypeptide is a coagulation factor selected from the group consisting of Factor VIII, Factor IX, or a functional variant thereof.

In some embodiments, the vector based on rAAV5 comprises a heterologous nucleic acid sequence encoding a product that is Factor VIII or a functional variant thereof.

In some embodiments, the vector based on rAAV5 comprises a heterologous nucleic acid sequence encoding a product that is Factor IX or a functional variant thereof.

In one aspect, the present invention relates to a pharmaceutical composition for the delivery of a gene product to a subject in need thereof, which comprises:

- a) the above vector based on rAAV5; and
- b) a pharmaceutically acceptable excipient.

In some embodiments, the pharmaceutical composition is used for the delivery of a gene product to a human in need thereof.

In one aspect, the present invention relates to a method for the delivery of a gene product to a subject in need thereof, which comprises administering to the subject the above vector based on rAAV5 or the above pharmaceutical composition.

In some embodiments, the method for the delivery of a gene product is used for the delivery of a gene product to a human in need thereof.

In one aspect, the present invention relates to the use of the above vector based on rAAV5 or the above pharmaceutical composition for the treatment of a disease in a subject in need thereof.

In some embodiments, the use is used for the treatment of a disease in a human in need thereof.

In some embodiments of the use, the disease is selected from the group comprising: blood diseases; central nervous system diseases; metabolic diseases; muscle diseases; hereditary diseases.

In some embodiments of the use, the disease is a blood disease.

In some embodiments of the use, the expression product of the heterologous nucleic acid sequence is Factor IX or a functional variant thereof.

In some embodiments of the use, the expression product of the heterologous nucleic acid sequence is Factor VIII or a functional variant thereof.

In some embodiments of the use, the disease is a muscle disease.

In some embodiments of the use, the disease is a hereditary disease.

In one aspect, the present invention relates to a method for the production of the above the vector based on rAAV5, which comprises the transfection of producer cells with the above nucleic acid encoding the above capsid.

Brief description of drawings

Fig. 1. Circular scheme of plasmid pAAV-linker intended for cloning the libraries of random variants of capsid gene of AAV serotype 5.

AmpR is a beta-lactamase gene that provides resistance to ampicillin,

pUC origin is a pUC replication origin in bacteria,

ITR is inverted terminal repeats,

CMV-Promoter is the promoter of cytomegalovirus early genes,

Poly A is a polyadenylation signal sequence, for increasing mRNA stability,

HBG Intron is human beta globine intron,

GFP is a green fluorescent protein gene,

T2A is a 2A self-cleaving peptide that allows co-expression of a target protein and reporter protein.

Fig. 2. Circular scheme of plasmid pAAV-Rep intended for producing recombinant viral products of wild-type AAV serotype 5 from the library of random variants.

AmpR is a beta-lactamase gene that provides resistance to ampicillin,

pUC origin is a pUC replication origin in bacteria,

Rep genes is a Rep gene sequence encoding AAV replication proteins.

Fig. 3. Circular scheme of plasmid pHelper intended for producing recombinant viral products of wild-type AAV serotype 5 from the library of random variants.

AmpR is a beta-lactamase gene that provides resistance to ampicillin,

Ori is a replication origin in bacteria,

Adeno E2A is a helper adenovirus gene sequence involved in viral DNA replication,

Adeno E4 is a helper adenovirus gene sequence involved in viral DNA replication,

Adeno VARNA is a helper adenovirus gene sequence responsible for the translation of both early and late viral genes.

Fig. 4. Analysis of efficiency of CHO-K1-S cell transduction with AAV5-GFP-based viral products, wherein VP1 protein of the wild-type AAV5 capsid includes one or more amino acid substitutions.

Fig. 5. Analysis of concentration of hFIX protein in the medium harvested from the CHO-K1-S cells 7 days post transduction with the AAV5-hFIX-based viral products, wherein VP1 protein of the wild-type AAV5 capsid includes one or more amino acid substitutions.

Fig. 6. Position of AAV5 capsid proteins in the genome.

2087-4258 bp – VP1

2495-4258 bp – VP2

2663-4258 bp – VP3

Description of invention

Definitions and general methods

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of ordinary skill in the art.

Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. Typically, the classification and methods of cell culture, molecular biology, immunology, microbiology, genetics, analytical chemistry, organic synthesis chemistry, medical and pharmaceutical chemistry, as well as hybridization and chemistry of protein and nucleic acids described herein are well known and widely used by those skilled in the art. Enzyme reactions and purification methods are performed according to the manufacturer's guidelines, as is common in the art, or as described herein.

"Isolated" means altered or removed from the natural state. For example, a nucleic acid or a peptide naturally present in an animal is not "isolated", but the same nucleic acid or peptide partially or completely separated from the coexisting materials of its natural state is "isolated". An isolated nucleic acid or protein can exist in substantially purified form, or can exist in a non-native environment such as, for example, a genetically modified cell.

The terms "naturally occurring," "native," or "wild-type" is used to describe an object that can be found in nature as distinct from being artificially produced. For example, a protein or nucleotide sequence present in an organism (including a virus), which can be isolated from a source in nature and that has not been intentionally modified by a person in the laboratory, is naturally occurring.

The term "genome" refers to the complete genetic material of an organism.

As used in the present description and claims that follow, unless otherwise dictated by the context, the words "include" and "comprise," or variations thereof such as "having," "includes", "including", "comprises," or "comprising," will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

Protein (Peptide)

As used in the present description, the terms "peptide", "polypeptide" and "protein" are used interchangeably, and they refer to a compound consisting of amino acid residues that are covalently linked by peptide bonds. A protein or peptide must contain at least two amino acids, and no limitation is placed on the maximum number of amino acids that can comprise a protein's or peptide's sequence. Polypeptides include any peptide or protein comprising two or more amino acids joined to each other by peptide bonds. As used in the present description, the term refers to both short chains, which also commonly are referred to in the art, for example, as peptides, oligopeptides and oligomers, and to longer chains, which generally are referred to in the art as proteins, of which there are many types. "Polypeptides" include, inter alia, for example, biologically active fragments, substantially homologous polypeptides, oligopeptides, homodimers, heterodimers, variants of polypeptides, modified polypeptides, derivatives, analogs, fusion proteins. The polypeptides include natural peptides, recombinant peptides, synthetic peptides, or a combination thereof.

The terms "transformation," "transfection," and "transduction" refer to any method or means by which a nucleic acid is introduced into a cell or host organism, and may be used

interchangeably to convey the same meaning. Such methods include, but are not limited to, transfection, electroporation, microinjection, infection, PEG-fusion, and the like.

Nucleic acid molecules

The terms "nucleic acid", "nucleic sequence", "nucleic acid sequence", "polynucleotide", "oligonucleotide", "polynucleotide sequence" and "nucleotide sequence", used interchangeably in the present description, mean a precise sequence of nucleotides, modified or not, determining a fragment or a region of a nucleic acid, containing unnatural nucleotides or not, and being either a double-stranded DNA or RNA, a single-stranded DNA or RNA, or transcription products of said DNAs.

One skilled in the art has the general knowledge that nucleic acids are polynucleotides that can be hydrolyzed to monomeric "nucleotides". Monomeric nucleotides can be hydrolyzed into nucleosides. As used in the present description, polynucleotides include, as non-limiting examples, all nucleic acid sequences which are obtained by any means available in the art, including, as non-limiting examples, recombinant means, i.e. the cloning of nucleic acid sequences from a recombinant library or a cell genome, using ordinary cloning technology and PCR and the like, and by synthetic means.

It should also be noted here that the present invention does not relate to nucleotide sequences in their natural chromosomal environment, i.e. in a natural state. The sequences of the present invention have been isolated and/or purified, i.e. they were sampled directly or indirectly, for example by a copy, their environment having been at least partially modified. Thus, isolated nucleic acids obtained by recombinant genetics, by means, for example, of host cells, or obtained by chemical synthesis should also be mentioned here.

An "isolated" nucleic acid molecule is one which is identified and separated from at least one nucleic acid molecule-impurity, which the former is typically bound to in the natural source of nucleic acid. An isolated nucleic acid molecule is different from the form or set in which it is found under natural conditions. Thus, an isolated nucleic acid molecule is different from a nucleic acid molecule that exists in cells under natural conditions. An isolated nucleic acid molecule however includes a nucleic acid molecule located in cells in which the nucleic acid is normally expressed, for example, if the nucleic acid molecule has a chromosomal localization that is different from its localization in cells under natural conditions.

Unless otherwise indicated, the term nucleotide sequence encompasses its complement. Thus, a nucleic acid having a particular sequence should be understood as one which encompasses the complementary strand thereof with the complementary sequence thereof.

Adeno-associated virus (AAV)

Viruses of the Parvoviridae family are small DNA-containing animal viruses. The Parvoviridae family may be divided into two subfamilies: the Parvovirinae, which members infect vertebrates, and the Densovirinae, which members infect insects. By 2006, there have been 11 serotypes of adeno-associated virus described (Mori, S. ET AL., 2004, "Two novel adeno-associated viruses from cynomolgus monkey: pseudotyping characterization of capsid protein", *Virology*, T. 330 (2): 375-83). All of the known serotypes can infect cells from multiple tissue types. Tissue specificity is determined by the capsid protein serotype; therefore, the adeno-associated virus-based vectors are constructed by assigning the desired serotype. Further information on parvoviruses and other members of the Parvoviridae is described in the literature (Kenneth I. Berns, "Parvoviridae: The Viruses and Their Replication", Chapter 69 in *Fields Virology* (3d Ed. 1996)).

The genomic organization of all known AAV serotypes is very similar. The genome of AAV is a linear, single-stranded DNA molecule that is less than about 5,000 nucleotides (nt) in length. Inverted terminal repeats (ITRs) flank the unique coding nucleotide sequences of replication of non-structural proteins (Rep) and structural proteins (Cap). The Cap gene encodes the VP proteins (the VP1, VP2, and VP3) which form the capsid. The terminal 145 nucleotides are self-complementary and are organized such that an energetically stable intramolecular duplex forming a T-shaped hairpin may be formed. Such hairpin structures function as an origin for virus DNA replication, serving as primers for the cellular DNA polymerase complex. Following wild-type AAV (wtAAV) infection in mammalian cells, the Rep genes (e.g. Rep78 and Rep52) are expressed using the P5 promoter and the P19 promoter, respectively, and the both Rep proteins have a certain function in the replication of the viral genome. A splicing event in the Rep open reading frame (Rep ORF) results in the expression of actually four Rep proteins (e.g. Rep78, Rep68, Rep52, and Rep40). However, it has been shown that the unspliced mRNA encoding Rep78 and Rep52 proteins is sufficient for AAV vector production in mammalian cells.

Recombinant adeno-associated virus (rAAV)-based vector

The term "vector" as used herein means a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked.

The term "recombinant AAV vector" (or "rAAV vector") as used in the present description refers to a vector comprising one or more polynucleotide sequences of interest, genes

of interest or "transgenes" that are flanked by parvoviral or inverted terminal repeat sequences (ITRs)

The terms "infection unit (iu)," "infectious particle," or "replication unit," as used in reference to a viral titer, refer to the number of infectious recombinant AAV vector particles as measured by the infectious center assay, also known as replication center assay, as described, for example, in McLaughlin et al., *J. Virol.* (1988) 62:1963-1973.

The term "heterologous" as it relates to nucleic acid sequences such as coding sequences and regulatory sequences, denotes sequences that are not normally joined together, and/or are not normally associated with a particular cell. Thus, a "heterologous" region of a nucleic acid construct or a vector is a fragment of nucleic acid within or attached to another nucleic acid molecule that is not found in association with the other molecule in nature. For example, a heterologous region of a nucleic acid construct may include a coding sequence flanked by sequences not found in association with the coding sequence in nature. Another example of a heterologous coding sequence is a construct where the coding sequence itself is not found in nature (e.g. synthetic sequences having codons different from the native gene).

As used herein, the term "operably linked" refers to a linkage of polynucleotide (or polypeptide) elements in a functional relationship. A nucleic acid is "operably linked" when it is present in functional relationship conditions with another nucleic acid sequence. For example, a transcription regulatory sequence is operably linked to a coding sequence if it affects the transcription of said coding sequence. The term "operably linked" means that the DNA sequences being linked are typically contiguous and, where it is necessary to join two protein coding regions, are also contiguous and are present in the reading frame.

As used in the present description, the term "promoter" or "transcription regulatory sequence" or "regulatory sequence" refers to a nucleic acid fragment that controls the transcription of one or more coding sequences, and that is located upstream with respect to the direction of reading relative to the direction of transcription from the transcription initiation site of the coding sequence, and is structurally identified by the presence of a binding site for DNA-dependent RNA polymerase, transcription initiation sites and any other DNA sequences, including, but not limited to transcription factor binding sites, repressor and activator protein binding sites, and any other sequences of nucleotides known to one of skill in the art that directly or indirectly regulate the level of transcription with said promoter. A "constitutive" promoter is a promoter that is active in most tissues under typical physiological and developmental conditions. An "inducible" promoter is a promoter that is physiologically or developmentally regulated, e.g.

under the influence of a chemical inducer. A "tissue specific" promoter is only active in specific types of tissues or cells.

The terms "enhancers" or "enhancer" as used herein can refer to a DNA sequence that is located adjacent to the DNA sequence that encodes a recombinant product. Enhancer elements are typically located in a 5' direction from a promoter element or can be located downstream of or within a coding DNA sequence (e.g. a DNA sequence transcribed or translated into a recombinant product or products). Hence, an enhancer element can be located 100 base pairs, 200 base pairs, or 300 or more base pairs upstream of a DNA sequence that encodes a recombinant product, or downstream of said sequence. Enhancer elements can increase the amount of a recombinant product being expressed from a DNA sequence above the level of expression associated with a single promoter element. Multiple enhancer elements are readily available to those of ordinary skill in the art.

The term "selectable marker gene" refers to a gene that when expressed confers a selectable phenotype, for example, antibiotic resistance, on a transformed cell.

As used in the present description, the term "expression" is defined as the transcription and/or translation of a particular nucleotide sequence driven by its promoter.

Use for treatment

"Gene therapy" is the insertion of genes into subject's cells and/or tissues to treat a disease, typically hereditary diseases, in which a defective mutant allele is replaced with a functional one.

"Treat", "treatment" and "therapy" refer to a method of alleviating or abrogating a biological disorder and/or at least one of attendant symptoms thereof. As used herein, to "alleviate" a disease, disorder or condition means reducing the severity and/or occurrence frequency of the symptoms of a disease, disorder, or condition. Further, references herein to "treatment" include references to curative, palliative and prophylactic treatment.

