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[Continued on next page]

- (54) **Title: RECOMBINANT AAV1, AAV5, AND AAV6 CAPSID MUTANTS AND USES THEREOF**

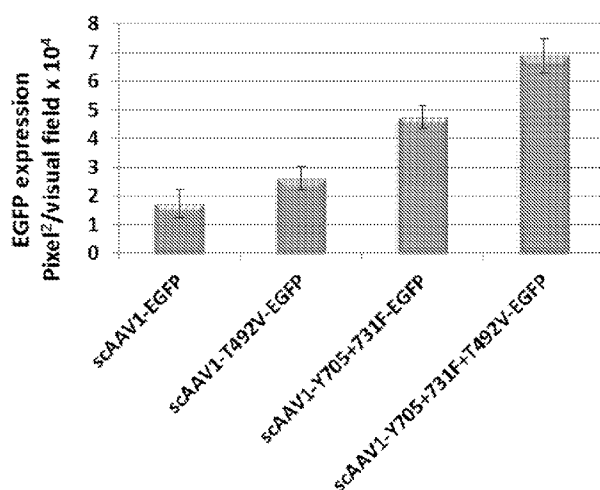
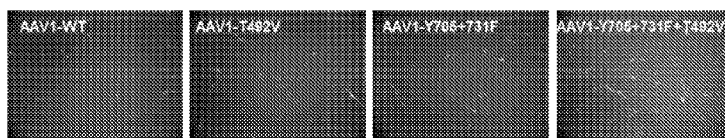


FIG. 1A

(57) **Abstract:** Provided herein are modified recombinant adeno-associated virus (rAAV) capsid proteins, such as modified rAAV1, rAAV5, and rAAV6 capsid proteins, rAAV particles comprising such capsid proteins, nucleic acid molecules encoding such capsid proteins, as well as compositions, kits and methods of use thereof.



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RECOMBINANT AAV1, AAV5, AND AAV6 CAPSID MUTANTS AND USES THEREOF

RELATED APPLICATIONS

This application claims priority under 35 U.S.C. § 119(e) to U.S. provisional application number 62/111,319, filed February 3, 2015, the content of which is incorporated herein by reference in its entirety.

GOVERNMENT SUPPORT

This invention was made with government support under R01 HL097088 and R21 EB-015684 awarded by the National Institutes of Health. The government has certain rights in the invention.

BACKGROUND OF INVENTION

Gene therapy using recombinant adeno-associated virus (rAAV) vectors has advanced significantly in the last decade. However, the transduction efficiency of rAAV vectors varies widely between different cells and tissues *in vitro* and *in vivo*.

SUMMARY OF THE INVENTION

As described herein, rAAV1, rAAV5, and rAAV6 capsid mutant-containing viral particles were shown to transduce different tissues and cells (*e.g.*, muscle, retina, airway epithelia, hematopoietic stem cells, dendritic cells, monocytes, airway epithelial cells, and microglial cells) with high efficiency, when compared to rAAV particles comprising wild-type capsid proteins.

Accordingly, the present disclosure provides AAV capsid proteins comprising modifications of a combination of one or more of the surface-exposed residues. Also provided are rAAV viral particles that comprise the modified AAV capsid proteins, as well as nucleic acid molecules and rAAV vectors encoding the modified AAV capsid proteins. Also disclosed herein are methods utilizing such proteins, viral particles, nucleic acid molecules and rAAV vectors.

In some embodiments, the present disclosure provides a nucleic acid molecule

comprising a nucleotide sequence encoding an AAV capsid protein (*e.g.*, an AAV1, AAV5, or AAV6 capsid protein), wherein the VP3 region of the capsid protein comprises modifications (*e.g.*, replacement of a tyrosine residue with a non-tyrosine residue and/or a threonine residue with a non-threonine residue) at positions corresponding to:

5 one or more of or each of Y705, Y731, and T492 of a wild-type AAV1 capsid protein (*e.g.*, SEQ ID NO: 1),

 one or more of or each of Y436, Y693, and Y719 of a wild-type AAV5 capsid protein (*e.g.*, SEQ ID NO: 2), or

 one or more of or each of Y705, Y731, and T492 of a wild-type AAV6 capsid protein
10 (*e.g.*, SEQ ID NO: 3).

 In some embodiments, the nucleotide sequence encodes an AAV capsid protein (*e.g.*, an AAV1, AAV5, or AAV6 capsid protein) comprising Y to F (tyrosine to phenylalanine) modifications or T to V (threonine to valine) modifications in the VP3 region of the capsid at positions corresponding to:

15 one or more of or each of Y705F, Y731F, and T492V of a wild-type AAV1 capsid protein (*e.g.*, SEQ ID NO: 1),

 one or more of or each of Y436F, Y693F, and Y719F of a wild-type AAV5 capsid protein (*e.g.*, SEQ ID NO: 2), or

 one or more of or each of Y705F, Y731F, and T492V of a wild-type AAV6 capsid
20 protein (*e.g.*, SEQ ID NO: 3).

 In some embodiments, the present disclosure provides an AAV capsid protein (*e.g.*, an AAV1, AAV5, or AAV6 capsid protein), wherein a VP3 region of the capsid protein comprises modifications (*e.g.*, replacement of a tyrosine residue with a non-tyrosine residue and/or a threonine residue with a non-threonine residue) at positions corresponding to:

25 one or more of or each of Y705, Y731, and T492 of a wild-type AAV1 capsid protein (*e.g.*, SEQ ID NO: 1),

 one or more of or each of Y436, Y693, and Y719 of a wild-type AAV5 capsid protein (*e.g.*, SEQ ID NO: 2), or

 one or more of or each of Y705, Y731, and T492 of a wild-type AAV6 capsid protein

(*e.g.*, SEQ ID NO: 3).

In some embodiments, the AAV capsid protein (*e.g.*, an AAV1, AAV5, or AAV6 capsid protein) comprises Y to F (tyrosine to phenylalanine) modifications or T to V (threonine to valine) modifications in the VP3 region of the capsid protein at positions
5 corresponding to:

one or more of or each of Y705F, Y731F, and T492V of a wild-type AAV1 capsid protein (*e.g.*, SEQ ID NO: 1),

one or more of or each of Y436F, Y693F, and Y719F of a wild-type AAV5 capsid protein (*e.g.*, SEQ ID NO: 2), or

10 one or more of or each of Y705F, Y731F, and T492V of a wild-type AAV6 capsid protein (*e.g.*, SEQ ID NO: 3).

In some embodiments, the present disclosure provides an rAAV particle comprising an AAV capsid protein (*e.g.*, an AAV1, AAV5, or AAV6 capsid protein), wherein the VP3 region of the capsid protein comprises modifications (*e.g.*, replacement of a tyrosine residue
15 with a non-tyrosine residue and/or a threonine residue with a non-threonine residue) at positions corresponding to:

one or more of or each of Y705, Y731, and T492 of a wild-type AAV1 capsid protein (*e.g.*, SEQ ID NO: 1),

one or more of or each of Y436, Y693, and Y719 of a wild-type AAV5 capsid protein
20 (*e.g.*, SEQ ID NO: 2), or

one or more of or each of Y705, Y731, and T492 of a wild-type AAV6 capsid protein (*e.g.*, SEQ ID NO: 3).