In one aspect, the subject of treatment, or patient, is a mammal, preferably a human subject. Said subject may be either male or female, of any age.

The term "disorder" means any condition that would benefit from treatment according to the present invention. This includes chronic and acute disorders or diseases including those pathological conditions that predispose the mammal to the disorder in question.

"Disease" is a state of health of an animal where the animal cannot maintain homeostasis, and where if the disease is not ameliorated then the animal's health continues to deteriorate.

The terms "subject," "patient," "individual," and the like are used interchangeably in the present description, and they refer to any animal amenable to the methods described in the present description. In certain non-limiting embodiments, the subject, patient or individual is a human.

"Therapeutically effective amount" refers to that amount of the therapeutic agent being administered during treatment which will relieve to some extent one or more of the symptoms of the disease being treated.

The term "chronic" use refers to continued (uninterrupted) use of agent(s) as opposed to acute (transient) route of administration so as to sustain the initial therapeutic effect (activity) for a long period of time.

"Intermittent" use refers to treatment that is not carried out consistently without interruptions, but which is rather periodic in nature.

Detailed description of invention

Isolated altered VP1 protein of adeno-associated virus serotype 5 (AAV5) capsid

In one aspect, the present invention relates to an isolated altered VP1 protein of adeno-associated virus serotype 5 (AAV5) capsid for highly-efficient transduction of target cells, comprising the amino acid sequence of a VP1 protein of the wild-type AAV5 capsid, which is encoded by the **Cap** gene, with one or more substitutions selected from the group comprising:

S651A,

S2A and T711S,

S2A, S651A, and T711S.

The amino acid S2A substitution is understood to mean the substitution of Serine (Ser, S) at position 2 of the VP1 protein of wild-type adeno-associated virus serotype 5 capsid for Alanine (Ala, A).

The amino acid S651A substitution is understood to mean the substitution of Serine (Ser, S) at position 651 of the VP1 protein of wild-type adeno-associated virus serotype 5 capsid for Alanine (Ala, A).

The amino acid T711S substitution is understood to mean the substitution of Threonine (Thr, T) at position 711 of the VP1 protein of wild-type adeno-associated virus serotype 5 capsid for Serine (Ser, S).

In some embodiments, the amino acid sequence of a VP1 protein of the wild-type AAV5 capsid has the amino acid sequence represented by MSFVDHPPDWLEEVGEGLEFLGLEAGPPKPKPNQQHQDQARGLVLPGYNYLGPGNGL DRGEPVNRADDEVAREHDISYNEQLEAGDNPYLKYNHADAEFQEKLADDTSFGGNLGK AVFQAKKRVLEPFGLVEEGAKTAPTGKRIDDHFPKRKKARTEEDSKPSTSSDAEAGPSG SQQQLQIPAQPASSLGADTMSAGGGGGLGDNNQGADGVGNASGDWHCDSTWMGDRVV TKSTRTWLPSYNNHQYREIKSGSVDGSNANAYFGYSTPWGYFDFNRFHSHWSPRDW QRLINNYWGFRPRSLRVKIFNIQVKEVTVQDSTTTIANNLTSTVQVFTDDDDYQLPYVVG NGTEGCLPAFPPQVFTLPQYGYATLNRDNTENPTERSSFFCLEYFPSKMLRTGNNFEFTY NFEEVPFHSSFAPSQNLFLKLANPLVDQYLYRFVSTNNTGGVQFNKNLAGRYANTYKNW FPGPMGRTQGWNLGSGVNRASVSFAFATTNRMELEGASYQVPPQPNGMTNNLQGSNTY ALENTMIFNSQPANPGTTATYLEGNMLITSESETQPVNRVAYNVGGQMATNNQSSTTAP ATGTYNLQEIVPGSVWMERDVYLQGPIWAKIPETGAHFHPSAMGGFGLKHPPPMMLI KNTVPVGNITSFSDVPVSSFITQYSTGQVTVEMEWELKKENSKRWNPEIQYTNNYNDPQ FVDFAPDSTGEYRTTRPIGTRYLTRPL (SEQ ID NO: 1).

In some embodiments, the isolated altered VP1 protein of the AAV5 capsid includes one substitution at S651A position.

In some embodiments, the isolated altered VP1 protein of the AAV5 capsid has the amino acid sequence represented by MSFVDHPPDWLEEVGEGLEFLGLEAGPPKPKPNQQHQDQARGLVLPGYNYLGPGNGL DRGEPVNRADDEVAREHDISYNEQLEAGDNPYLKYNHADAEFQEKLADDTSFGGNLGK AVFQAKKRVLEPFGLVEEGAKTAPTGKRIDDHFPKRKKARTEEDSKPSTSSDAEAGPSG SQQQLQIPAQPASSLGADTMSAGGGGGLGDNNQGADGVGNASGDWHCDSTWMGDRVV TKSTRTWLPSYNNHQYREIKSGSVDGSNANAYFGYSTPWGYFDFNRFHSHWSPRDW QRLINNYWGFRPRSLRVKIFNIQVKEVTVQDSTTTIANNLTSTVQVFTDDDDYQLPYVVG NGTEGCLPAFPPQVFTLPQYGYATLNRDNTENPTERSSFFCLEYFPSKMLRTGNNFEFTY NFEEVPFHSSFAPSQNLFLKLANPLVDQYLYRFVSTNNTGGVQFNKNLAGRYANTYKNW FPGPMGRTQGWNLGSGVNRASVSFAFATTNRMELEGASYQVPPQPNGMTNNLQGSNTY ALENTMIFNSQPANPGTTATYLEGNMLITSESETQPVNRVAYNVGGQMATNNQSSTTAP ATGTYNLQEIVPGSVWMERDVYLQGPIWAKIPETGAHFHPSAMGGFGLKHPPPMMLI KNTVPVGNITSFADVPVSSFITQYSTGQVTVEMEWELKKENSKRWNPEIQYTNNYNDPQ FVDFAPDSTGEYRTTRPIGTRYLTRPL (SEQ ID NO: 2).

In some embodiments, the isolated altered VP1 protein of the AAV5 capsid includes the S2A and T711S substitutions.

In some embodiments, the isolated altered VP1 protein of the AAV5 capsid has the amino acid sequence represented by

MAFVDHPPDWLEEVGEGREFLGLEAGPPKPKPNQQHQDQARGLVLPGYNYLGPGNG
 LDRGEPVNRADDEVAREHDISYNEQLEAGDNPYLKYNHADADEFQEKLADDTSFGGNLGK
 AVFQAKKRVLEPFGLVEEGAKTAPTGKRIDDHFPKRKKARTEEDSKPSTSSDAEAGPSG
 SQQQLQIPAQPASSLGADTMSAGGGGGLGDNNQGADGVGNASGDWHCDSTWMGDRVV
 TKSTRTWLPSYNNHQYREIKSGSVDGSNANAYFGYSTPWGYFDFNRFHSHWSPRDW
 QRLINNYWGFRPRSLRVKIFNIQVKEVTVQDSTTTIANNTSTVQVFTDDDDYQLPYVVG
 NGTEGCLPAFPQVFTLPQYGYATLNRDNTENPTERSSFFCLEYFPSKMLRTGNNFEFTY
 NFEEVPFHSSFAPSQNLFLKLANPLVDQYLYRFVSTNNTGGVQFNKNLAGRYANTYKNW
 FPGPMGRTQGWNLGSGVNRASVSFAFATTNRMELEGASYQVPPQPNGMTNNLQGSNTY
 ALENTMIFNSQPANPGTTATYLEGNMLITSESETQPVNRVAYNVGGQMATNNQSSTTAP
 ATGTYNLQEIVPGSVWMERDVYLQGPIWAKIPETGAHFHPSPAMGGFGLKHPPPMMLI
 KNTVPVGNITSFSDVPVSSFITQYSTGQVTVEMEWELKKENSKRWNPEIQYTNNYNDPQ
 FVDFAPDSTGEYRSTRPIGTRYLTRPL (SEQ ID NO: 3).

In some embodiments, the isolated altered VP1 protein of the AAV5 capsid includes the S2A, S651A, and T711S substitutions.

In some embodiments, the isolated altered VP1 protein of the AAV5 capsid has the amino acid sequence represented by

MAFVDHPPDWLEEVGEGREFLGLEAGPPKPKPNQQHQDQARGLVLPGYNYLGPGNG
 LDRGEPVNRADDEVAREHDISYNEQLEAGDNPYLKYNHADADEFQEKLADDTSFGGNLGK
 AVFQAKKRVLEPFGLVEEGAKTAPTGKRIDDHFPKRKKARTEEDSKPSTSSDAEAGPSG
 SQQQLQIPAQPASSLGADTMSAGGGGGLGDNNQGADGVGNASGDWHCDSTWMGDRVV
 TKSTRTWLPSYNNHQYREIKSGSVDGSNANAYFGYSTPWGYFDFNRFHSHWSPRDW
 QRLINNYWGFRPRSLRVKIFNIQVKEVTVQDSTTTIANNTSTVQVFTDDDDYQLPYVVG
 NGTEGCLPAFPQVFTLPQYGYATLNRDNTENPTERSSFFCLEYFPSKMLRTGNNFEFTY
 NFEEVPFHSSFAPSQNLFLKLANPLVDQYLYRFVSTNNTGGVQFNKNLAGRYANTYKNW
 FPGPMGRTQGWNLGSGVNRASVSFAFATTNRMELEGASYQVPPQPNGMTNNLQGSNTY
 ALENTMIFNSQPANPGTTATYLEGNMLITSESETQPVNRVAYNVGGQMATNNQSSTTAP
 ATGTYNLQEIVPGSVWMERDVYLQGPIWAKIPETGAHFHPSPAMGGFGLKHPPPMMLI
 KNTVPVGNITSFADVPVSSFITQYSTGQVTVEMEWELKKENSKRWNPEIQYTNNYNDPQ
 FVDFAPDSTGEYRSTRPIGTRYLTRPL (SEQ ID NO: 4).

Isolated altered VP2 and VP3 proteins of adeno-associated virus serotype 5 (AAV5) capsid

The "right side" of a (+)-chain of genomic DNA of adeno-associated virus comprises overlapping sequences encoding three capsid proteins, VP1, VP2 and VP3. Transcription of these genes starts from one promoter, p40. The molecular weights of the corresponding proteins are 87, 72, and 62 kDa, respectively. All of the three proteins are translated from a single mRNA. After transcription, pre-mRNA can be spliced in two different manners, where either longer or shorter intron is excised to form mRNAs of 2300 or 2600 nucleotide long.

Thus, the introduction of mutations into the *Cas* gene will affect not only the VP1 protein of AAV5 capsid, but also the VP2 and VP3 proteins of AAV5 capsid.

Figure 6 is a schematic representation of the position of the AAV5 capsid proteins in AAV genome:

2087-4258 bp – VP1

2495-4258 bp – VP2

2663-4258 bp – VP3.

It follows from the above that a mutation similar to the mutation S2A in VP1 will be absent in VP2 and VP3, whereas mutations similar to the mutations S651A and/or T711S in VP1 will be present in both VP2 and VP3.

With the consideration of an overlapping sequence encoding the three capsid proteins, VP1, VP2 and VP3, the amino acid substitution S651A in VP1 will correspond to:

the amino acid substitution at position S515A in VP2;

the amino acid substitution at position S459A in VP3.

With the consideration of an overlapping sequence encoding the three capsid proteins, VP1, VP2 and VP3, the amino acid T711S substitution will correspond to:

the amino acid substitution at position T575S in VP2;

the amino acid substitution at position T519S in VP3.

Further, the applicant considers it appropriate to specify the environment of the mutations that have been found, by indicating a short amino acid sequence including said mutations in VP1/VP2/VP3:

For S2A in VP1 (not present in VP2 and VP3) – M**S**FDHP;

For S651A in VP1 (S515A in VP2/ S459A in VP3) – TSF**S**DVP;

For T711S in VP1 (T575S in VP2/ T519S in VP3) – EYR**T**TRP.

In some embodiments, the amino acid sequence of the VP2 protein of the wild-type AAV5 capsid has the amino acid sequence represented by

TAPTGKRIDDHFPKRKKARTEEDSKPSTSSDAEAGPSGSQQQLQIPAQPASSLGA
DTMSAGGGGGLGDNNQGADGVGNASGDWHCDSTWMGDRVVTKSTRTWVLPSYN
NHQYREIKSGSVDGSNANAYFGYSTPWGYFDFNRFHSHWSPRDWQRLINNYWGFR
PRSLRVKIFNIQVKEVTVDSTTTIANNTSTVQVFTDDDDYQLPYVVGNGTEGCLPAF
PPQVFTLPQYGYATLNRDNTENPTERSSFFCLEYFPSKMLRTGNNFEFTYNFEEVPFH
SSFAPSQNLFKLANPLVDQYLYRFVSTNNTGGVQFNKNLAGRYANTYKNWFPGPM
GRTQGWNLGSGVNRASVSASFATTNRMELEGASYQVPPQPNGMTNNLQGSNTYALE
NTMIFNSQPANPGTTATYLEGNMLITSESETQPVNRVAYNVGGQMATNNQSSTTAP
ATGTYNLQEIVPGSVWMERDVYLQGPIWAKIPETGAHFHPSPAMGGFGLKHPPPM
LIKNTVPVGNITSFSDVPVSSFITQYSTGQVTVEMEWELKKENSKRWNPEIQYTNNYN
DPQFVDFAPDSTGEYRTRPIGTRYLTRPL (SEQ ID NO: 8)

In some embodiments, the isolated altered VP2 protein of the AAV5 capsid includes the T575S substitution.

In some embodiments, the isolated altered VP2 protein of the AAV5 capsid has the amino acid sequence represented by

TAPTGKRIDDHFPKRKKARTEEDSKPSTSSDAEAGPSGSQQQLQIPAQPASSLGA
DTMSAGGGGGLGDNNQGADGVGNASGDWHCDSTWMGDRVVTKSTRTWVLPSYN
NHQYREIKSGSVDGSNANAYFGYSTPWGYFDFNRFHSHWSPRDWQRLINNYWGFR
PRSLRVKIFNIQVKEVTVDSTTTIANNTSTVQVFTDDDDYQLPYVVGNGTEGCLPAF
PPQVFTLPQYGYATLNRDNTENPTERSSFFCLEYFPSKMLRTGNNFEFTYNFEEVPFH
SSFAPSQNLFKLANPLVDQYLYRFVSTNNTGGVQFNKNLAGRYANTYKNWFPGPM
GRTQGWNLGSGVNRASVSASFATTNRMELEGASYQVPPQPNGMTNNLQGSNTYALE
NTMIFNSQPANPGTTATYLEGNMLITSESETQPVNRVAYNVGGQMATNNQSSTTAP
ATGTYNLQEIVPGSVWMERDVYLQGPIWAKIPETGAHFHPSPAMGGFGLKHPPPM
LIKNTVPVGNITSFSDVPVSSFITQYSTGQVTVEMEWELKKENSKRWNPEIQYTNNYN
DPQFVDFAPDSTGEYRSTRPIGTRYLTRPL (SEQ ID NO: 9)

In some embodiments, the isolated altered VP2 protein of the AAV5 capsid includes the S515A and T575S substitutions.

In some embodiments, the isolated altered VP2 protein of the AAV5 capsid has the amino acid sequence represented by

TAPTGKRIDDHFPKRKKARTEEDSKPSTSSDAEAGPSGSQQQLQIPAQPASSLGA
DTMSAGGGGGLGDNNQGADGVGNASGDWHCDSTWMGDRVVTKSTRTWVLPSYN

NHQYREIKSGSVDGSNANAYFGYSTPWGYFDFNRFHSHWSPRDWQRLINNYWGFR
PRSLRVKIFNIQVKEVTVQDSTTTIANNLTSTVQVFTDDDDYQLPYVVGNGTEGCLPAF
PPQVFTLPQYGYATLNRDNTENPTERSSFFCLEYFPSKMLRTGNNFEFTYNFEEVPPFH
SSFAPSQNLFLKLANPLVDQYLYRFVSTNNTGGVQFNKNLAGRYANTYKNWFPGPM
GRTQGWNLGSGVNRASVSAFATTNRMELEGASYQVPPQPNGMTNNLQGSNTYALE
NTMIFNSQPANPGTTATYLEGNMLITSESETQPVNRVAYNVGGQMATNNQSSTTAP
ATGTYNLQEIVPGSVWMERDVYLQGPIWAKIPETGAHFHPSPAMGGFGLKHPPPM
LIKNTVPVGNITSFADVPVSSFITQYSTGQVTVEMEWELKKENSKRWNPEIQYTNNY
NDPQFVDFAPDSTGEYRSTRPIGTRYLTRPL (SEQ ID NO: 10)

In some embodiments, the amino acid sequence of the VP3 protein of the wild-type AAV5 capsid has the amino acid sequence represented by

MSAGGGGPLGDNNQGADGVGNASGDWHCDSTWMGDRVVTKSTRTWVLPSYN
NHQYREIKSGSVDGSNANAYFGYSTPWGYFDFNRFHSHWSPRDWQRLINNYWGFRPRS
LRVKIFNIQVKEVTVQDSTTTIANNLTSTVQVFTDDDDYQLPYVVGNGTEGCLPAFPPQVF
TLPQYGYATLNRDNTENPTERSSFFCLEYFPSKMLRTGNNFEFTYNFEEVPPFHSSFAPSQ
NLFLKLANPLVDQYLYRFVSTNNTGGVQFNKNLAGRYANTYKNWFPGPMGRTQGWNL
GSGVNRASVSAFATTNRMELEGASYQVPPQPNGMTNNLQGSNTYALENTMIFNSQPAN
PGTTATYLEGNMLITSESETQPVNRVAYNVGGQMATNNQSSTTAPATGTYNLQEIVPGS
VWMERDVYLQGPIWAKIPETGAHFHPSPAMGGFGLKHPPPMMLIKNTVPVGNITSFSDV
PVSSFITQYSTGQVTVEMEWELKKENSKRWNPEIQYTNNYNDPQFVDFAPDSTGEYRTT
RPIGTRYLTRPL (SEQ ID NO: 11)

In some embodiments, the isolated altered VP3 protein of the AAV5 capsid includes the T519S substitution.

In some embodiments, the isolated altered VP3 protein of the AAV5 capsid has the amino acid sequence represented by

MSAGGGGPLGDNNQGADGVGNASGDWHCDSTWMGDRVVTKSTRTWVLPS
YNNHQYREIKSGSVDGSNANAYFGYSTPWGYFDFNRFHSHWSPRDWQRLINNYWG
FRPRSLRVKIFNIQVKEVTVQDSTTTIANNLTSTVQVFTDDDDYQLPYVVGNGTEGCLP
AFPPQVFTLPQYGYATLNRDNTENPTERSSFFCLEYFPSKMLRTGNNFEFTYNFEEVP
FHSSFAPSQNLFLKLANPLVDQYLYRFVSTNNTGGVQFNKNLAGRYANTYKNWFPGP
MGRTQGWNLGSGVNRASVSAFATTNRMELEGASYQVPPQPNGMTNNLQGSNTYAL
ENTMIFNSQPANPGTTATYLEGNMLITSESETQPVNRVAYNVGGQMATNNQSSTTAP
ATGTYNLQEIVPGSVWMERDVYLQGPIWAKIPETGAHFHPSPAMGGFGLKHPPPM

LIKNTVPVGNITSFSDVPVSSFITQYSTGQVTVEMEWELKKENSKRWNPEIQYTNNYN
DPQFVDFAPDSTGEYRSTRPIGTRYLTRPL (SEQ ID NO: 12)

In some embodiments, the isolated altered VP3 protein of the AAV5 capsid includes the S459A and T519S substitutions.

In some embodiments, the isolated altered VP3 protein of the AAV5 capsid has the amino acid sequence represented by

MSAGGGGPLGDNNQGADGVGNASGDWHCDSTWMGDRVVTKSTRTWVLPS
YNNHQYREIKSGSVDGSNANAYFGYSTPWGYFDFNRFHSHWSPRDWQRLINNYWG
FRPRSLRVKIFNIQVKEVTVQDSTTTIANNLSTVQVFTDDDDYQLPYVVGNNGTEGCLP
AFPPQVFTLPQYGYATLNRDNTENPTERSSFFCLEYFPSKMLRTGNNFEFTYNFEEVP
FHSSFAPSQNLFKLANPLVDQYLYRFVSTNNTGGVQFNKNLAGRYANTYKNWFPGP
MGRTQGWNLGSGVNRASVSAFATTNRMELEGASYQVPPQPNGMTNNLQGSNTYAL
ENTMIFNSQPANPGTTATYLEGNMLITSESETQPVNRVAYNVGGQMATNNQSSTTAP
ATGTYNLQEIVPGSVWMERDVYLQGPIWAKIPETGAHFHPSAMGGFGLKHPPPM
LIKNTVPVGNITSFADVPVSSFITQYSTGQVTVEMEWELKKENSKRWNPEIQYTNNY
NDPQFVDFAPDSTGEYRSTRPIGTRYLTRPL (SEQ ID NO: 13)

Capsid

In one aspect, the present invention relates to an isolated capsid for highly-efficient transduction of target cells, which includes the above altered VP1 protein of adeno-associated virus serotype 5 (AAV5) capsid.

In one embodiment, the isolated capsid includes the above altered VP1 protein of adeno-associated virus serotype 5 (AAV5) capsid, a VP2 protein of AAV5 capsid or an altered variant thereof, and a VP3 protein of AAV5 capsid or an altered variant thereof.

Particularly preferred embodiments include substitutions that are conservative in nature, i.e. substitutions which take place within a family of amino acids that are joined in their side chains. In particular, amino acids are typically divided into four families: (1) acidic amino acids are aspartate and glutamate; (2) basic amino acids are lysine, arginine, histidine; (3) non-polar amino acids are alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan, and (4) uncharged polar amino acids are glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine. Phenylalanine, tryptophan, and tyrosine are sometimes classified as aromatic amino acids. For example, it is reasonably predictable that an isolated substitution of leucine for isoleucine or valine, an aspartate for a glutamate, a threonine for a serine, or a similar

conservative substitution of an amino acid for a structurally related amino acid, will not have a major effect on the biological activity. For example, the polypeptide of interest may include up to about 5-10 conservative or non-conservative amino acid substitutions, or even up to about 15-25 or 50 conservative or non-conservative amino acid substitutions, or any integer between 5-50, so long as the desired function of the molecule remains intact.

In one embodiment, the isolated capsid includes a VP2 protein of the wild-type AAV5 capsid.

In one embodiment, the isolated capsid includes the VP2 protein of the wild-type AAV5 capsid, which has the amino acid sequence represented by SEQ ID NO: 8.

In one embodiment, the isolated capsid includes the altered VP2 protein of adeno-associated virus serotype 5 (AAV5) capsid.

In one embodiment, the isolated capsid includes the altered VP2 protein of AAV5 capsid, which comprises the T575S substitution.

In one embodiment, the isolated capsid includes the altered VP2 protein of AAV5 capsid, which comprises the T575S substitution, and has the amino acid sequence represented by SEQ ID NO: 9.

In one embodiment, the isolated capsid includes the altered VP2 protein of AAV5 capsid, which comprises the S515A and T575S substitutions.

In one embodiment, the isolated capsid includes the altered VP2 protein of AAV5 capsid, which comprises the S515A and T575S substitutions, and has the amino acid sequence represented by SEQ ID NO: 10.

In one embodiment, the isolated capsid includes a VP3 protein of the wild-type AAV5 capsid.

In one embodiment, the isolated capsid includes the VP3 protein of the wild-type AAV5 capsid, which has the amino acid sequence represented by SEQ ID NO: 11.

In one embodiment, the isolated capsid includes an altered VP3 protein of adeno-associated virus serotype 5 (AAV5) capsid.

In one embodiment, the isolated capsid includes an altered VP3 protein of AAV5 capsid, which comprises the T519S substitution.

In one embodiment, the isolated capsid includes an altered VP3 protein of AAV5 capsid, which comprises the T519S substitution, and has the amino acid sequence represented by SEQ ID NO: 12.

In one embodiment, the isolated capsid includes an altered VP3 protein of AAV5 capsid, which comprises the S459A and T519S substitutions.

In one embodiment, the isolated capsid includes an altered VP3 protein of AAV5 capsid, which comprises the S459A and T519S substitutions, and has the amino acid sequence represented by SEQ ID NO: 13.

Isolated nucleic acid

In one aspect, the present invention relates to an isolated nucleic acid encoding the above altered VP1 protein of adeno-associated virus serotype 5 (AAV5) capsid, which is used for highly efficient transduction of target cells.

In some embodiments, the isolated nucleic acid encoding the altered VP1 protein of adeno-associated virus serotype 5 (AAV5) capsid with the amino acid S651A substitution is represented by the nucleic acid sequence

ATGTCTTTTGGTTGATCACCTCCAGATTGGTTGGAAGAAGTTGGTGAAGGTCTTCGC
GAGTTTTTTGGGCCTTGAAGCGGGCCACCGAAACCAAAACCCAATCAGCAGCATCA
AGATCAAGCCCGTGGTCTTGTGCTGCCTGGTTATAACTATCTCGGACCCGGAAACGG
TCTCGATCGAGGAGAGCCTGTCAACAGGGCAGACGAGGTCGCGCGAGAGCACGACA
TCTCGTACAACGAGCAGCTTGAGGCGGGAGACAACCCCTACCTCAAGTACAACCAC
GCGGACGCCGAGTTTCAGGAGAAGCTCGCCGACGACACATCCTTCGGGGGAAACCT
CGGAAAGGCAGTCTTTCAGGCCAAGAAAAGGGTTCTCGAACCTTTTGGCCTGGTTGA
AGAGGGTGCTAAGACGGCCCCTACCGGAAAGCGGATAGACGACCACTTTCCAAAAA
GAAAGAAGGCTCGGACCGAAGAGGACTCCAAGCCTTCCACCTCGTCAGACGCCGAA
GCTGGACCCAGCGGATCCCAGCAGCTGCAAATCCCAGCCCAACCAGCCTCAAGTTT
GGGAGCTGATACAATGTCTGCGGGAGGTGGCGGGCCATTGGGCGACAATAACCAAG
GTGCCGATGGAGTGGGCAATGCCTCGGGAGATTGGCATTGCGATTCCACGTGGATG
GGGGACAGAGTCGTCACCAAGTCCACCCGAACCTGGGTGCTGCCCAGCTACAACAA
CCACCAGTACCGAGAGATCAAAAGCGGCTCCGTCGACGGAAGCAACGCCAACGCCT
ACTTTGGATACAGCACCCCCTGGGGGTACTTTGACTTTAACCGCTTCCACAGCCACT
GGAGCCCCCGAGACTGGCAAAGACTCATCAACAATACTGGGGCTTCAGACCCCGG
TCCCTCAGAGTCAAAATCTTCAACATTCAAGTCAAAGAGGTCACGGTGCAGGACTCC
ACCACCACCATCGCCAACAACCTCACCTCCACCGTCCAAGTGTTTACGGACGACGAC

TACCAGCTGCCCTACGTCGTCGGCAACGGGACCGAGGGATGCCTGCCGGCCTTCCCT
 CCGCAGGTCTTTACGCTGCCGCAGTACGGTTACGCGACGCTGAACCGCGACAACAC
 AGAAAATCCCACCGAGAGGAGCAGCTTCTTCTGCCTAGAGTACTTTCCCAGCAAGAT
 GCTGAGAACGGGCAACAACCTTTGAGTTTACCTACAACCTTTGAGGAGGTGCCCTTCCA
 CTCCAGCTTCGCTCCCAGTCAGAACCTGTTCAAGCTGGCCAACCCGCTGGTGGACCA
 GTACTTGTACCGCTTCGTGAGCACAAATAACACTGGCGGAGTCCAGTTCAACAAGA
 ACCTGGCCGGGAGATACGCCAACACCTACAAAACTGGTTCCCGGGGGCCCATGGGC
 CGAACCCAGGGCTGGAACCTGGGCTCCGGGGTCAACCGCGCCAGTGTGAGCGCCTT
 CGCCACGACCAATAGGATGGAGCTCGAGGGCGCGAGTTACCAGGTGCCCCCGCAGC
 CGAACGGCATGACCAACAACCTCCAGGGCAGCAACACCTATGCCCTGGAGAACACT
 ATGATCTTCAACAGCCAGCCGGCGAACCCGGGCACCACCGCCACGTACCTCGAGGG
 CAACATGCTCATCACCAGCGAGAGCGAGACGCAGCCGGTGAACCGCGTGGCGTACA
 ACGTCGGCGGGCAGATGGCCACCAACAACCAGAGCTCCACCACTGCCCCCGCGACC
 GGCACGTACAACCTCCAGGAAATCGTGCCCGGCAGCGTGTGGATGGAGAGGGACGT
 GTACCTCCAAGGACCCATCTGGGCCAAGATCCCAGAGACGGGGGGCGCACTTTCACC
 CCTCTCCGGCCATGGGCGGATTCGGACTCAAACACCCACCGCCCATGATGCTCATCA
 AGAACACGCCTGTGCCCGGAAATATCACCAGCTTCgCGGACGTGCCCGTCAGCAGCT
 TCATCACCCAGTACAGCACCGGGCAGGTACCGTGGAGATGGAGTGGGAGCTCAAG
 AAGGAAAACCTCCAAGAGGTGGAACCCAGAGATCCAGTACACAAACAACACTACAACG
 ACCCCCAGTTTGTGGACTTTGCCCCGGACAGCACCGGGGAATACAGAACCACCAGA
 CCTATCGGAACCCGATACCTTACCCGACCCCTTTAA (SEQ ID NO: 5) or by any other
 sequence encoding the corresponding amino acid sequence of the altered protein of adeno-
 associated virus serotype 5 (AAV5) capsid with the amino acid S651A substitution.