In some embodiments, the rAAV particle comprises an AAV capsid protein (*e.g.*, an AAV1, AAV5, or AAV6 capsid protein) comprising Y to F modifications or T to V
25 modifications in the VP3 region at positions corresponding to:

one or more of or each of Y705F, Y731F, and T492V of a wild-type AAV1 capsid protein (*e.g.*, SEQ ID NO: 1),

one or more of or each of Y436F, Y693F, and Y719F of a wild-type AAV5 capsid protein (*e.g.*, SEQ ID NO: 2), or

one or more of or each of Y705F, Y731F, and T492V of a wild-type AAV6 capsid protein (*e.g.*, SEQ ID NO: 3).

In related embodiments, the disclosure provides methods for using the viral particles, nucleic acids, vectors, proteins and compositions disclosed herein, and further provides processes for the transduction of one or more cells, one or more tissues, and/or one or more organs of interest, and particularly those of a mammalian animal using the disclosed viral particles. In an overall and general sense, such methods generally include at least the step of contacting a suitable host cell of interest with at least a first composition that comprises, consists essentially of, or alternatively consists of, an effective amount of a rAAV viral particle described herein, in an amount and for a time sufficient to transform at least a first cell or a first population of cells with a nucleic acid segment contained within the particle. In some embodiments, the viral particles of the present disclosure are preferably useful as vectors for introducing one or more nucleic acid segments to a selected host cell of interest. Preferably the host cell is a mammalian host cell, with human host cells being particularly preferred as targets for the rAAV particles described herein. In certain embodiments, such rAAV particles will comprise one or more isolated nucleic acid segments (*e.g.*, DNA segments) encoding a selected therapeutic and/or diagnostic agent, including, for example one or more polynucleotides comprising one or more genes of interest or other therapeutic agent(s) that are capable of being expressed in a mammalian host cell that has been transformed by one or more of the rAAV viral particles described herein. Exemplary therapeutic agents include a polypeptide, a peptide, an antibody, an antigen binding fragment, a ribozyme, a peptide nucleic acid, a siRNA, an RNAi, an antisense oligonucleotide and an antisense polynucleotide.

In some embodiments, a method is provided, comprising:

contacting a host cell (*e.g.*, a muscle cell) with an rAAV particle comprising an AAV1 capsid protein, wherein the AAV1 capsid protein comprises modifications (*e.g.*, replacement of a tyrosine residue with a non-tyrosine residue and/or a threonine residue with a non-threonine residue) in a VP3 region of the capsid protein at positions corresponding to Y705, Y731, and T492 of a wild-type AAV1 (*e.g.*, SEQ ID NO: 1). In some embodiments,

the AAV1 capsid protein comprises Y to F (tyrosine to phenylalanine) modifications or T to V (threonine to valine) modifications in the VP3 region of the capsid protein at positions corresponding to Y705F, Y731F, and T492V of a wild-type AAV1 capsid protein (*e.g.*, SEQ ID NO: 1). The contacting may be *in vitro* (*e.g.*, by administering to a cell in a dish or well) or *in vivo* (*e.g.*, by administering the rAAV particle, *e.g.*, as a composition comprising the rAAV particle, to a subject).

In some embodiments, a method is provided, comprising:

contacting a host cell (*e.g.*, a retinal or airway epithelial cell) with an rAAV particle comprising an AAV5 capsid protein, wherein the AAV5 capsid protein comprises modifications (*e.g.*, replacement of a tyrosine residue with a non-tyrosine residue and/or a threonine residue with a non-threonine residue) in a VP3 region of the capsid protein at positions corresponding to Y436, Y693, and Y719 of a wild-type AAV5 capsid protein (*e.g.*, SEQ ID NO: 2). In some embodiments, the AAV5 capsid protein comprises Y to F (tyrosine to phenylalanine) modifications in the VP3 region of the capsid protein at positions corresponding to Y436F, Y693F, and Y719F of a wild-type AAV5 capsid protein (*e.g.*, SEQ ID NO: 2). The contacting may be *in vitro* (*e.g.*, by administering to a cell in a dish or well) or *in vivo* (*e.g.*, by administering the rAAV particle, *e.g.*, as a composition comprising the rAAV particle, to a subject).

In some embodiments, a method is provided, comprising:

contacting a host cell (*e.g.*, a hematopoietic stem cell, a dendritic cell, a monocyte, airway an epithelial cell, a muscle cell, a liver cell, a pancreas cell or a microglial cell) with an rAAV particle comprising an AAV6 capsid protein, wherein the AAV6 capsid protein comprises modifications (*e.g.*, replacement of a tyrosine residue with a non-tyrosine residue and/or a threonine residue with a non-threonine residue) in a VP3 region of the capsid protein at positions corresponding to Y705, Y731, and T492 of a wild-type AAV6 capsid protein (*e.g.*, SEQ ID NO: 3). In some embodiments, the VP3 region of the AAV6 capsid protein comprises Y to F (tyrosine to phenylalanine) modifications or T to V (threonine to valine) modifications at positions corresponding to Y705F, Y731F, and T492V of a wild-type AAV6 capsid protein (*e.g.*, SEQ ID NO: 3). The contacting may be *in vitro* (*e.g.*, by administering

the rAAV particle to a cell in a dish or well) or *in vivo* (e.g., by administering the rAAV particle, e.g., as a composition comprising the rAAV particle, to a subject).

In another aspect, the disclosure further provides compositions comprising rAAV particles, and pharmaceutical formulations thereof, useful in methods for delivering genetic material encoding one or more beneficial or therapeutic product(s) to mammalian cells and tissues. In particular, the compositions and methods of the disclosure provide a significant advancement in the art through their use in the treatment, prevention, and/or amelioration of symptoms of one or more mammalian diseases. It is contemplated that human gene therapy will particularly benefit from the present teachings by providing new and improved rAAV particles for use in the treatment of a number of diverse diseases, disorders, and dysfunctions.

In another aspect, the disclosure concerns a rAAV particle as described herein comprising a rAAV nucleic acid vector that encodes one or more mammalian therapeutic agents for the prevention, treatment, and/or amelioration of one or more disorders in the mammal into which the vector construct is delivered. In particular, the disclosure provides rAAV particles comprising rAAV-based nucleic acid expression constructs that encode one or more mammalian therapeutic agent(s) (including, but not limited to, for example, protein(s), polypeptide(s), peptide(s), enzyme(s), a ribozyme, a peptide nucleic acid, a siRNA, an RNAi, an antisense oligonucleotide, an antisense polynucleotide, antibodies, antigen binding fragments, as well as variants, and/or active fragments thereof, for use in the treatment, prophylaxis, and/or amelioration of one or more symptoms of a mammalian disease, dysfunction, injury, and/or disorder). polypeptide, a peptide, an antibody, an antigen binding fragment,.

In some embodiments, the disclosure provides rAAV particles as described herein comprising rAAV nucleic acid vectors that comprise at least a first nucleic acid segment that encodes one or more therapeutic agents that alter, inhibit, reduce, prevent, eliminate, or impair the activity of one or more endogenous biological processes in the cell. In particular embodiments, such therapeutic agents may be those that selectively inhibit or reduce the effects of one or more metabolic processes, dysfunctions, disorders, or diseases. In certain embodiments, the defect may be caused by injury or trauma to the mammal for which

treatment is desired. In other embodiments, the defect may be caused by the over-expression of an endogenous biological compound, while in other embodiments still, the defect may be caused by the under-expression or even lack of one or more endogenous biological compounds.