"Other sequence encoding the corresponding amino acid sequence of the altered VP1 protein of adeno-associated virus serotype 5 (AAV5) capsid with the amino acid S651A substitution" means a nucleic sequence that is alternative to the nucleic sequence with SEQ ID NO: 5, as, due to the degeneracy of genetic code, a wide range of different DNA sequences can encode the amino acid sequence disclosed herein as SEQ ID NO: 2. It is well within the skill of a person trained in the art to create these alternative DNA sequences encoding the same amino acid sequences. Such variant DNA sequences are within the scope of the present invention.

In some embodiments, the isolated nucleic acid encoding the altered VP1 protein of adeno-associated virus serotype 5 (AAV5) capsid with the amino acid S2A and T711S substitutions, which is represented by the nucleic sequence

ATGGCTTTTGTTGATCACCTCCAGATTGGTTGGAAGAAGTTGGTGAAGGTCTTCGC
GAGTTTTTGGGCCTTGAAGCGGGCCACCGAAACCAAAACCCAATCAGCAGCATCA
AGATCAAGCCCGTGGTCTTGTGCTGCCTGGTTATAACTATCTCGGACCCGGAAACGG
TCTCGATCGAGGAGAGCCTGTCAACAGGGCAGACGAGGTCGCGCGAGAGCACGACA
TCTCGTACAACGAGCAGCTTGAGGCGGGAGACAACCCCTACCTCAAGTACAACCAC
GCGGACGCCGAGTTTCAGGAGAAGCTCGCCGACGACACATCCTTCGGGGGAAACCT
CGGAAAGGCAGTCTTTCAGGCCAAGAAAAGGGTTCTCGAACCTTTTGGCCTGGTTGA
AGAGGGTGCTAAGACGGCCCCTACCGGAAAGCGGATAGACGACCACTTTCCAAAAA
GAAAGAAGGCTCGGACCGAAGAGGACTCCAAGCCTTCCACCTCGTCAGACGCCGAA
GCTGGACCCAGCGGATCCCAGCAGCTGCAAATCCCAGCCCAACCAGCCTCAAGTTT
GGGAGCTGATACAATGTCTGCGGGAGGTGGCGGCCCATTTGGGCGACAATAACCAAG
GTGCCGATGGAGTGGGCAATGCCTCGGGAGATTGGCATTGCGATTCCACGTGGATG
GGGGACAGAGTCGTCACCAAGTCCACCCGAACCTGGGTGCTGCCCAGCTACAACAA
CCACCAGTACCGAGAGATCAAAAGCGGCTCCGTCGACGGAAGCAACGCCAACGCCT
ACTTTGGATACAGCACCCCCTGGGGGTACTTTGACTTTAACCGCTTCCACAGCCACT
GGAGCCCCCGAGACTGGCAAAGACTCATCAACAATACTACTGGGGCTTCAGACCCCGG
TCCCTCAGAGTCAAAATCTTCAACATTCAAGTCAAAGAGGTCACGGTGCAGGACTCC
ACCACCACCATCGCCAACAACCTCACCTCCACCGTCCAAGTGTTTACGGACGACGAC
TACCAGCTGCCCTACGTCGTCGGCAACGGGACCGAGGGATGCCTGCCGGCCTTCCCT
CCGCAGGTCTTTACGCTGCCGCAGTACGGTTACGCGACGCTGAACCGCGACAACAC
AGAAAATCCCACCGAGAGGAGCAGCTTCTTCTGCCTAGAGTACTTTCCCAGCAAGAT
GCTGAGAACGGGCAACAACCTTTGAGTTTACCTACAACCTTTGAGGAGGTGCCCTTCCA
CTCCAGCTTCGCTCCCAGTCAGAACCTCTTCAAGCTGGCCAACCCGCTGGTGGACCA
GTA CT TGTACCGCTTCGTGAGCACAAATAACACTGGCGGAGTCCAGTTCAACAAGA
ACCTGGCCGGGAGATACGCCAACACCTACAAAAACTGGTTCCCGGGGGCCCATGGGC
CGAACCCAGGGCTGGAACCTGGGCTCCGGGGGTCAACCGCGCCAGTGTCAGCGCCTT
CGCCACGACCAATAGGATGGAGCTCGAGGGCGCGAGTTACCAGGTGCCCCCGCAGC
CGAACGGCATGACCAACAACCTCCAGGGCAGCAACACCTATGCCCTGGAGAACACT
ATGATCTTCAACAGCCAGCCGGCGAACCCGGGCACCACCGCCACGTACCTCGAGGG
CAACATGCTCATCACCAGCGAGAGCGAGACGCAGCCGGTGAACCGCGTGGCGTACA
ACGTCGGCGGGCAGATGGCCACCAACAACCAGAGCTCCACCACTGCCCCCGCGACC
GGCACGTACAACCTCCAGGAAATCGTGCCCGGCAGCGTGTGGATGGAGAGGGACGT
GTACCTCCAAGGACCCATCTGGGCCAAGATCCCAGAGACGGGGGGCGCACTTTCACC
CCTCTCCGGCCATGGGCGGATTCGGACTCAAACACCCACCGCCCATGATGCTCATCA
AGAACACGCCTGTGCCCGGAAATATCACCAGCTTCTCGGACGTGCCCGTCAGCAGCT

TCATCACCCAGTACAGCACCGGGCAGGTCACCGTGGAGATGGAGTGGGAGCTCAAG
AAGGAAAACCTCCAAGAGGTGGAACCCAGAGATCCAGTACACAAACAACCTACAACG
ACCCCCAGTTTGTGGACTTTGCCCCGGACAGCACCGGGGAATACAGAAGCACCAGA
CCTATCGGAACCCGATACCTTACCCGACCCCTTTAA (SEQ ID NO: 6) or by any other
sequence encoding the corresponding amino acid sequence of the altered VP1 protein of adeno-
associated virus serotype 5 (AAV5) capsid with the amino acid S2A and T711S substitutions.

"Other sequence encoding the corresponding amino acid sequence of the altered
VP1 protein of adeno-associated virus serotype 5 (AAV5) capsid with the amino acid S2A
and T711S substitutions" means a nucleic sequence that is alternative to the nucleic
sequence with SEQ ID NO: 6, as, due to the degeneracy of genetic code, a wide range of
different DNA sequences can encode the amino acid sequence disclosed herein as SEQ ID
NO: 3. It is well within the skill of a person trained in the art to create these alternative
DNA sequences encoding the same amino acid sequences. Such variant DNA sequences are
within the scope of the present invention.

In some embodiments, the isolated nucleic acid encoding the altered VP1 protein of
adeno-associated virus serotype 5 (AAV5) capsid with the amino acid S2A, S651A and T711S
substitutions, which is represented by the nucleic sequence
ATGGCTTTTGTGATCACCTCCAGATTGGTTGGAAGAAGTTGGTGAAGGTCTTCGC
GAGTTTTTGGGCCTTGAAGCGGGCCACCGAAACCAAAACCCAATCAGCAGCATCA
AGATCAAGCCCGTGGTCTTGTGCTGCCTGGTTATAACTATCTCGGACCCGGAAACGG
TCTCGATCGAGGAGAGCCTGTCAACAGGGCAGACGAGGTCGCGCGAGAGCACGACA
TCTCGTACAACGAGCAGCTTGAGGCGGGAGACAACCCCTACCTCAAGTACAACCAC
GCGGACGCCGAGTTTCAGGAGAAGCTCGCCGACGACACATCCTTCGGGGGAAACCT
CGGAAAGGCAGTCTTTCAGGCCAAGAAAAGGGTTCTCGAACCTTTTGGCCTGGTTGA
AGAGGGTGCTAAGACGGCCCCTACCGGAAAGCGGATAGACGACCACTTTCCAAAAA
GAAAGAAGGCTCGGACCGAAGAGGACTCCAAGCCTTCCACCTCGTCAGACGCCGAA
GCTGGACCCAGCGGATCCCAGCAGCTGCAAATCCCAGCCCAACCAGCCTCAAGTTT
GGGAGCTGATACAATGTCTGCGGGAGGTGGCGGCCCATTGGGCGACAATAACCAAG
GTGCCGATGGAGTGGGCAATGCCTCGGGAGATTGGCATTGCGATTCCACGTGGATG
GGGGACAGAGTCGTCACCAAGTCCACCCGAACCTGGGTGCTGCCAGCTACAACAA
CCACCAGTACCGAGAGATCAAAAGCGGCTCCGTCGACGGAAGCAACGCCAACGCCT
ACTTTGGATACAGCACCCCCTGGGGGTACTTTGACTTTAACCGCTTCCACAGCCACT
GGAGCCCCCGAGACTGGCAAAGACTCATCAACAATACTGGGGCTTCAGACCCCGG
TCCCTCAGAGTCAAAATCTTCAACATTCAAGTCAAAGAGGTCACGGTGCAGGACTCC

ACCACCACCATCGCCAACAACCTCACCTCCACCGTCCAAGTGTTTACGGACGACGAC
TACCAGCTGCCCTACGTGCTCGGCAACGGGACCGAGGGATGCCTGCCGGCCTTCCCT
CCGCAGGTCTTTACGCTGCCGCAGTACGGTTACGCGACGCTGAACCGCGACAACAC
AGAAAATCCCACCGAGAGGAGCAGCTTCTTCTGCCTAGAGTACTTTCCCAGCAAGAT
GCTGAGAACGGGCAACAACCTTTGAGTTTACCTACAACCTTTGAGGAGGTGCCCTTCCA
CTCCAGCTTCGCTCCCAGTCAGAACCTCTTCAAGCTGGCCAACCCGCTGGTGGACCA
GTACTTGTACCGCTTCGTGAGCACAAATAACACTGGCGGAGTCCAGTTCAACAAGA
ACCTGGCCGGGAGATACGCCAACACCTACAAAACTGGTTCCCGGGGGCCCATGGGC
CGAACCCAGGGCTGGAACCTGGGCTCCGGGGTCAACCGCGCCAGTGTGAGCGCCTT
CGCCACGACCAATAGGATGGAGCTCGAGGGCGCGAGTTACCAGGTGCCCCCGCAGC
CGAACGGCATGACCAACAACCTCCAGGGCAGCAACACCTATGCCCTGGAGAACACT
ATGATCTTCAACAGCCAGCCGGGCGAACCCGGGCACCAACCGCCACGTACCTCGAGGG
CAACATGCTCATCACCAGCGAGAGCGAGACGCAGCCGGTGAACCGCGTGGCGTACA
ACGTCGGCGGGCAGATGGCCACCAACAACCAGAGCTCCACCACTGCCCCCGCGACC
GGCACGTACAACCTCCAGGAAATCGTGCCCGGCAGCGTGTGGATGGAGAGGGACGT
GTACCTCCAAGGACCCATCTGGGCCAAGATCCCAGAGACGGGGGGCGCACTTTTACC
CCTCTCCGGCCATGGGCGGATTCGGACTCAAACACCCACCGCCCATGATGCTCATCA
AGAACACGCCTGTGCCCGGAAATATCACCAGCTTCgCGGACGTGCCCGTCAGCAGCT
TCATCACCCAGTACAGCACCGGGCAGGTCACCGTGGAGATGGAGTGGGAGCTCAAG
AAGGAAAACCTCCAAGAGGTGGAACCCAGAGATCCAGTACACAAACAACCTACAACG
ACCCCCAGTTTGTGGACTTTGCCCCGGACAGCACCGGGGAATACAGAAGCACCAGA
CCTATCGGAACCCGATACCTTACCCGACCCCTTTAA (SEQ ID NO: 7) or by any other
sequence encoding the corresponding amino acid sequence of the altered VP1 protein of adeno-
associated virus serotype 5 (AAV5) capsid with the amino acid S2A, S651A and T711S
substitutions.

"Other sequence encoding the corresponding amino acid sequence of the altered VP1 protein of adeno-associated virus serotype 5 (AAV5) capsid with the amino acid S2A, S651A and T711S substitutions" means a nucleic sequence that is alternative to the nucleic sequence with SEQ ID NO: 7, as, due to the degeneracy of genetic code, a wide range of different DNA sequences can encode the amino acid sequence disclosed herein as SEQ ID NO: 4. It is well within the skill of a person trained in the art to create these alternative DNA sequences encoding the same amino acid sequences. Such variant DNA sequences are within the scope of the present invention.

The isolated nucleic acid encoding the above VP1 of wild-type adeno-associated virus serotype 5 (AAV5) capsid is represented by the nucleic sequence

ATGTCTTTTGTGATCACCTCCAGATTGGTTGGAAGAAGTTGGTGAAGGTCT
TCGCGAGTTTTTGGGCCTTGAAGCGGGCCCACCGAAACCAAAACCCAATCAGCAGC
ATCAAGATCAAGCCCGTGGTCTTGTGCTGCCTGGTTATAACTATCTCGGACCCGGAA
ACGGTCTCGATCGAGGAGAGCCTGTCAACAGGGCAGACGAGGTTCGCGCGAGAGCAC
GACATCTCGTACAACGAGCAGCTTGAGGCGGGAGACAACCCCTACCTCAAGTACAA
CCACGCGGACGCCGAGTTTCAGGAGAAGCTCGCCGACGACACATCCTTCGGGGGAA
ACCTCGGAAAGGCAGTCTTTCAGGCCAAGAAAAGGGTTCTCGAACCTTTTGGCCTGG
TTGAAGAGGGTGCTAAGACGGCCCCCTACCGGAAAGCGGATAGACGACCACTTTCCA
AAAAGAAAGAAGGCTCGGACCGAAGAGGACTCCAAGCCTTCCACCTCGTCAGACGC
CGAAGCTGGACCCAGCGGATCCCAGCAGCTGCAAATCCCAGCCCAACCAGCCTCAA
GTTTGGGAGCTGATACAATGTCTGCGGGAGGTGGCGGCCCCATTGGGCGACAATAAC
CAAGGTGCCGATGGAGTGGGCAATGCCTCGGGAGATTGGCATTGCGATTCCACGTG
GATGGGGGACAGAGTCGTCACCAAGTCCACCCGAACCTGGGTGCTGCCCAGCTACA
ACAACCACCAGTACCGAGAGATCAAAAGCGGCTCCGTCGACGGAAGCAACGCCAAC
GCCTACTTTGGATACAGCACCCCCTGGGGGTACTTTGACTTTAACCGCTTCCACAGC
CACTGGAGCCCCCGAGACTGGCAAAGACTCATCAACAATACTGGGGCTTCAGACC
CCGGTCCCTCAGAGTCAAAATCTTCAACATTCAAGTCAAAGAGGTACGGTGCAGG
ACTCCACCACCACCATCGCCAACAACCTCACCTCCACCGTCCAAGTGTTTACGGACG
ACGACTACCAGCTGCCCTACGTGTCGGCAACGGGACCGAGGGATGCCTGCCGGCC
TTCCCTCCGCAGGTCTTTACGCTGCCGCAGTACGGTTACGCGACGCTGAACCGCGAC
AACACAGAAAATCCCACCGAGAGGAGCAGCTTCTTCTGCCTAGAGTACTTTCCCAGC
AAGATGCTGAGAACGGGCAACAACCTTTGAGTTTACCTACAACCTTTGAGGAGGTGCC
CTTCCACTCCAGCTTCGCTCCCAGTCAGAACCTCTTCAAGCTGGCCAACCCGCTGGT
GGACCAGTACTTGTACCGCTTCGTGAGCACAAATAACACTGGCGGAGTCCAGTTCA
ACAAGAACCTGGCCGGGAGATACGCCAACACCTACAAAACTGGTTCCCGGGGGCCC
ATGGGGCCGAACCCAGGGCTGGAACCTGGGCTCCGGGGTCAACCGCGCCAGTGTCAG
CGCCTTCGCCACGACCAATAGGATGGAGCTCGAGGGCGCGAGTTACCAGGTGCCCC
CGCAGCCGAACGGCATGACCAACAACCTCCAGGGCAGCAACACCTATGCCCTGGAG
AACACTATGATCTTCAACAGCCAGCCGGCGAACCCGGGCACCAACCGCCACGTACCT
CGAGGGCAACATGCTCATCACCAGCGAGAGCGAGACGCAGCCGGTGAACCGCGTGG
CGTACAACGTCGGCGGGCAGATGGCCACCAACAACCAGAGCTCCACCACTGCCCCC
GCGACCGGCACGTACAACCTCCAGGAAATCGTGCCCGGCAGCGTGTGGATGGAGAG

GGACGTGTACCTCCAAGGACCCATCTGGGCCAAGATCCCAGAGACGGGGGCGCACT
TTCACCCCTCTCCGGCCATGGGCGGATTCGGACTCAAACACCCACCGCCCATGATGC
TCATCAAGAACACGCCTGTGCCCCGGAATATCACCAGCTTCTCGGACGTGCCCCGTCA
GCAGCTTCATCACCCAGTACAGCACCGGGCAGGTCACCGTGGAGATGGAGTGGGAG
CTCAAGAAGGAAAACCTCCAAGAGGTGGAACCCAGAGATCCAGTACACAAACAAC
ACAACGACCCCCAGTTTGTGGACTTTGCCCCGGACAGCACCGGGGAATACAGAACC
ACCAGACCTATCGGAACCCGATACCTTACCCGACCCCTTTAA (SEQ ID NO: 14) or by
any other sequence encoding the corresponding amino acid sequence of the VP1 protein of wild-
type adeno-associated virus serotype 5 (AAV5) capsid.

"Other sequence encoding the corresponding amino acid sequence of the VP1 protein of wild-type adeno-associated virus serotype 5 (AAV5) capsid" means a nucleic sequence that is alternative to the nucleic sequence with SEQ ID NO: 14, as, due to the degeneracy of genetic code, a wide range of different DNA sequences can encode the amino acid sequence disclosed herein as SEQ ID NO: 1. It is well within the skill of a person trained in the art to create these alternative DNA sequences encoding the same amino acid sequences. Such variant DNA sequences are within the scope of the present invention.

In one aspect, the present invention relates to an isolated nucleic acid encoding the above altered VP2 protein of adeno-associated virus serotype 5 (AAV5) capsid.

In some embodiments, the isolated nucleic acid encoding the altered VP2 protein of adeno-associated virus serotype 5 (AAV5) capsid with the amino acid T575S substitution is represented by the nucleic sequence

ACGGCCCCTACCGGAAAGCGGATAGACGACCACTTTCCAAAAAGAAAGAAGGCTCG
GACCGAAGAGGACTCCAAGCCTTCCACCTCGTCAGACGCCGAAGCTGGACCCAGCG
GATCCCAGCAGCTGCAAATCCCAGCCCAACCAGCCTCAAGTTTGGGAGCTGATACA
ATGTCTGCGGGAGGTGGCGGCCCATTTGGGCGACAATAACCAAGGTGCCGATGGAGT
GGGCAATGCCTCGGGAGATTGGCATTGCGATTCCACGTGGATGGGGGACAGAGTCG
TCACCAAGTCCACCCGAACCTGGGTGCTGCCCAGCTACAACAACCACCAGTACCGA
GAGATCAAAAGCGGCTCCGTCGACGGAAGCAACGCCAACGCCTACTTTGGATACAG
CACCCCCTGGGGGTACTTTGACTTTAACCGCTTCCACAGCCACTGGAGCCCCCGAGA
CTGGCAAAGACTCATCAACAACCTACTGGGGCTTCAGACCCCGGTCCCTCAGAGTCA
AAATCTTCAACATTCAAGTCAAAGAGGTACGGTGCAGGACTCCACCACCACCATC
GCCAACAACCTCACCTCCACCGTCCAAGTGTTTACGGACGACGACTACCAGCTGCCC
TACGTCGTCGGCAACGGGACCGAGGGATGCCTGCCGGCCTTCCCTCCGCAGGTCTTT

ACGCTGCCGCAGTACGGTTACGCGACGCTGAACCGCGACAACACAGAAAATCCCAC
CGAGAGGAGCAGCTTCTTCTGCCTAGAGTACTTTCCCAGCAAGATGCTGAGAACGG
GCAACAACCTTTGAGTTTACCTACAACCTTTGAGGAGGTGCCCTTCCACTCCAGCTTCG
CTCCCAGTCAGAACCTCTTCAAGCTGGCCAACCCGCTGGTGGACCAGTACTTGTACC
GCTTCGTGAGCACAAATAACACTGGCGGAGTCCAGTTCAACAAGAACCTGGCCGGG
AGATACGCCAACACCTACAAAAACTGGTTCCCGGGGGCCCATGGGCCGAACCCAGGG
CTGGAACCTGGGCTCCGGGGTCAACCGCGCCAGTGTTCAGCGCCTTCGCCACGACCA
ATAGGATGGAGCTCGAGGGCGCGAGTTACCAGGTGCCCCCGCAGCCGAACGGCATG
ACCAACAACCTCCAGGGCAGCAACACCTATGCCCTGGAGAACACTATGATCTTCAA
CAGCCAGCCGGCGAACCCGGGCACACCGCCACGTACCTCGAGGGCAACATGCTCA
TCACCAGCGAGAGCGAGACGCAGCCGGTGAACCGCGTGGCGTACAACGTCGGCGGG
CAGATGGCCACCAACAACCAGAGCTCCACCACTGCCCCCGCGACCGGCACGTACAA
CCTCCAGGAAATCGTGCCCGGCAGCGTGTGGATGGAGAGGGACGTGTACCTCCAAG
GACCCATCTGGGCCAAGATCCCAGAGACGGGGGCGCACTTTCACCCCTCTCCGGCC
ATGGGCGGATTTCGGACTCAAACACCCACCGCCCATGATGCTCATCAAGAACACGCC
TGTGCCCCGGAATATCACCAGCTTCTCGGACGTGCCCCGTCAGCAGCTTCATCACCCA
GTACAGCACCGGGCAGGTCACCGTGGAGATGGAGTGGGAGCTCAAGAAGGAAAAC
TCCAAGAGGTGGAACCCAGAGATCCAGTACACAAACAACACTACAACGACCCCCAGTT
TGTGGACTTTGCCCCGGACAGCACCGGGGAATACAGAAGCACCAGACCTATCGGAA
CCCGATACCTTACCCGACCCCTTTAA (SEQ ID NO: 15) or by any other sequence encoding
the corresponding amino acid sequence of the altered VP2 protein of adeno-associated virus
serotype 5 (AAV5) capsid with the amino acid T575S substitution.

"Other sequence encoding the corresponding amino acid sequence of the altered VP2 protein of adeno-associated virus serotype 5 (AAV5) capsid with the amino acid T575S substitution " means a nucleic sequence that is alternative to the nucleic sequence with SEQ ID NO: 15, as, due to the degeneracy of genetic code, a wide range of different DNA sequences can encode the amino acid sequence disclosed herein as SEQ ID NO: 9. It is well within the skill of a person trained in the art to create these alternative DNA sequences encoding the same amino acid sequences. Such variant DNA sequences are within the scope of the present invention.

In some embodiments, the isolated nucleic acid encoding the altered VP2 protein of adeno-associated virus serotype 5 (AAV5) capsid with the amino acid S515A and T575S substitutions has any nucleic acid sequence that encodes the amino acid sequence disclosed herein as SEQ ID NO: 10. It is well within the skill of a person trained in the art to create these

alternative DNA sequences encoding the same amino acid sequences. Such variant DNA sequences are within the scope of the present invention.

The isolated nucleic acid encoding the above VP2 of wild-type adeno-associated virus serotype 5 (AAV5) capsid is represented by the nucleic sequence

ACGGCCCCTACCGGAAAGCGGATAGACGACCACTTTCCAAAAAGAAAGAAGGCTCG
GACCGAAGAGGACTCCAAGCCTTCCACCTCGTCAGACGCCGAAGCTGGACCCAGCG
GATCCCAGCAGCTGCAAATCCCAGCCCAACCAGCCTCAAGTTTGGGAGCTGATACA
ATGTCTGCGGGAGGTGGCGGCCCATTTGGGCGACAATAACCAAGGTGCCGATGGAGT
GGGCAATGCCTCGGGAGATTGGCATTGCGATTCCACGTGGATGGGGGACAGAGTCG
TCACCAAGTCCACCCGAACCTGGGTGCTGCCAGCTACAACAACCACCAGTACCGA
GAGATCAAAAGCGGCTCCGTCGACGGAAGCAACGCCAACGCCTACTTTGGATACAG
CACCCCCTGGGGGTACTTTGACTTTAACCGCTTCCACAGCCACTGGAGCCCCCGAGA
CTGGCAAAGACTCATCAACAATACTGTTGGGCTTCAGACCCCGGTCCCTCAGAGTCA
AAATCTTCAACATTCAAGTCAAAGAGGTCACGGTGCAGGACTCCACCACCACCATC
GCCAACAACCTCACCTCCACCGTCCAAGTGTTTACGGACGACGACTACCAGCTGCCC
TACGTCGTTCGGCAACGGGACCGAGGGATGCCTGCCGGCCTTCCCTCCGCAGGTCTTT
ACGCTGCCGCAGTACGGTTACGCGACGCTGAACCGCGACAACACAGAAAATCCCAC
CGAGAGGAGCAGCTTCTTCTGCCTAGAGTACTTTCCCAGCAAGATGCTGAGAACGG
GCAACAACCTTTGAGTTTACCTACAACCTTTGAGGAGGTGCCCTTCCACTCCAGCTTCG
CTCCCAGTCAGAACCTCTTCAAGCTGGCCAACCCGCTGGTGGACCAGTACTTGTACC
GCTTCGTGAGCACAAATAACACTGGCGGAGTCCAGTTCAACAAGAACCTGGCCGGG
AGATACGCCAACACCTACAAAACTGGTTCCCGGGGGCCCATGGGCGCAACCCAGGG
CTGGAACCTGGGCTCCGGGGTCAACCGCGCCAGTGTCAGCGCCTTCGCCACGACCA
ATAGGATGGAGCTCGAGGGCGCGAGTTACCAGGTGCCCCCGCAGCCGAACGGCATG
ACCAACAACCTCCAGGGCAGCAACACCTATGCCCTGGAGAACACTATGATCTTCAA
CAGCCAGCCGGCGAACCCGGGCACCACCGCCACGTACCTCGAGGGCAACATGCTCA
TCACCAGCGAGAGCGAGACGCAGCCGGTGAACCGCGTGGCGTACAACGTCGGCGGG
CAGATGGCCACCAACAACCAGAGCTCCACCACTGCCCCCGCAGCCGGCACGTACAA
CCTCCAGGAAATCGTGCCCGGCAGCGTGTGGATGGAGAGGGACGTGTACCTCCAAG
GACCCATCTGGGCCAAGATCCCAGAGACGGGGGCGCACTTTCACCCCTCTCCGGCC
ATGGGCGGATTTCGGAATCAAACACCCACCGCCCATGATGCTCATCAAGAACACGCC
TGTGCCCCGGAATATCACCAGCTTCTCGGACGTGCCCGTCAGCAGCTTCATCACCCA
GTACAGCACCGGGCAGGTACCGTGGAGATGGAGTGGGAGCTCAAGAAGGAAAAC
TCCAAGAGGTGGAACCCAGAGATCCAGTACACAAACAATACTACAACGACCCCCAGTT

TGTGGACTTTGCCCCGGACAGCACCGGGGAATACAGAACCACCAGACCTATCGGAA
CCCGATACCTTACCCGACCCCTTTAA (SEQ ID NO: 16) or by any other sequence encoding
the corresponding amino acid sequence of the VP2 protein of wild-type adeno-associated virus
serotype 5 (AAV5) capsid.