5 When the use of such nucleic acid vectors is contemplated for introduction of one or more exogenous proteins, polypeptides, peptides, ribozymes, siRNAs, and/or antisense oligonucleotides, to a particular cell transfected with the nucleic acid vector, one may employ the AAV nucleic acid vectors disclosed herein by incorporating into the vector at least a first exogenous polynucleotide operably positioned downstream and under the control of at least a
10 first heterologous promoter that expresses the polynucleotide in a cell comprising the vector to produce the encoded therapeutic agent, including for example, peptides, proteins, polypeptides, antibodies, ribozymes, siRNAs, and antisense oligo- or polynucleotides. Such constructs may employ one or more heterologous promoters to express the therapeutic agent of interest. Such promoters may be constitutive, inducible, or even cell- or tissue-specific.
15 Exemplary promoters include, but are not limited to, a CMV promoter, a β -actin promoter, a hybrid CMV promoter, a hybrid β -actin promoter, an EF1 promoter, a U1a promoter, a U1b promoter, a Tet-inducible promoter, a VP16-LexA promoter, human parvovirus B19 promoter, a joint-specific promoter and a human-specific promoter.

 The rAAV nucleic acid vectors of the disclosure may also further comprise a second
20 nucleic acid segment that comprises, consists essentially of, or consists of, one or more enhancers, regulatory elements, transcriptional elements, to alter or effect transcription of the heterologous gene cloned in the rAAV nucleic acid vectors. For example, the rAAV nucleic acid vectors of the disclosure may further comprise a second nucleic acid segment that comprises, consists essentially of, or consists of, at least a first CMV enhancer, a synthetic
25 enhancer, or a cell- or tissue-specific enhancer. The second nucleic acid segment may also further comprise, consist essentially of, or consist of one or more intron sequences, post-transcriptional regulatory elements, or such like. The nucleic acid vectors of the disclosure may also optionally further comprise a third nucleic acid segment that comprises, consists essentially of, or consists of, one or more polylinker or multiple restriction sites/cloning

region(s) to facilitate insertion of one or more selected genetic elements, polynucleotides, and the like into the rAAV nucleic acid vectors at a convenient restriction site.

In aspects of the disclosure, the exogenous polynucleotides that are comprised within one or more of the rAAV nucleic acid vectors disclosed herein are preferably of mammalian origin, with polynucleotides encoding nucleic acids, polypeptides and peptides of human, primate, murine, porcine, bovine, ovine, feline, canine, equine, epine, caprine, or lupine origin being particularly preferred.

As described herein, the exogenous polynucleotide will preferably encode one or more proteins, polypeptides, peptides, enzymes, antibodies, siRNAs, ribozymes, antisense polynucleotides or oligonucleotides, PNA molecules, or a combination of two or more of these therapeutic agents. In fact, the exogenous polynucleotide may encode two or more such molecules, or a plurality of such molecules as may be desired. When combinational gene therapies are desired, two or more different molecules may be produced from a single rAAV expression system, or alternatively, a selected host cell may be transfected with two or more unique rAAV expression systems, each of which may comprise one or more distinct polynucleotides that encode a therapeutic agent.

In other embodiments, the disclosure also provides rAAV nucleic acid vectors that are comprised within an infectious rAAV viral particle (*e.g.*, an rAAV viral particle comprising a modified capsid protein as described herein), or pluralities of such particles, which themselves may also be comprised within one or more diluents, buffers, physiological solutions or pharmaceutical vehicles, formulated for administration to a mammal such as a human for therapeutic, and/or prophylactic gene therapy regimens. Such nucleic acid vectors or rAAV particles, and pluralities thereof may also be provided in excipient formulations that are acceptable for veterinary administration to selected livestock, exotic or domesticated animals, companion animals (including pets and such like), as well as non-human primates, zoological or otherwise captive specimens, and such like, wherein the use of such nucleic acid vectors and rAAV particles and related gene therapy is indicated to produce a beneficial effect upon administration to such an animal.

The disclosure also concerns host cells that comprise at least one of the disclosed rAAV particles or rAAV vectors. Such host cells include mammalian host cells, with human host cells being preferred, and may be either isolated, in cell or tissue culture. In the case of genetically modified animal models (*e.g.*, a mouse or dog), the transformed host cells may be
5 comprised within the body of a non-human animal itself.

Also provided herein is a method for the production of the rAAV particles described herein. In some embodiments, it is contemplated that one very significant advantage of the disclosed viral particles will be the ability to utilize lower titers of viral particles in mammalian transduction protocols, yet still retain transfection rates at a suitable level.

10 Compositions comprising one or more of the disclosed rAAV particles, rAAV nucleic acid vectors or host cells are also provided, and particularly those compositions that further comprise at least a first pharmaceutically-acceptable excipient for use in therapy, and for use in the manufacture of medicaments for the treatment of one or more mammalian diseases, disorders, dysfunctions, or trauma. Such pharmaceutical compositions may optionally further
15 comprise one or more diluents, buffers, liposomes, a lipid, a lipid complex, or the rAAV particles may be comprised within a microsphere or a nanoparticle. Pharmaceutical formulations suitable for intramuscular, intravenous, or direct injection into an organ or tissue or a plurality of cells or tissues of a human or other mammal are particularly preferred, however, the compositions disclosed herein may also find utility in administration to discreet
20 areas of the mammalian body, including for example, formulations that are suitable for direct injection into one or more organs, tissues, or cell types in the body. Such injection sites include, but are not limited to, a tissue such as a muscle or epithelium, or an organ such as the eye, or other site within a subject's body.

Also provided by the disclosure are kits comprising one or more of the disclosed
25 rAAV particles, vectors, proteins, transformed host cells or pharmaceutical compositions comprising such; and instructions for using the kit in a therapeutic, diagnostic, or clinical embodiment. Such kits may further comprise one or more reagents, restriction enzymes, peptides, therapeutics, pharmaceutical compounds, or means for delivery of the composition(s) to host cells, or to an animal (*e.g.*, syringes, injectables, and the like). Such

kits may be therapeutic kits for treating, preventing, or ameliorating the symptoms of a disease, deficiency, dysfunction, and/or injury, and may comprise one or more of the nucleic acid vectors, proteins, rAAV particles, or a plurality of such particles, and instructions for using the kit in a therapeutic and/or diagnostic medical regimen. Such kits may also be used
5 in large-scale production methodologies to produce large quantities of the viral particles themselves for commercial sale, or for use by others, including *e.g.*, virologists, medical professionals, and the like.

Another important aspect of the present disclosure concerns methods of use of the disclosed rAAV particles, nucleic acid vectors, protein compositions, and host cells described
10 herein in the preparation of medicaments for preventing, treating or ameliorating the symptoms of various diseases, dysfunctions, or deficiencies in an animal, such as a vertebrate mammal. Such methods generally involve administration to a mammal, such as a human in need thereof, one or more of the disclosed viral particles, nucleic acid vectors, host cells, compositions, or pluralities thereof, in an amount and for a time sufficient to prevent, treat, or
15 lessen the symptoms of such a disease, dysfunction, or deficiency in the affected animal. The methods may also encompass prophylactic treatment of animals suspected of having such conditions, or administration of such compositions to those animals at risk for developing such conditions either following diagnosis, or prior to the onset of symptoms.

In other embodiments, the disclosure also provides the disclosed rAAV nucleic acid
20 vectors comprised within a rAAV particle, comprised within one or more pharmaceutical carriers, and may be formulated for administration to a mammal such as a human for therapeutic, and/or prophylactic gene therapy regimens. Such vectors may also be provided in pharmaceutical formulations that are acceptable for veterinary administration to selected livestock, domesticated animals, pets, and the like.

25 Another aspect of the present disclosure concerns methods of use of the disclosed vectors, viral particles, compositions, and host cells described herein in the preparation of medicaments for treating or ameliorating the symptoms of various polypeptide deficiencies in a mammal. Such methods generally involve administration to a mammal, or human in need thereof, one or more of the disclosed nucleic acid vectors, viral particles, host cells, or

compositions, in an amount and for a time sufficient to treat or ameliorate the symptoms of such a deficiency in the affected mammal. The methods may also encompass prophylactic treatment of animals suspected of having such conditions, or administration of such compositions to those animals at risk for developing such conditions either following
 5 diagnosis, or prior to the onset of symptoms.

The details of one or more embodiments of the disclosure are set forth in the description below. Other features or advantages of the present disclosure will be apparent
 10 from the following drawings and detailed description of several embodiments, and also from the appending claims.

BRIEF DESCRIPTION OF THE SEQUENCES

SEQ ID NO:1 is an exemplary amino acid sequence of the capsid protein of the wild-
 15 type adeno-associated virus serotype 1 (AAV1);

SEQ ID NO:2 is an exemplary amino acid sequence of the capsid protein of the wild-
 type adeno-associated virus serotype 5 (AAV5);

SEQ ID NO:3 is an exemplary amino acid sequence of the capsid protein of the wild-
 type adeno-associated virus serotype 6 (AAV6).

20 SEQ ID NO: 1 (exemplary positions Y705, Y731, and T492 are each bolded, underlined, and italicized)

1 MAADGYLPDW LEDNLSEGIR EWWDLKPGAP KPKANQQKQD DGRGLVLPGY
 51 KYLGPFNGLD KGEPVNAADA AALEHDKAYD QQLKAGDNPY LRYNHADA EF
 25 101 QERLQEDTSF GGNLGRAVFQ AKKRVLEPLG LVEEGAKTAP GKKRPVEQSP
 151 QEPDSSSGIG KTGQQPAKKR LNFGQTGDSE SVPDPQPLGE PPATPAAVGP
 201 TTMASGGGAP MADNNEGADG VGNASGNWHC DSTWLGDRVI TTSTRTWALP
 251 TYNNHLYKQI SSASTGASND NHYFGYSTPW GYFDFNRFHC HFSPRDWQRL
 301 INNNWGFRPK RLNFKLFNIQ VKEVTTNDGV TTIANNLTST VQVFSDSEYQ
 30 351 LPYVLGSAHQ GCLPPFPADV FMIPQYGYLT LNNGSQAVGR SSFYCLEYFP

401 SQMLRTGNNF TFSYTFEEVP FHSSYAHSQS LDRLMNPLID QYLYYLNRTQ
 451 NQSGSAQNKD LLFSRGSPAG MSVQPKNWLP GPCYRQQRVS **K**TKTDNNNSN
 501 FTWTGASKYN LNGRESIINP GTAMASHKDD EDKFFPMSGV MIFGKESAGA
 551 SNTALDNVMI TDEEEIKATN PVATERFGTV AVNFQSSSTD PATGDVHAMG
 5 601 ALPGMVWQDR DVYLQGPIWA KIPHTDGHFH PSPLMGGFGL KNPPQILIK
 651 NTPVPANPPA EFSATKFASF ITQYSTGQVS VEIEWELQKE NSKRWNPEVQ
 701 YTSN**Y**AKSAN VDFTVDNNGL YTEPRPIGTR **Y**LTRPL

SEQ ID NO: 2 (exemplary positions Y436, Y693, and Y719 are each bolded, underlined, and
 10 italicized)

1 MSFVDHPPDW LEEVGEGFRE FLGLEAGPPK PKPNQQHQDQ ARGLVLPGYN
 51 YLPGNGGLDR GEPVNRADDEV AREHDISYNE QLEAGDNPYL KYNHADADEFQ
 101 EKLADDTSGF GNLGKAVFQA KKRVLPEFGL VEEGAKTAPT GKRIDDHFPK
 151 RKKARTEEDS KPSTSSDAEA GPSGSQQLQI PAQPASSLGA DTMSAGGGGP
 15 201 LGDNNQGADG VGNASGDWHC DSTWMGDRVV TKSTRTWVLP SYNHNHQRREI
 251 KSGSVDGSNA NAYFGYSTPW GYFDFNRFHS HWSRPDWQRL INNYWGFRPR
 301 SLRVKIFNIQ VKEVTVQDST TTIANNLTST VQVFTDDDDYQ LPYVVGNGTE
 351 GCLPAFPQV FTLPPQYGYAT LNRDNTENPT ERSSFFCLEY FPSKMLRTGN
 401 NFEFTYNFEE VPFHSSFAPS QNLFKLANPL VDQYL**Y**RFVS TNNTGGVQFN
 20 451 KNLAGRYANT YKNWFPGPMG RTQGWNLGSG VNRASVSFAA TTNRMEELEGA
 501 SYQVPPQPNG MTNNLQGSNT YALENTMIFN SQPANPGTTA TYLEGNMLIT
 551 SESETQPVNR VAYNVGGQMA TNNQSSTTAP ATGTYNLQEI VPGSVWMERD
 601 VYLQGPIWAK IPETGAHFHP SPAMGGFGLK HPPPMMLIKN TPVPGNITSF
 651 SDVPVSSFIT QYSTGQVTVE MEWELKKENS KRWNPEIQYT NN**Y**NDPQFVD
 25 701 FAPDSTGEYR TTRPIGTR**Y**L TRPL

SEQ ID NO: 3 (exemplary positions Y705, Y731, and T492 are each bolded, underlined, and
 italicized)

1 MAADGYLPDW LEDNLSEGIR EWWDLKPGAP KPKANQQKQD DGRGLVLPGY

51 KYLGPFNGLD KGEPVNAADA AALEHDKAYD QQLKAGDNPY LRYNHADAEF
 101 QERLQEDTSF GGNLGRAVFQ AKKRVLEPFG LVEEGAKTAP GKKRPVEQSP
 151 QEPDSSSGIG KTGQQPAKKR LNFGQTGDSE SVPDPQPLGE PPATPAAVGP
 201 TTMASGGGAP MADNNEGADG VGNASGNWHC DSTWLGDRVI TTSTRTWALP
 5 251 TYNNHLYKQI SSASTGASND NHYFGYSTPW GYFDFNRFHC HFSPRDWQRL
 301 INNNWGFRPK RLNFKLFNIQ VKEVTTNDGV TTIANNLTST VQVFSDSEYQ
 351 LPYVLGSAHQ GCLPPFPADV FMIPQYGYLT LNNGSQAVGR SSFYCLEYFP
 401 SQMLRTGNNF TFSYTFEDVP FHSSYAHSQS LDRLMNPLID QYLYFLNRTQ
 451 NQSGSAQNKD LLFSRGSPAG MSVQPKNWLP GPCYRQQRVS K7KTDNNNSN
 10 501 FTWTGASKYN LNGRESIINP GTAMASHKDD KDKFFPMSGV MIFGKESAGA
 551 SNTALDNVMI TDEEEIKATN PVATERFGTV AVNLQSSSTD PATGDVHVMG
 601 ALPGMVWQDR DVYLQGPIWA KIPHTDGHFH PSPLMGGFGL KHPPPQILIK
 651 NTPVPANPPA EFSATKFASF ITQYSTGQVS VEIEWELQKE NSKRWNPEVQ
 701 YTSNYAKSAN VDFTVDNNGL YTEPRPIGTR YLTRPL