"Other sequence encoding the corresponding amino acid sequence of the VP2
protein of wild-type adeno-associated virus serotype 5 (AAV5) capsid" means a nucleic
sequence that is alternative to the nucleic sequence with SEQ ID NO: 16, as, due to the
degeneracy of genetic code, a wide range of different DNA sequences can encode the
amino acid sequence disclosed herein as SEQ ID NO: 8. It is well within the skill of a
person trained in the art to create these alternative DNA sequences encoding the same
amino acid sequences. Such variant DNA sequences are within the scope of the present
invention.

In one aspect, the present invention relates to an isolated nucleic acid encoding the above
altered VP3 protein of adeno-associated virus serotype 5 (AAV5) capsid.

In some embodiments, the isolated nucleic acid encoding the altered VP3 protein of
adeno-associated virus serotype 5 (AAV5) capsid with the amino acid T519S substitution is
represented by the nucleic sequence
ATGTCTGCGGGAGGTGGCGGCCATTGGGCGACAATAACCAAGGTGCCGATGGAGT
GGGCAATGCCTCGGGAGATTGGCATTGCGATTCCACGTGGATGGGGGACAGAGTCG
TCACCAAGTCCACCCGAACCTGGGTGCTGCCAGCTACAACAACCACCAGTACCGA
GAGATCAAAAGCGGCTCCGTCGACGGAAGCAACGCCAACGCCTACTTTGGATACAG
CACCCCCTGGGGGTACTTTGACTTTAACCGCTTCCACAGCCACTGGAGCCCCCGAGA
CTGGCAAAGACTCATCAACAACACTACTGGGGCTTCAGACCCCGGTCCCTCAGAGTCA
AAATCTTCAACATTCAAGTCAAAGAGGTCACGGTGCAGGACTCCACCACCACCATC
GCCAACAACCTCACCTCCACCGTCCAAGTGTTTACGGACGACGACTACCAGCTGCCC
TACGTCGTCGGCAACGGGACCGAGGGATGCCTGCCGGCCTTCCCTCCGCAGGTCTTT
ACGCTGCCGCAGTACGGTTACGCGACGCTGAACCGCGACAACACAGAAAATCCCAC
CGAGAGGAGCAGCTTCTTCTGCCTAGAGTACTTTCCCAGCAAGATGCTGAGAACGG
GCAACAACCTTTGAGTTTACCTACAACCTTTGAGGAGGTGCCCTTCCACTCCAGCTTCG
CTCCCAGTCAGAACCTCTTCAAGCTGGCCAACCCGCTGGTGGACCAGTACTTGTACC
GCTTCGTGAGCACAATAACACTGGCGGAGTCCAGTTCAACAAGAACCTGGCCGGG
AGATACGCCAACACCTACAAAACTGGTTCCCGGGGCCCATGGGCGCAACCCAGGG
CTGGAACCTGGGCTCCGGGGTCAACCGCGCCAGTGTCAGCGCCTTCGCCACGACCA
ATAGGATGGAGCTCGAGGGCGCGAGTTACCAGGTGCCCCCGCAGCCGAACGGCATG

ACCAACAACCTCCAGGGCAGCAACACCTATGCCCTGGAGAACACTATGATCTTCAA
CAGCCAGCCGGCGAACCCGGGCACCACCGCCACGTACCTCGAGGGCAACATGCTCA
TCACCAGCGAGAGCGAGACGCAGCCGGTGAACCGCGTGGCGTACAACGTCGGCGGG
CAGATGGCCACCAACAACCAGAGCTCCACCCTGCCCCCGCGACCGGCACGTACAA
CCTCCAGGAAATCGTGCCCGGCAGCGTGTGGATGGAGAGGGACGTGTACCTCCAAG
GACCCATCTGGGCCAAGATCCCAGAGACGGGGGGCGCACTTTCACCCCTCTCCGGCC
ATGGGCGGATTTCGGACTCAAACACCCACCGCCCATGATGCTCATCAAGAACACGCC
TGTGCCCCGGAATATCACCAGCTTCTCGGACGTGCCCCGTCAGCAGCTTCATCACCCA
GTACAGCACCGGGCAGGTCACCGTGGAGATGGAGTGGGAGCTCAAGAAGGAAAAC
TCCAAGAGGTGGAACCCAGAGATCCAGTACACAAACAACACTACAACGACCCCCAGTT
TGTGGACTTTGCCCCGGACAGCACCGGGGAATACAGAAGCACCAGACCTATCGGAA
CCCGATACCTTACCCGACCCCTTTAA (SEQ ID NO: 17) or by any other sequence encoding
the corresponding amino acid sequence of the altered VP3 protein of adeno-associated virus
serotype 5 (AAV5) capsid with the amino acid T519S substitution.

"Other sequence encoding the corresponding amino acid sequence of the altered
VP3 protein of adeno-associated virus serotype 5 (AAV5) capsid with the amino acid
T519S substitution" means a nucleic sequence that is alternative to the nucleic sequence
with SEQ ID NO: 17, as, due to the degeneracy of genetic code, a wide range of different
DNA sequences can encode the amino acid sequence disclosed herein as SEQ ID NO: 12. It
is well within the skill of a person trained in the art to create these alternative DNA
sequences encoding the same amino acid sequences. Such variant DNA sequences are
within the scope of the present invention.

In some embodiments, the isolated nucleic acid encoding the altered VP3 protein of
adeno-associated virus serotype 5 (AAV5) capsid with the amino acid S459A and T519S
substitutions has any nucleic acid sequence that encodes the amino acid sequence disclosed
herein as SEQ ID NO: 13. It is well within the skill of a person trained in the art to create these
alternative DNA sequences encoding the same amino acid sequences. Such variant DNA
sequences are within the scope of the present invention.

The isolated nucleic acid encoding the above VP3 of wild-type adeno-associated virus
serotype 5 (AAV5) capsid is represented by the nucleic sequence
ATGTCTGCGGGAGGTGGCGGCCCATTTGGGCGACAATAACCAAGGTGCCGATGGAGT
GGGCAATGCCTCGGGAGATTGGCATTGCGATTCCACGTGGATGGGGGACAGAGTCG
TCACCAAGTCCACCCGAACCTGGGTGCTGCCCAGCTACAACAACCACAGTACCGA
GAGATCAAAAGCGGCTCCGTCGACGGAAGCAACGCCAACGCCTACTTTGGATACAG

CACCCCCTGGGGGTACTTTGACTTTAACCGCTTCCACAGCCACTGGAGCCCCCGAGA
CTGGCAAAGACTCATCAACAACACTACTGGGGCTTCAGACCCCGGTCCCTCAGAGTCA
AAATCTTCAACATTCAAGTCAAAGAGGTCACGGTGCAGGACTCCACCACCACCATC
GCCAACAACCTCACCTCCACCGTCCAAGTGTTTACGGACGACGACTACCAGCTGCCC
TACGTCGTCGGCAACGGGACCGAGGGATGCCTGCCGGCCTTCCCTCCGCAGGTCTTT
ACGCTGCCGCAGTACGGTTACGCGACGCTGAACCGCGACAACACAGAAAATCCCAC
CGAGAGGAGCAGCTTCTTCTGCCTAGAGTACTTTCCCAGCAAGATGCTGAGAACGG
GCAACAACCTTTGAGTTTACCTACAACCTTTGAGGAGGTGCCCTTCCACTCCAGCTTCG
CTCCCAGTCAGAACCTCTTCAAGCTGGCCAACCCGCTGGTGGACCAGTACTTGTACC
GCTTCGTGAGCACAAATAACACTGGCGGAGTCCAGTTCAACAAGAACCTGGCCGGG
AGATACGCCAACACCTACAAAACTGGTTCCCGGGGCCCATGGGCGCAACCCAGGG
CTGGAACCTGGGCTCCGGGGTCAACCGCGCCAGTGTCAGCGCCTTCGCCACGACCA
ATAGGATGGAGCTCGAGGGCGCGAGTTACCAGGTGCCCCCGCAGCCGAACGGCATG
ACCAACAACCTCCAGGGCAGCAACACCTATGCCCTGGAGAACACTATGATCTTCAA
CAGCCAGCCGGCGAACCCGGGCACCAACCGCCACGTACCTCGAGGGCAACATGCTCA
TCACCAGCGAGAGCGAGACGCAGCCGGTGAACCGCGTGGCGTACAACGTCGGCGGG
CAGATGGCCACCAACAACCAGAGCTCCACCACTGCCCCCGCGACCGGCACGTACAA
CCTCCAGGAAATCGTGCCCGGCAGCGTGTGGATGGAGAGGGACGTGTACCTCCAAG
GACCCATCTGGGCCAAGATCCCAGAGACGGGGGCGCACTTTCACCCCTCTCCGGCC
ATGGGCGGATTTCGGACTIONAACACCCACCGCCCATGATGCTCATCAAGAACACGCC
TGTGCCCCGGAATATCACCAGCTTCTCGGACGTGCCCGTCAGCAGCTTCATCACCCA
GTACAGCACCGGGCAGGTCACCGTGGAGATGGAGTGGGAGCTCAAGAAGGAAAAC
TCCAAGAGGTGGAACCCAGAGATCCAGTACACAAACAACACTACAACGACCCCCAGTT
TGTGGACTTTGCCCCGGACAGCACCGGGGAATACAGAACCACCAGACCTATCGGAA
CCCGATACCTTACCCGACCCCTTTAA (SEQ ID NO: 18) or by any other sequence encoding
the corresponding amino acid sequence of the VP3 protein of wild-type adeno-associated virus
serotype 5 (AAV5) capsid.

"Other sequence encoding the corresponding amino acid sequence of the VP3
protein of wild-type adeno-associated virus serotype 5 (AAV5) capsid" means a nucleic
sequence that is alternative to the nucleic sequence with SEQ ID NO: 18, as, due to the
degeneracy of genetic code, a wide range of different DNA sequences can encode the
amino acid sequence disclosed herein as SEQ ID NO: 11. It is well within the skill of a
person trained in the art to create these alternative DNA sequences encoding the same

amino acid sequences. Such variant DNA sequences are within the scope of the present invention.

In one aspect, the present invention relates to an isolated nucleic acid encoding the above capsid, which is used for highly-efficient transduction of target cells.

In some embodiments, the isolated nucleic acid encoding the above capsid includes any of the above nucleic acid sequences.

Vector based on recombinant adeno-associated virus serotype 5 (rAAV5)

In one aspect, the present invention relates to a vector based on recombinant adeno-associated virus serotype 5 (rAAV5) for delivery to a subject of a heterologous nucleic acid sequence, which includes:

- 1) the above capsid, and
- 2) a heterologous nucleic acid sequence comprising regulatory sequences that promote the expression of the target product encoded by the heterologous nucleic acid sequence, in target cells.

The rAAV vector of the invention does not comprise nucleotide sequences of genes encoding non-structural proteins (Rep) and structural proteins (Cap).

The capsid is characterized in detail in the above section of the description.

In some embodiments, the vector based on rAAV5 has an expression product of the heterologous nucleic acid sequence, which is a therapeutic polypeptide or a reporter polypeptide.

In some embodiments, the vector based on rAAV5 comprises a heterologous nucleic acid sequence encoding a product that is a therapeutic polypeptide, wherein the therapeutic polypeptide is a coagulation factor selected from the group consisting of Factor VIII, Factor IX, or a functional variant thereof.

In some embodiments, the vector based on rAAV5 comprises a heterologous nucleic acid sequence encoding a product that is Factor VIII or a functional variant thereof.

In some embodiments, the vector based on rAAV5 comprises a heterologous nucleic acid sequence encoding a product that is Factor IX or a functional variant thereof.

Pharmaceutical composition

In one aspect, the present invention relates to a pharmaceutical composition for the delivery of a gene product to a subject in need thereof, which comprises:

- a) the above vector based on rAAV5; and
- b) a pharmaceutically acceptable excipient.

In some embodiments, the pharmaceutical composition is used for the delivery of a gene product to a human in need thereof.

In particular embodiments, the present invention relates to a pharmaceutical composition comprising the rAAV5 viral particle of the invention in a pharmaceutically acceptable carrier or other medicinal agents, pharmaceutical agents, carriers, adjuvants, diluents, etc. For injection, the carrier will typically be a liquid carrier. For other methods of administration, the carrier may be either solid or liquid, such as sterile pyrogen-free water or sterile pyrogen-free phosphate-buffered saline solution. For inhalation administration, the carrier is respirable, and preferably is in a solid or liquid particulate form. As an injection medium, it is preferred to use water that contains the additives that are common for injection solutions, such as stabilizing agents, salts or saline, and/or buffers.

In other embodiments, the present invention relates to a pharmaceutical composition comprising a cell, in which vector based on rAAV5 is integrated into the genome, in a pharmaceutically acceptable carrier or other medicinal agents, pharmaceutical agents, carriers, adjuvants, diluents, etc.

"Pharmaceutical composition" means a composition comprising the above vector based on rAAV5 of the invention and at least one of components selected from the group consisting of pharmaceutically acceptable and pharmacologically compatible excipients, such as fillers, solvents, diluents, carriers, auxiliary, distributing agents, delivery agents, preservatives, stabilizers, emulsifiers, suspending agents, thickeners, prolonged delivery controllers, the choice and proportions of which depend on the type and route of administration and dosage. Pharmaceutical compositions of the present invention and methods for preparation thereof will be undoubtedly apparent to those skilled in the art. Pharmaceutical compositions should preferably be manufactured in compliance with the GMP (Good Manufacturing Practice) requirements. A composition may comprise a buffer composition, tonicity agents, stabilizers and solubilizers.

"Pharmaceutically acceptable" means a material that does not have biological or other negative side effects, for example, the material can be administered to a subject without causing any undesirable biological effects. Thus, such pharmaceutical compositions may be used, for example, in transfection of a cell ex vivo or in administration in vivo of a viral particle or a cell directly to a subject.

The term "excipient" is used herein to describe any ingredient other than the above ingredients of the invention. These are substances of inorganic or organic nature which are used in the pharmaceutical manufacturing in order to give drug products the necessary physicochemical properties.

"Stabilizer" refers to an excipient or a mixture of two or more excipients that provide the physical and/or chemical stability of the active agent.

The term "buffer", "buffer composition", "buffering agent" refers to a solution, which is capable of resisting changes in pH by the action of its acid-base conjugate components, which allows the vector based on rAAV5 product to resist changes in pH. Generally, the pharmaceutical composition preferably has a pH in the range from 4.0 to 8.0. Examples of buffers that can be used include, but are not limited to, acetate, phosphate, citrate, histidine, succinate, etc. buffer solutions.