BRIEF DESCRIPTION OF THE DRAWINGS

The following drawings form part of the present specification and are included to
 further demonstrate certain aspects of the present disclosure, which can be better understood
 20 by reference to one or more of these drawings in combination with the detailed description of
 specific embodiments presented herein.

FIG. 1A is a series of photographs and a graph showing EGFP expression in a murine
 muscle cell line treated with wild-type (WT) or mutant AAV1 vectors.

FIG. 1B is a graph showing the total Alpha-1 antitrypsin (AAT) concentration in
 25 muscle from mice injected with wild-type AAV1 vectors or mutant AAV1
 Y705F+Y731F+T492V vector (AAV1m).

FIG. 2 is a photograph showing mCherry expression in major organs of mice
 following injection of wild-type (WT) or mutant AAV6 vectors.

FIG. 3 is a series of photographs showing EGFP expression in mouse pancreas at the
 30 interior or edge following injection with wild-type (WT) or mutant AAV6 vectors.

FIG. 4 is a series of photographs showing EGFP expression in primary murine glial cells cultured from mice injected with wild-type (WT) or mutant AAV6 vectors.

FIG. 5 is a series of photographs showing EGFP expression in murine glial cells in mice injected with wild-type (WT) or mutant AAV6 vectors.

FIG. 6 is a series of photographs showing EGFP expression in neurons and astrocytes in brains from mice injected with mutant AAV6 Y705+731F+T492V vectors.

FIG. 7 is a series of photographs and a graph showing EGFP expression in human airway epithelial cells transduced with wild-type (WT) or mutant AAV1, AAV2, AAV5, or AAV6 vectors.

FIG. 8A is a series of photographs and a graph showing EGFP expression in primary human monocyte-derived dendritic cells transduced with wild-type (WT) or mutant AAV2 vectors.

FIG. 8B is a series of photographs and a graph showing EGFP expression in primary human monocyte-derived dendritic cells transduced with wild-type (WT) AAV6 or mutant AAV2 or AAV6 vectors.

DETAILED DESCRIPTION OF THE INVENTION

The present disclosure provides AAV capsid proteins comprising modifications of one or more of a combination of the surface-exposed threonine and/or tyrosine residues in the VP3 region. Also provided are rAAV particles that include one or more of the AAV capsid protein mutations disclosed herein, as well as nucleic acid molecules encoding the AAV capsid proteins disclosed herein. Advantageously, the rAAV particles of the present disclosure have improved transduction efficiency in a variety of cells, tissues and organs of interest, when compared to rAAV viral particles comprising wild-type capsid proteins. In particular, as described herein, it was found that rAAV particles comprising a modified AAV1 capsid protein having three mutations at Y705F+Y731F+T492V efficiently transduced muscle. It was also found that rAAV particles comprising a modified AAV5 capsid protein having three mutations at Y436F+Y693F+Y719F efficiently transduced retinal

cells and airway epithelial cells. Lastly, it was found that rAAV particles comprising a modified AAV6 capsid protein having three mutations at Y705F+Y731F+T492V efficiently transduced multiple cells and tissues including hematopoietic stem cells, dendritic cells, monocytes, airway epithelial cells, muscle, liver, pancreas and microglial cells. Accordingly, aspects of the disclosure relate to rAAV particles, capsid proteins, nucleic acid vectors, host cells, compositions, kits, and uses thereof.

RECOMBINANT AAV VECTORS AND VIRAL PARTICLES

One aspect of the disclosure provides AAV capsid proteins, such as AAV VP3 capsid proteins, or VP1 or VP2 capsid proteins comprising a VP3 region, comprising modifications of a combination of the surface-exposed threonine and/or tyrosine residues. Also provided are rAAV particles comprising the AAV capsid proteins, as well as nucleic acid molecules encoding the AAV capsid proteins of the present disclosure. Advantageously, the rAAV particles described herein have improved efficiency in transduction of a variety of cells, tissues and organs of interest, when compared to rAAV particles comprising wild-type AAV capsid proteins.

In some embodiments, the present disclosure provides a nucleic acid molecule comprising a nucleotide sequence encoding an AAV capsid protein (*e.g.*, an AAV1, AAV5, or AAV6 capsid protein), wherein the AAV capsid protein comprises modifications of a combination of the surface-exposed threonine and/or tyrosine residues at positions within the VP3 region.

In one embodiment, the nucleic acid molecule comprises a nucleotide sequence encoding an AAV capsid protein, the AAV capsid protein comprising one of the following modifications in the VP3 region:

(i) a non-tyrosine residue at one or more of or at each of the positions that correspond to Y705 and Y731 of a wild-type AAV1 capsid protein (*e.g.*, SEQ ID NO: 1) and a non-threonine residue at a position that corresponds to T492 in the wild-type AAV1 protein;

(ii) a chemically-modified tyrosine residue at one or more of or at each of the positions that correspond to Y705 and Y731 of a wild-type AAV1 capsid protein (*e.g.*, SEQ

ID NO: 1) and a chemically-modified threonine residue at a position that corresponds to T492 of the wild-type AAV1 capsid protein;

(iii) a non-tyrosine residue at one or more of or at each of the positions that correspond to Y436F, Y693F, and Y719F of a wild-type AAV5 capsid protein (*e.g.*, SEQ ID NO: 2);

(iv) a chemically-modified tyrosine residue at one or more of or at each of the positions that correspond to Y436F, Y693F, and Y719F of a wild-type AAV5 capsid protein (*e.g.*, SEQ ID NO: 2);

(v) a non-tyrosine residue at one or more of or at each of the positions that correspond to Y705 and Y731 of a wild-type AAV6 capsid protein (*e.g.*, SEQ ID NO: 3) and a non-threonine residue at a position that corresponds to T492 of the wild-type AAV6 capsid protein; or

(vi) a chemically-modified tyrosine residue at one or more of or at each of the positions that correspond to Y705 and Y731 of a wild-type AAV6 capsid protein (*e.g.*, SEQ ID NO: 3) and a chemically-modified threonine residue at a position that corresponds to T492 of the wild-type AAV6 capsid protein.

In some embodiments, the present disclosure provides an AAV capsid protein (*e.g.*, an AAV1, AAV5, or AAV6 capsid protein), wherein the AAV capsid protein comprises modifications of a combination of the surface-exposed threonine and/or tyrosine residues at positions within the VP3 region.

In one embodiment, the AAV capsid protein comprises one of the following modifications in the VP3 region:

(i) a non-tyrosine residue at one or more of or at each of the positions that correspond to Y705 and Y731 of a wild-type AAV1 capsid protein (*e.g.*, SEQ ID NO: 1) and a non-threonine residue at a position that corresponds to T492 in the wild-type AAV1 protein;

(ii) a chemically-modified tyrosine residue at one or more of or at each of the positions that correspond to Y705 and Y731 of a wild-type AAV1 capsid protein (*e.g.*, SEQ ID NO: 1) and a chemically-modified threonine residue at a position that corresponds to T492 of the wild-type AAV1 capsid protein;

(iii) a non-tyrosine residue at one or more of or at each of the positions that correspond to Y436F, Y693F, and Y719F of a wild-type AAV5 capsid protein (*e.g.*, SEQ ID NO: 2);

(iv) a chemically-modified tyrosine residue at one or more of or at each of the positions that correspond to Y436F, Y693F, and Y719F of a wild-type AAV5 capsid protein (*e.g.*, SEQ ID NO: 2);

(v) a non-tyrosine residue at one or more of or at each of the positions that correspond to Y705 and Y731 of a wild-type AAV6 capsid protein (*e.g.*, SEQ ID NO: 3) and a non-threonine residue at a position that corresponds to T492 of the wild-type AAV6 capsid protein; or

(vi) a chemically-modified tyrosine residue at one or more of or at each of the positions that correspond to Y705 and Y731 of a wild-type AAV6 capsid protein (*e.g.*, SEQ ID NO: 3) and a chemically-modified threonine residue at a position that corresponds to T492 of the wild-type AAV6 capsid protein.

In some embodiments, the present disclosure provides an rAAV particle comprising an AAV capsid protein (*e.g.*, an AAV1, AAV5, or AAV6 capsid protein), wherein the AAV capsid protein comprises modifications of a combination of the surface-exposed threonine and/or tyrosine residues at positions within the VP3 region.

In one embodiment, the rAAV particle comprises an AAV capsid protein, the AAV capsid protein comprising one of the following modifications in the VP3 region:

(i) a non-tyrosine residue at one or more of or at each of the positions that correspond to Y705 and Y731 of a wild-type AAV1 capsid protein (*e.g.*, SEQ ID NO: 1) and a non-threonine residue at a position that corresponds to T492 in the wild-type AAV1 protein;

(ii) a chemically-modified tyrosine residue at one or more of or at each of the positions that correspond to Y705 and Y731 of a wild-type AAV1 capsid protein (*e.g.*, SEQ ID NO: 1) and a chemically-modified threonine residue at a position that corresponds to T492 of the wild-type AAV1 capsid protein;

(iii) a non-tyrosine residue at one or more of or at each of the positions that correspond to Y436F, Y693F, and Y719F of a wild-type AAV5 capsid protein (*e.g.*, SEQ ID NO: 2);

(iv) a chemically-modified tyrosine residue at one or more of or at each of the positions that correspond to Y436F, Y693F, and Y719F of a wild-type AAV5 capsid protein (*e.g.*, SEQ ID NO: 2);

(v) a non-tyrosine residue at one or more of or at each of the positions that correspond to Y705 and Y731 of a wild-type AAV6 capsid protein (*e.g.*, SEQ ID NO: 3) and a non-threonine residue at a position that corresponds to T492 of the wild-type AAV6 capsid protein; or

(vi) a chemically-modified tyrosine residue at one or more of or at each of the positions that correspond to Y705 and Y731 of a wild-type AAV6 capsid protein (*e.g.*, SEQ ID NO: 3) and a chemically-modified threonine residue at a position that corresponds to T492 of the wild-type AAV6 capsid protein.

In some embodiments, modified AAV capsid protein does not result in phosphorylation and/or ubiquitination of an rAAV particle comprising the capsid protein and/or increases the efficiency of transduction of such a viral particle into a cell or tissue compared to a rAAV particle comprising a corresponding wild-type capsid protein (*e.g.*, a AAV1, AAV5, or AAV6 wild-type capsid protein, such as SEQ ID NOs:1-3).

In one embodiment, the nucleic acid molecule comprises a nucleotide sequence encoding an AAV capsid protein (*e.g.*, a VP3 capsid protein), wherein the AAV serotype is selected from AAV1, AAV5, and AAV6. In certain embodiments, the wild-type AAV capsid protein has an amino acid sequence selected from SEQ ID NOs: 1-3.

In some embodiments, the nucleic acid molecule comprises a nucleotide sequence encoding an AAV1 capsid protein. The adeno-associated virus 1 (AAV1) is a non-pathogenic human parvovirus. Recombinant AAV1 vectors are currently in use in Phase I/II clinical trials for gene therapy of a number of diseases such as alpha-1 antitrypsin deficiency, LPL deficiency, Pompe's disease and muscular dystrophy. In some embodiments, the nucleic acid molecule comprises a nucleotide sequence encoding an AAV5 capsid protein.

Recombinant AAV5 vectors are currently in use in Phase I/II clinical trials for gene therapy of diseases such as Acute Intermittent Porphyria. In some embodiments, the nucleic acid molecule comprises a nucleotide sequence encoding an AAV6 capsid protein. Recombinant AAV6 vectors are currently in use in Phase I/II clinical trials for gene therapy of diseases such as severe heart failure.

In one embodiment, a surface-exposed threonine residue corresponding to a threonine residue of a wild-type AAV capsid sequence described herein (*e.g.*, SEQ ID NOs:1-3) is modified into a non-threonine residue and/or is chemically modified so that said non-threonine residue or said modified threonine residue does not result in phosphorylation and/or ubiquitination of an AAV viral particle. In some embodiments, the surface-exposed threonine residue of the AAV capsid is modified into valine (V).

In some embodiments, a surface-exposed tyrosine residue corresponding to a tyrosine residue of a wild-type AAV capsid sequence described herein (*e.g.*, SEQ ID NOs:1-3) is modified into a non-tyrosine residue and/or is chemically modified so that said non-tyrosine residue or said modified tyrosine residue does not result in phosphorylation and/or ubiquitination of an AAV viral particle. In some embodiments, the surface-exposed tyrosine residue of the AAV capsid is modified into phenylalanine (F).

In some embodiments, the disclosure provides a rAAV particle comprising an AAV capsid protein described herein (*e.g.*, a Y705F+Y731F+T492V AAV1 modified capsid protein, a Y436F+Y693F+Y719F AAV5 modified capsid protein, or a Y705F+Y731F+T492V AAV6 modified capsid protein). In some embodiments, a rAAV particle comprises a AAV nucleic acid vector described herein and a capsid comprising the modified capsid protein (*e.g.*, a Y705F+Y731F+T492V AAV1 modified capsid protein, a Y436F+Y693F+Y719F AAV5 modified capsid protein, or a Y705F+Y731F+T492V AAV6 modified capsid protein), wherein the capsid encapsidates the AAV nucleic acid vector. In one embodiment, the rAAV particle has enhanced transduction efficiency, when compared to the wild-type rAAV particle. In another embodiment, the rAAV particle is capable of efficient transduction of cells, tissues, and/or organs of interest.