A pharmaceutical composition is "stable" if the active agent retains physical stability and/or chemical stability and/or biological activity thereof during the specified shelf life at storage temperature, for example, of 2-8 °C. Preferably, the active agent retains both physical and chemical stability, as well as biological activity. Storage period is adjusted based on the results of stability test in accelerated or natural aging conditions.

A pharmaceutical composition of the invention can be manufactured, packaged, or widely sold in the form of a single unit dose or a plurality of single unit doses in the form of a ready formulation. The term "single unit dose" as used herein refers to a discrete quantity of a pharmaceutical composition containing a predetermined quantity of an active ingredient. The quantity of the active ingredient typically equals the dose of the active ingredient to be administered in a subject, or a convenient portion of such dose, for example, half or a third of such dose.

Method for delivery of gene product

In one aspect, the present invention relates to a method for the delivery of a gene product to a subject in need thereof, which comprises administering to the subject the above vector based on rAAV5 or the above pharmaceutical composition.

In some embodiments, the method for the delivery of a gene product is used for the delivery of a gene product to a human in need thereof.

Any method for administering vector based on rAAV5, which is recognized in the art, can be suitably used for the above vector based on rAAV5 of the present invention.

The rAAV5-based recombinant viral vectors are preferably administered to a cell in a biologically-effective amount. A "biologically-effective" amount of the viral vector is an amount that is sufficient to cause infection (or transduction) and expression of the heterologous nucleic acid sequence in the cell. If the virus is administered to a cell in vivo (e.g. the virus is administered to a subject, as described below), a "biologically-effective" amount of the viral vector is an amount that is sufficient to cause the transduction and expression of the heterologous nucleic acid sequence in a target cell.

The cell for administering the rAAV5 viral vector of the invention may be a cell of any type, including but not limited to neural cells (including cells of the peripheral and central nervous systems, in particular, brain cells), lung cells, epithelial cells (e.g. gut and respiratory epithelial cells), muscle cells, pancreatic cells (including islet cells), hepatic cells, myocardial cells, bone cells (e.g. bone marrow stem cells), hematopoietic stem cells, spleen cells, keratinocytes, fibroblasts, endothelial cells, prostate cells, germ cells, and the like. Alternatively, the cell for administering the rAAV5 viral vector may be any progenitor cell. As a further alternative, the cells can be stem cells (e.g. neural stem cells, liver stem cells). Furthermore, the cells can be from any species of origin, as specified above.

Use

In one aspect, the present invention relates to the use of the above vector based on rAAV5 or the above pharmaceutical composition for the treatment of a disease in a subject in need thereof.

In some embodiments, the use is used for the treatment of a disease in a human in need thereof.

Administration of the vector based on rAAV5 of the present invention to a human subject or an animal in need thereof can be by any means known in the art for administering viral vectors.

Exemplary modes of administration include local, oral, rectal, transmucosal, transdermal, inhalation, parenteral administration (e.g. intravenous, subcutaneous, intradermal, intramuscular, and intraarticular administration), and the like, as well as direct tissue or organ injection, and, alternatively, intrathecal, direct intramuscular, intraventricular, intravenous, intraperitoneal, intranasal, or intraocular injections. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for the preparation of solution or suspensions in liquid prior to injection, or as emulsions. Alternatively, one may administer the vector based on rAAV5 in a local rather than systemic manner, for example in a depot or sustained-release formulation.

In some embodiments of the use, the disease is selected from the group comprising: blood diseases; central nervous system diseases; metabolic diseases; muscle diseases; hereditary diseases.

In some embodiments of the use, the disease is a blood disease.

In some embodiments of the use, the disease is a muscle disease.

In some embodiments of the use, the disease is a hereditary disease.

In particular embodiments of the present invention, the nucleotide sequence of interest is delivered by the vector based on rAAV5 to the liver of the subject. Administration to the liver may be performed by any method known in the art, including, but not limited to intravenous administration, intraportal administration, intrabiliary administration, intra-arterial administration, and direct injection into the liver parenchyma.

Preferably, the cells (e.g. liver cells) are infected with the vector based on rAAV5 encoding a peptide or protein, the cells express the encoded peptide or protein and secrete it into the blood circulatory system in a therapeutically effective amount (as described below). Alternatively, the vector is delivered to and expressed by another cell or tissue, including but not limited to, brain, pancreas, spleen or muscles.

A "therapeutically-effective amount" is understood to mean an amount that is sufficient to alleviate (e.g. mitigate, decrease, reduce) at least one of the symptoms associated with a disease state. Alternatively stated, a "therapeutically-effective" amount is an amount that is sufficient to provide some improvement in the condition of the subject.

In some embodiments of the use, the expression product of the heterologous nucleic acid sequence is Factor IX or a functional variant thereof.

In some embodiments of the use, the expression product of the heterologous nucleic acid sequence is Factor VIII or a functional variant thereof.

In other preferred embodiments, the vector based on rAAV5 of the invention is administered intramuscularly, more preferably by intramuscular injection or by local administration (as described above). In other preferred embodiments, the parvovirus particles of the present invention are administered to the lungs.

The vector based on rAAV5 disclosed in the invention may be administered to the lungs of a subject by any suitable means, but is preferably administered in the form of an aerosol suspension of respirable particles comprised of the vector based on rAAV5 of the invention, which the subject inhales. The respirable particles may be liquid or solid. Aerosols of liquid particles comprising the parvovirus rAAV5 vectors of the invention may be produced by any suitable means, such as with a pressure-driven aerosol nebulizer or an ultrasonic nebulizer, as is known to those skilled in the art. Aerosols of solid particles comprising the viral rAAV5 vectors of the invention may also be produced with any solid particulate medicament aerosol generator by techniques known in the pharmaceutical art.

Dosages of the parvovirus rAAV5 particles of the invention will depend on the mode of administration, the disease or condition to be treated, the subject's condition, the particular viral vector, and the gene to be delivered and can be determined in a routine manner. Exemplary doses for achieving therapeutic effects are viral titers of at least about 10^5 , 10^6 , 10^7 , 10^8 , 10^9 , 10^{10} , 10^{11} , 10^{12} , 10^{13} , 10^{14} , 10^{15} , 10^{16} transducing units or more, preferably about 10^8 to 10^{13} transducing units, yet more preferably 10^{12} transducing units.

Thus, the parvovirus vectors based on rAAV5, reagents, and methods of the present invention can be used to direct a nucleic acid to either dividing or non-dividing cells, and to stably express the heterologous nucleic acid therein. Using this vector system, it is now possible to introduce into cells under in vivo conditions the genes that encode proteins that affect cell physiology. The vectors of the present invention can thus be useful in gene therapy for disease states.

In general, the present invention may be employed to deliver any foreign nucleic acid with a biological effect to treat or ameliorate the symptoms associated with any disorder related to gene expression. Exemplary disease states include, but are not limited to: cystic fibrosis (and other lung diseases), hemophilia A, hemophilia B, thalassemia, anemia and other blood coagulation disorders, AIDs, Alzheimer's disease, Parkinson's

disease, Huntington's disease, amyotrophic lateral sclerosis, epilepsy, and other neurological disorders, diabetes mellitus, muscular dystrophies (e.g. Duchenne, Becker), Gaucher's disease, Hurler's disease, adenosine deaminase deficiency, glycogen storage diseases and other metabolic defects, diseases of solid organs (e.g. brain, liver, kidney, heart), and the like.

Gene transfer has substantial potential use in understanding and providing therapy for disease states. There are a number of hereditary diseases for which defective genes are known and have been cloned. In some cases, the function of these cloned genes is known. In general, the above disease states fall into two classes: deficiency states, typically enzyme deficiency, which are generally inherited in a recessive manner, and unbalanced states, sometimes involving at least regulatory or structural proteins, which are inherited in a dominant manner. For deficiency state diseases, gene transfer could be used to bring a normal gene into affected tissues for replacement therapy. For unbalanced disease states, gene transfer could be used to create a disease state in a model system, which could then be used in efforts to counteract the disease state. Thus, the methods of the present invention permit to treat genetic diseases. According to the invention, a disease state is treated by partially or wholly remedying the deficiency or imbalance that causes the disease or makes it more severe. The use of site-specific integration of nucleic sequences to induce mutations or to correct deficiencies is also possible.

Method for producing rAAV5-based vector

In one aspect, the present invention relates to a method for the production of the above vector based on rAAV5, which comprises the transfection of producer cells with the above nucleic acid comprising a sequence encoding a capsid including an altered VP1 capsid protein of adeno-associated virus serotype 5 (AAV5).

In some embodiments of the method for producing the vector based on rAAV5, used is the above nucleic acid which comprises a sequence encoding the above altered VP1 protein of adeno-associated virus serotype 5 (AAV5) capsid, a VP2 protein of AAV5 capsid or an altered variant thereof, and a VP3 protein of AAV5 capsid or an altered variant thereof.

Altered variants of the VP2 protein of the wild-type AAV5 capsid and the VP3 protein of AAV5 capsid protein are understood to mean the variants of the VP2 protein of the wild-type AAV5 capsid and VP3 protein of the wild-type AAV5 capsid, which include one or more amino acid substitutions.

Particularly preferred embodiments include substitutions that are conservative in nature, i.e. substitutions which take place within a family of amino acids that are joined in their side chains. In particular, amino acids are typically divided into four families: (1) acidic amino acids are aspartate and glutamate; (2) basic amino acids are lysine, arginine, histidine; (3) non-polar amino acids are alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan, and (4) uncharged polar amino acids are glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine. Phenylalanine, tryptophan, and tyrosine are sometimes classified as aromatic amino acids. For example, it is reasonably predictable that an isolated substitution of leucine for isoleucine or valine, an aspartate for a glutamate, a threonine for a serine, or a similar conservative substitution of an amino acid for a structurally related amino acid, will not have a major effect on the biological activity. For example, the polypeptide of interest may include up to about 5-10 conservative or non-conservative amino acid substitutions, or even up to about 15-25 or 50 conservative or non-conservative amino acid substitutions, or any integer between 5-50, so long as the desired function of the molecule remains intact.

In some embodiments of the method for producing the vector based on rAAV5, used is the above nucleic acid which comprises a sequence encoding the above altered VP1 protein of adeno-associated virus serotype 5 (AAV5) capsid, the VP2 protein of the wild-type AAV5 capsid and VP3 protein of the wild-type AAV5 capsid

The altered variants of the VP2 and VP3 proteins of AAV5 capsids, as well as nucleic acids encoding them, are disclosed in detail in the corresponding sections of the description.

Examples

The following examples are provided for better understanding of the invention. These examples are for purposes of illustration only and are not to be construed as limiting the scope of the invention in any manner.

All publications, patents, and patent applications cited in this specification are incorporated herein by reference. Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that

certain changes and modifications may be made thereto without departing from the spirit or scope of the appended embodiments.

Materials and general methods

Recombinant DNA techniques

DNA manipulations were carried out by standard techniques as described by Sambrook J. et al, Molecular cloning: A laboratory manual; Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 1989. The molecular biological reagents were used according to the manufacturer instructions.

Gene synthesis

Desired gene segments were prepared from oligonucleotides made by chemical synthesis. The gene segments of 300-4000 bp long, which were flanked by singular restriction sites, were assembled by annealing and ligation of oligonucleotides including PCR amplification and subsequently cloned via the specified restriction sites. The DNA sequences of the subcloned gene fragments were confirmed by DNA sequencing.

DNA sequence determination

DNA sequences were determined by Sanger sequencing.

DNA and protein sequence analysis and sequence data management

The Infomax's Vector NTI Advance suite version 8.0 and SnapGene Viewer were used for sequence creation, mapping, analysis, annotation and illustration.

Example 1. Production of libraries of AAV5 capsid variants

Libraries of AAV5 capsid variants were produced by random mutagenesis of the Cap gene sequence (Davidsson M. et al., 2016). Briefly, the wild-type sequence of the Cap gene of serotype 5 (GenBank ID AF085716.1) was assembled de novo, the synthesized wild-type AAV5 capsid gene was thereafter fragmented using uracil-DNA glycosylase, the resulting fragments were assembled to a full-length Cap gene using DNA polymerase not having a proofreading activity (as a result, random mutations emerged in the sequence). The full-length mutant variants were cloned into the carrier plasmid pAAV-linker (Fig. 1) at AscI/EcoRI restriction sites in a common reading frame with green fluorescent protein (GFP), thereby producing a diverse

random library of AAV5 capsids, which library was thereafter used to select capsid variants having increased transduction activity.

Positive selection of viral particles having increased transduction activity was performed in vitro on CHO-K1-S cells. Thereby, for transduction, we used particles that were purified using ultracentrifugation in a iodixanol gradient. After 48 hours, the cells were harvested and genomic DNA was isolated for subsequent amplification of the viral genome sequences capable of efficient transduction. The resulting sequences were thereafter re-cloned and re-produced for subsequent selection iterations to enrich the library with variants having the highest transduction efficiency. After 5 rounds of selection, the capsid genes of 30 clones were sequenced to determine the most successful mutations and combinations thereof. The sequencing results showed that the predominant combinations of mutations were S2A, T711S in AAV5 VP1 and capsid variants containing S2A, T711S, S651A in AAV5 VP1, which were about 20% of the clones. Capsid variants comprising the mutation S651A in AAV5 VP1 were also selected. These capsid variants were cloned into vectors for producing viral particles and further used for visualizing and comparing the transduction profiles relative to wild-type AAV5.

Example 2. Production and subsequent selection of recombinant viral particles from resulting sequence library

To produce and subsequently select recombinant viral particles from the resulting sequence library, a series of plasmids was developed as follows: a carrier plasmid, a plasmid comprising the Rep gene sequence, as well as a construct comprising adenoviral genes that are required for the replication of viral particles.

The carrier plasmid pAAV-linker (Fig. 1), intended for cloning the libraries of random variants of the capsid gene of AAV serotype 5 into one reading frame with the reporter protein, was produced by substituting the sequence of a modified green fluorescent protein in the original construct pAAV-GFP Control plasmid (VPK-402) from CellBiolab (USA), using the restrictase-ligase method of cloning at HindIII/EcoRI sites, for the sequence T2A-GFP synthesized *de novo* with the addition of an EcoRI restriction site from the 5' end and a HindIII restriction site from the 3' end.