In some embodiments, the rAAV nucleic acid vector comprises inverted terminal repeat sequences (ITRs), such as those derived from a wild-type AAV genome, such as the AAV2 genome. In some embodiments, the rAAV nucleic acid vector further comprises a transgene (also referred to as a heterologous nucleic acid molecule) operably linked to a promoter and optionally, other regulatory elements, wherein the ITRs flank the transgene. In some embodiments, the rAAV nucleic acid vector comprises a transgene, wherein the transgene is a gene of interest operatively linked to a promoter (*e.g.*, a heterologous promoter, for example, a promoter sequence non-native to the gene of interest) and flanked by ITRs. In one embodiment, the transgene encodes a therapeutic agent of interest.

Exemplary promoters include one or more heterologous, tissue-specific, constitutive or inducible promoters, including, but not limited to, a promoter selected from the group consisting of cytomegalovirus (CMV) promoters, desmin (DES), beta-actin promoters, insulin promoters, enolase promoters, BDNF promoters, NGF promoters, EGF promoters, growth factor promoters, axon-specific promoters, dendrite-specific promoters, brain-specific promoters, hippocampal-specific promoters, kidney-specific promoters, elafin promoters, cytokine promoters, interferon promoters, growth factor promoters, alpha-1 antitrypsin promoters, brain-specific promoters, neural cell-specific promoters, central nervous system cell-specific promoters, peripheral nervous system cell-specific promoters, interleukin promoters, serpin promoters, hybrid CMV promoters, hybrid beta-actin promoters, EF1 promoters, U1a promoters, U1b promoters, Tet-inducible promoters and VP16-LexA promoters. In exemplary embodiments, the promoter is a mammalian or avian beta-actin promoter.

Exemplary enhancer sequences include, but are not limited to, one or more selected from the group consisting of CMV enhancers, synthetic enhancers, liver-specific enhancers, vascular-specific enhancers, brain-specific enhancers, neural cell-specific enhancers, lung-specific enhancers, muscle-specific enhancers, kidney-specific enhancers, pancreas-specific enhancers, and islet cell-specific enhancers.

Exemplary therapeutic agents include, but are not limited to, an agent selected from the group consisting of polypeptides, peptides, antibodies, antigen binding fragments,

ribozymes, peptide nucleic acids, siRNA, RNAi, antisense oligonucleotides and antisense polynucleotides.

In exemplary embodiments, the rAAV nucleic acid vectors of the disclosure encode a therapeutic protein or polypeptide (*e.g.*, as a transgene operatively linked to a heterologous promoter, for example, a promoter sequence non-native to the transgene) selected from the group consisting of adrenergic agonists, anti-apoptosis factors, apoptosis inhibitors, cytokine receptors, cytokines, cytotoxins, erythropoietic agents, glutamic acid decarboxylases, glycoproteins, growth factors, growth factor receptors, hormones, hormone receptors, interferons, interleukins, interleukin receptors, kinases, kinase inhibitors, nerve growth factors, netrins, neuroactive peptides, neuroactive peptide receptors, neurogenic factors, neurogenic factor receptors, neuropilins, neurotrophic factors, neurotrophins, neurotrophin receptors, N-methyl-D-aspartate antagonists, plexins, proteases, protease inhibitors, protein decarboxylases, protein kinases, protein kinase inhibitors, proteolytic proteins, proteolytic protein inhibitors, semaphorin a semaphorin receptors, serotonin transport proteins, serotonin uptake inhibitors, serotonin receptors, serpins, serpin receptors, and tumor suppressors.

In exemplary embodiments, the rAAV nucleic acid vectors may comprise a nucleic acid segment that encodes a polypeptide selected from the group consisting of BDNF, CNTF, CSF, EGF, FGF, G-SCF, GM-CSF, gonadotropin, IFN, IFG-1, M-CSF, NGF, PDGF, PEDF, TGF, TGF-B2, TNF, VEGF, prolactin, somatotropin, XIAP1, IL-1, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-10(187A), viral IL-10, IL-11, IL-12, IL-13, IL-14, IL-15, IL-16, IL-17, and IL-18. Such therapeutic agents may be of human, murine, avian, porcine, bovine, ovine, feline, canine, equine, epine, caprine, lupine or primate origin.

Exemplary rAAV nucleic acid vectors useful according to the disclosure include single-stranded (ss) or self-complementary (sc) AAV nucleic acid vectors.

The rAAV nucleic acid vectors or rAAV particles of the present disclosure may also be within an isolated mammalian host cell, including for example, human, primate, murine, feline, canine, porcine, ovine, bovine, equine, epine, caprine and lupine host cells. The rAAV nucleic acid vectors or rAAV particles may be within an isolated mammalian host cell such

as a human endothelial, epithelial, vascular, liver, lung, heart, pancreas, intestinal, kidney, cardiac, cancer or tumor, muscle, bone, neural, blood, or brain cell.

The rAAV particle may be of any AAV serotype, including any derivative or pseudotype (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13, or pseudotypes/derivatives thereof).

5 For example, any ITR sequence derived or modified from an AAV serotype (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13) can be used with viral particles comprising capsid proteins of a different serotype (*e.g.*, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, or 13, or derivatives thereof).

Methods of producing rAAV particles and nucleic acid vectors are also known in the art and commercially available (see, *e.g.*, Zolotukhin et al. Production and purification of
10 serotype 1, 2, and 5 recombinant adeno-associated viral vectors. *Methods* 28 (2002) 158–167; and U.S. Patent Publication Numbers US20070015238 and US20120322861, which are incorporated herein by reference; and plasmids and kits available from ATCC and Cell Biolabs, Inc.). For example, a plasmid containing the nucleic acid vector may be combined with one or more helper plasmids, *e.g.*, that contain a rep gene (*e.g.*, encoding Rep78, Rep68,
15 Rep52 and Rep40) and a cap gene (encoding VP1, VP2, and VP3, including a modified VP3 region as described herein), and transfected into a producer cell line such that the rAAV particle can be packaged and subsequently purified.

In some embodiments, the one or more helper plasmids is a first helper plasmid comprising a rep gene and a cap gene and a second helper plasmid comprising a E1a gene, a
20 E1b gene, a E4 gene, a E2a gene, and a VA gene. In some embodiments, the rep gene is a rep gene derived from AAV2 and the cap gene is derived from AAV1, AAV5, and AAV6 and includes modifications to the gene in order to produce the modified capsid protein described herein. Helper plasmids, and methods of making such plasmids, are known in the art and commercially available (see, *e.g.*, pDM, pDG, pDP1rs, pDP2rs, pDP3rs, pDP4rs,
25 pDP5rs, pDP6rs, pDG(R484E/R585E), and pDP8.ape plasmids from PlasmidFactory, Bielefeld, Germany; other products and services available from Vector Biolabs, Philadelphia, PA; Cell Biolabs, San Diego, CA; Agilent Technologies, Santa Clara, Ca; and Addgene, Cambridge, MA; pxx6; Grimm et al. (1998), *Novel Tools for Production and Purification of Recombinant Adenoassociated Virus Vectors*, Human Gene Therapy, Vol. 9, 2745-2760;

Kern, A. et al. (2003), Identification of a Heparin-Binding Motif on Adeno-Associated Virus Type 2 Capsids, *Journal of Virology*, Vol. 77, 11072-11081.; Grimm et al. (2003), Helper Virus-Free, Optically Controllable, and Two-Plasmid-Based Production of Adeno-associated Virus Vectors of Serotypes 1 to 6, *Molecular Therapy*, Vol. 7, 839-850; Kronenberg et al. (2005), A Conformational Change in the Adeno-Associated Virus Type 2 Capsid Leads to the Exposure of Hidden VP1 N Termini, *Journal of Virology*, Vol. 79, 5296-5303; and Moullier, P. and Snyder, R.O. (2008), International efforts for recombinant adeno-associated viral vector reference standards, *Molecular Therapy*, Vol. 16, 1185-1188).