The plasmid pAAV-Rep comprising the Rep gene sequence (Fig. 2) was produced by *de novo* cloning of the synthesized sequence of the AAV serotype 2 Rep gene (GenBank ID AF043303.1) at PciI/PsiI restriction sites (New England Biolabs, USA) with subsequent

treatment with T4 DNA Polymerase (New England Biolabs, USA) to generate blunt ends into the plasmid pGem-T Easy (Promega, USA) also treated with PciI/PsiI restriction enzymes (New England Biolabs, USA).

The adenoviral genes for producing the recombinant viral particles were sourced from the construct pHelper (Fig. 3) from the commercial kit AAV-2 Packaging System (VPK-402) from CellBiolab (USA), comprising AmpR - a beta-lactamase gene that provides resistance to ampicillin, Ori - a replication origin in bacteria, Adeno E2A - a helper adenovirus gene sequence involved in viral DNA replication, Adeno E4 - a helper adenovirus gene sequence involved in viral DNA replication, Adeno VARNA - a helper adenovirus gene sequence responsible for the translation of both early and late viral genes

Example 3. Method for producing vectors based on altered adeno-associated virus serotype 5 (rAAV)

To produce rAAV particles with an altered serotype 5 capsid, producer cells were transfected simultaneously with 3 plasmids as follows:

- 1) With a plasmid comprising adenovirus nucleotide sequences encoding proteins and RNAs required for assembly of rAAV particles (helper plasmid);

- 2) With a plasmid comprising the natural nucleotide sequence of the Rep gene of adeno-associated virus serotype 2, as well as the sequence of an altered Cap gene, which is selected from the group comprising: the nucleotide sequence of SEQ ID NOs: 5, 6 or 7 or any other nucleotide sequence encoding the VP1 protein with the amino acid sequences of SEQ ID Nos: 2, 3 or 4, and the VP2 and VP3 proteins from alternative reading frames of the nucleotide sequence being used, wherein

the VP2 can have any of the amino acid sequences of SEQ ID Nos: 8, 9, or 10;

and the VP3 can have any of the amino acid sequences of SEQ ID Nos: 11, 12, or 13;

- 3) With a plasmid comprising the heterologous genome of the rAAV particle, encoding a target gene intended for delivery into patient's cells.

This set of genes provides assembly of the rAAV viral particles and encapsidation therein of the target genome within 72 hours. 72 hours following transfection, the producer cells are lysed to release rAAV particles for purification by subsequent filtration and chromatography steps. The titer of the purified rAAV particles is verified by enzyme linked immunosorbent assay and quantitative PCR.

Example 4. Increasing the efficiency of cell transduction with rAAV5-based products in the presence of mutations S2A, S651A, T711S in VP1 protein of the wild-type AAV5 capsid.

Experimental design:

CHO-K1-S cells were plated into the wells of 12-well plates. Seeding was made into the following growth medium: DMEM/F12 supplemented with glutamine, glucose content was 4.5 g/l, 5% bovine serum. Cell seeding density was 10,000 cell/cm². During the transduction run, pre-prepared cells were transduced at MOI of 100,000 vg/cell. All samples were run in triplicates. Intact cells were used as a negative control.

Analysis of transduction efficiency was performed using the Guava EasyCyteflow cytometer and the GuavaSoft software.

The inventors have surprisingly found that the presence of one or more mutations selected from the group comprising S2A, S651A or T711S in VP1 protein of the wild-type AAV5 capsid caused a significant increase in the efficiency of transgene delivery by the rAAV vectors with the above mutations. For example, the flow cytometry method showed a change in the amount of GFP-positive cells 48 hours post transduction of the CHO-K1-S line with the rAAV-based products containing the VP1 protein of the wild-type AAV5 capsid or the VP1 protein of the wild-type AAV5 capsid bearing one or more mutations selected from the group comprising: S2A, S651A, T711S (Fig. 4.).

When the mutation S651A (AAV5-01Mut-GFP) was present, the amount of GFP-expressing cells increased 2.2-fold from 22.54% to 49.45% as compared to control AAV5 with a wild-type VP1 capsid protein (AAV5-NullMut-GFP).

When both the mutations S2A and T711S (AAV5-02Mut-GFP) were simultaneously present, the amount of GFP-expressing cells increased 2.6-fold from 22.54% to 58.51% as compared to control AAV5 with a wild-type VP1 capsid protein (AAV5-NullMut-GFP).

When the mutations S2A, S651A and T711S (AAV5-03Mut-GFP) were simultaneously present, the amount of GFP-expressing cells increased 1.7-fold from 22.54% to 38.27% as compared to control AAV5 with a wild-type VP1 capsid protein (AAV5-NullMut-GFP).

Example 5. Increasing the production of target protein encoded by transgene following cell transduction with rAAV5-based products in the presence of mutations S2A, S651A, T711S in the VP1 protein of the wild-type AAV5 capsid.

Experimental design:

CHO-K1-S cells were plated into the wells of 12-well plates. Seeding was made into the following growth medium: DMEM/F12 supplemented with glutamine, glucose content was 4.5 g/l, 5% bovine serum. Cell seeding density was 10,000 cell/cm². During the transduction run, pre-prepared cells were transduced at MOI of 100,000 vg/cell. All samples were run in triplicates. Intact cells were used as a negative control.

The amount of FIX protein in the culture liquid 7 days post transduction was assessed using the Human Factor IX ELISA Kit. We used a 1:25 dilution of the samples. The procedure was carried out according to the manufacturer's instructions.

The inventors have surprisingly found that the presence of one or more mutations selected from the group comprising S2A, S651A, T711S in the VP1 protein of the wild-type AAV5 capsid caused a significant increase in the production of hFIX protein post transduction of the CHO-K1-S cells with the vectors based on rAAV with the above mutations. For example, the enzyme-linked immunosorbent assay (ELISA) method showed an increase in the amount of hFIX protein in the culture medium 7 days post transduction of the CHO-K1-S cells with the rAAV products with a wild-type AAV5 VP1 capsid protein or the VP1 protein of the wild-type AAV5 capsid bearing one or more mutations selected from the group comprising: S2A, S651A, T711S (Fig. 5.).

When the mutation S651A (AAV5-01Mut-FIX) was present, the amount of protein being produced increased 4.6-fold from 0.17 ng/ml to 0.74 ng/ml as compared to control AAV5 with a wild-type VP1 capsid protein (AAV5-NullMut-GFP).

When both the mutations S2A and T711S (AAV5-02Mut-GFP) were simultaneously present, the amount of protein being produced increased 7.1-fold from 0.17 ng/ml to 1.24 ng/ml as compared to control AAV5 with a wild-type VP1 capsid protein (AAV5-NullMut-GFP).

When the mutations S2A, S651A and T711S (AAV5-03Mut-GFP) were simultaneously present, the amount of protein being produced increased 3.3-fold from 0.17 ng/ml to 0.57 ng/ml as compared to control AAV5 with a wild-type VP1 capsid protein (AAV5-NullMut-GFP).

Claims:

1. An isolated altered VP1 protein of adeno-associated virus serotype 5 (AAV5) capsid for highly-effective transduction of target cells, comprising the amino acid sequence of the VP1 protein of a wild-type AAV5 capsid, encoded by the *Cap* gene, comprising one or more substitutions selected from the group comprising:

S651A,

S2A and T711S,

S2A, S651A and T711S.

2. The isolated altered VP1 protein of the AAV5 capsid of Claim 1, wherein the amino acid sequence of the VP1 protein of a wild-type AAV5 capsid has the amino acid sequence represented by SEQ ID NO: 1.

3. The isolated altered VP1 protein of the AAV5 capsid of Claim 1, which comprises one substitution at the S651A position.

4. The isolated altered VP1 protein of the AAV5 capsid of Claim 3, which has the amino acid sequence represented by SEQ ID NO: 2.

5. The isolated altered VP1 protein of the AAV5 capsid of Claim 1, which comprises the S2A and T711S substitutions.

6. The isolated altered VP1 protein of the AAV5 capsid of Claim 5, which has the amino acid sequence represented by SEQ ID NO: 3.

7. The isolated altered VP1 protein of the AAV5 capsid of Claim 1, which comprises the substitutions S2A, S651A and T711S.

8. The isolated altered AAV5 VP1 capsid protein of Claim 7, which has the amino acid sequence represented by SEQ ID NO: 4.

9. An isolated nucleic acid encoding the altered VP1 protein of adeno-associated virus serotype 5 (AAV5) capsid of any one of Claims 1 to 8, which is used for highly efficient transduction of target cells.

10. The isolated nucleic acid of Claim 9 encoding an altered VP1 protein of adeno-associated virus serotype 5 (AAV5) capsid comprising the amino acid S651A substitution, which is represented by the nucleic sequence of SEQ ID NO: 5 or by any other sequence encoding the corresponding amino acid sequence.

11. The isolated nucleic acid of Claim 9 encoding an altered VP1 protein of adeno-associated virus serotype 5 (AAV5) capsid comprising the amino acid S2A and T711S substitutions, which is represented by the nucleic sequence of SEQ ID NO: 6 or any other sequence encoding the corresponding amino acid sequence.

12. The isolated nucleic acid of Claim 9 encoding an altered VP1 protein of adeno-associated virus serotype 5 (AAV5) capsid comprising the amino acid S2A, S651A and T711S substitutions, which is represented by the nucleic sequence of SEQ ID NO: 7 or any other sequence encoding the corresponding amino acid sequence.

13. An isolated capsid for highly efficient transduction of target cells comprising the altered VP1 protein of adeno-associated virus serotype 5 (AAV5) capsid of any one of Claims 1 to 8.

14. The isolated capsid of Claim 13 comprising the altered VP1 protein of adeno-associated virus serotype 5 (AAV5) capsid of any one of Claims 1 to 8, a VP2 protein of AAV5 capsid or an altered variant thereof, and a VP3 protein of AAV5 capsid or an altered variant thereof.

15. The isolated capsid of Claim 14 comprising the VP2 protein of a wild-type AAV5 capsid.

16. The isolated capsid of Claim 15, comprising the VP2 protein of a wild-type AAV5 capsid protein having the amino acid sequence represented by SEQ ID NO: 8.

17. The isolated capsid of Claim 14 comprising the altered VP2 protein of adeno-associated virus serotype 5 (AAV5) capsid.

18. The isolated capsid of Claim 17 comprising the altered VP2 protein of AAV5 capsid comprising the T575S substitution.

19. The isolated capsid of Claim 18 comprising the altered VP2 protein of AAV5 capsid comprising the T575S substitution and having the amino acid sequence represented by SEQ ID NO: 9.

20. The isolated capsid of Claim 18 comprising the altered VP2 protein of AAV5 capsid comprising the S515A and T575S substitutions.

21. The isolated capsid of Claim 20 comprising a the altered VP2 protein of AAV5 capsid comprising the S515A and T575S substitutions and having the amino acid sequence represented by SEQ ID NO: 10.

22. The isolated capsid of Claim 14 comprising the VP3 protein of a wild-type AAV5 capsid.

23. The isolated capsid of Claim 22, comprising the VP3 protein of a wild-type AAV5 capsid having the amino acid sequence represented by SEQ ID NO: 11.

24. The isolated capsid of Claim 14 comprising the altered VP3 protein of adeno-associated virus serotype 5 (AAV5) capsid.

25. The isolated capsid of Claim 24 comprising the altered VP3 protein of AAV5 capsid comprising the T519S substitution.

26. The isolated capsid of Claim 25 comprising the altered VP3 protein of AAV5 capsid comprising the T519S substitution and having the amino acid sequence represented by SEQ ID NO: 12.

27. The isolated capsid of Claim 24 comprising the altered VP3 protein of AAV5 capsid comprising the S459A and T519S substitutions.

28. The isolated capsid of Claim 27 comprising the altered VP3 protein of AAV5 capsid comprising the S459A and T519S substitutions and having the amino acid sequence represented by SEQ ID NO: 13.

29. An isolated nucleic acid encoding the capsid of any one of Claims 13 to 28, which is used for highly efficient transduction of target cells.

30. A vector based on recombinant adeno-associated virus serotype 5 (rAAV5) for delivery to a subject of a heterologous nucleic acid sequence, which comprises:

1) the capsid of any one of Claims 13 to 28, and

2) a heterologous nucleic acid sequence comprising regulatory sequences that promote the expression of the product encoded by the heterologous nucleic acid sequence, in target cells.

31. The vector based on rAAV5 of Claim 30, wherein the expression product of the heterologous nucleic acid sequence is a therapeutic polypeptide or a reporter polypeptide.

32. The vector based on rAAV5 of Claim 31, wherein the therapeutic polypeptide is a coagulation factor selected from the group consisting of Factor VIII, Factor IX, or a functional variant thereof.

33. The vector based on rAAV5 of Claim 32, wherein the therapeutic peptide is Factor VIII or a functional variant thereof.

34. The vector based on rAAV5 of Claim 32, wherein the therapeutic peptide is Factor IX or a functional variant thereof.

35. A pharmaceutical composition for the delivery of a gene product to a subject in need thereof, comprising:

- a) the vector based on rAAV5 of any one of Claims 30 to 34; and
- b) a pharmaceutically acceptable excipient.

36. The pharmaceutical composition of Claim 35, wherein the subject is a human subject.

37. A method for the delivery of a gene product to a subject in need thereof, comprising administering to the subject the vector based on rAAV5 of any one of Claims 30 to 34 or the pharmaceutical composition of Claim 35.

38. The method for the delivery of a gene product of Claim 37, wherein the subject is a human subject.

39. A use of the vector based on rAAV5 of any one of Claims 30 to 34 or the pharmaceutical composition of Claim 35 for the treatment of a disease in a subject in need thereof.

40. The use of Claim 39, wherein the subject is a human subject.

41. The use of Claim 39, wherein the disease is selected from the group comprising: blood diseases; central nervous system diseases; metabolic diseases; muscle diseases; hereditary diseases.

42. The use of Claim 41, wherein the disease is a blood disease.

43. The use of Claim 42, wherein the expression product of the heterologous nucleic acid sequence is Factor IX or a functional variant thereof.

44. The use of Claim 42, wherein the expression product of the heterologous nucleic acid sequence is Factor VIII or a functional variant thereof.

45. The use of Claim 41, wherein the disease is a muscle disease.

46. The use of Claim 41, wherein the disease is a hereditary disease.

47. A method of obtaining of the vector based on rAAV5 of any one of Claims 30 to 34 comprising the transfection of producer cells with the nucleic acid of Claim 29.