An exemplary, non-limiting, rAAV particle production method is described next.

One or more helper plasmids are produced or obtained, which comprise rep and cap ORFs for the desired AAV serotype and the adenoviral VA, E2A (DBP), and E4 genes under the transcriptional control of their native promoters. The cap ORF may also comprise one or more modifications to produce a modified capsid protein as described herein. HEK293 cells (available from ATCC®) are transfected via CaPO₄-mediated transfection, lipids or polymeric molecules such as Polyethylenimine (PEI) with the helper plasmid(s) and a plasmid containing a nucleic acid vector described herein. The HEK293 cells are then incubated for at least 60 hours to allow for rAAV particle production. Alternatively, in another example Sf9-based producer stable cell lines are infected with a single recombinant baculovirus containing the nucleic acid vector. As a further alternative, in another example HEK293 or BHK cell lines are infected with a HSV containing the nucleic acid vector and optionally one or more helper HSVs containing rep and cap ORFs as described herein and the adenoviral VA, E2A (DBP), and E4 genes under the transcriptional control of their native promoters. The HEK293, BHK, or Sf9 cells are then incubated for at least 60 hours to allow for rAAV particle production. The rAAV particles can then be purified using any method known the art or described herein, *e.g.*, by iodixanol step gradient, CsCl gradient, chromatography, or polyethylene glycol (PEG) precipitation.

Exemplary nucleic acid sequences for producing mutated AAV1, AAV5 and AAV6 capsid proteins as described herein are provided below.

AAV1 (SEQ ID NO: 4):

ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACAACCTCTCTGAGGGCA
TTCGCGAGTGGTGGGACTTGAAACCTGGAGCCCCGAAGCCCAAAGCCAACCAGC
5 AAAAGCAGGACGACGGCCGGGGTCTGGTGCTTCCTGGCTACAAGTACCTCGGAC
CCTTCAACGGACTCGACAAGGGGGGAGCCCGTCAACGCGGCGGACGCAGCGGCCC
TCGAGCACGACAAGGCCTACGACCAGCAGCTCAAAGCGGGTGACAATCCGTACC
TGCGGTATAACCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAGATACGT
CTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAGAAGCGGGTTCTCG
10 AACCTCTCGGTCTGGTTGAGGAAGGCGCTAAGACGGCTCCTGGAAAGAAACGTC
CGGTAGAGCAGTCGCCACAAGAGCCAGACTCCTCCTCGGGCATCGGCAAGACAG
GCCAGCAGCCCGCTAAAAAGAGACTCAATTTTGGTCAGACTGGCGACTCAGAGT
CAGTCCCCGATCCACAACCTCTCGGAGAACCTCCAGCAACCCCCGCTGCTGTGGG
ACCTACTACAATGGCTTCAGGCGGTGGCGCACCAATGGCAGACAATAACGAAGG
15 CGCCGACGGAGTGGGTAATGCCTCAGGAAATTGGCATTGCGATTCCACATGGCT
GGGCGACAGAGTCATCACCACCAGCACCCGCACCTGGGCCTTGCCCCACCTACAA
TAACCACCTCTACAAGCAAATCTCCAGTGCTTCAACGGGGGCCAGCAACGACAA
CCACTACTTCGGCTACAGCACCCCCTGGGGGTATTTTGATTTCACAGATTCCAC
TGCCACTTTTACCACGTGACTGGCAGCGACTCATCAACAACAATTGGGGATTCC
20 GGCCCAAGAGACTCAACTTCAAACCTCTTCAACATCCAAGTCAAGGAGGTCACGA
CGAATGATGGCGTCACAACCATCGCTAATAACCTTACCAGCACGGTTCAAGTCTT
CTCGGACTCGGAGTACCAGCTTCCGTACGTCCTCGGCTCTGCGCACCAAGGGCTGC
CTCCCTCCGTTCCCGGCGGACGTGTTTCATGATTCCGCAATACGGCTACCTGACGC
TCAACAATGGCAGCCAAGCCGTGGGACGTTTCATCCTTTTACTGCCTGGAATATTT
25 CCCTTCTCAGATGCTGAGAACGGGCAACAACCTTTACCTTCAGCTACACCTTTGAG
GAAGTGCCTTTCCACAGCAGCTACGCGCACAGCCAGAGCCTGGACCGGCTGATG
AATCCTCTCATCGACCAATACCTGTATTACCTGAACAGAACTCAAAATCAGTCCG
GAAGTGCCCAAAACAAGGACTTGCTGTTTAGCCGTGGGTCTCCAGCTGGCATGTC
TGTTTCAGCCCAAAAACCTGGCTACCTGGACCCTGTTATCGGCAGCAGCGCGTTTCT

AAAGTAAAAACAGACAACAACAGCAATTTTACCTGGACTGGTGCTTCAAAA
TATAACCTCAATGGGCGTGAATCCATCATCAACCCTGGCACTGCTATGGCCTCAC
ACAAAGACGACGAAGACAAGTTCTTTCCCATGAGCGGTGTCATGATTTTTGGAAA
AGAGAGCGCCGGAGCTTCAAACACTGCATTGGACAATGTCATGATTACAGACGA
5 AGAGGAAATTAAAGCCACTAACCCTGTGGCCACCGAAAGATTTGGGACCGTGGC
AGTCAATTTCCAGAGCAGCAGCACAGACCCTGCGACCGGAGATGTGCATGCTAT
GGGAGCATTACCTGGCATGGTGTGGCAAGATAGAGACGTGTACCTGCAGGGTCC
CATTTGGGCCAAAATTCCTCACACAGATGGACACTTTCACCCGTCTCCTCTTATG
GGCGGCTTTGGACTCAAGAACCCGCCTCCTCAGATCCTCATCAAAAACACGCCTG
10 TTCCTGCGAATCCTCCGGCGGAGTTTTCAGCTACAAAGTTTGCTTCATTCATCACC
CAATACTCCACAGGACAAGTGAGTGTGGAAATTGAATGGGAGCTGCAGAAAGAA
AACAGCAAGCGCTGGAATCCCGAAGTGCAGTACACATCCAATTTTGCAAAATCT
GCCAATGTTGATTTTACTGTGGACAACAATGGACTTTTATACTGAGCCTCGCCCCA
TTGGCACGCGTTTCCTTACCCGTCCCCTGTAA

AAV5 (SEQ ID NO: 5):

ATGTCTTTTGTGATCACCCCTCCAGATTGGTTGGAAGAAGTTGGTGAAGGTCTTC
GCGAGTTTTTGGGCCTTGAAGCGGGCCACCGAAACCAAAACCCAATCAGCAGC
ATCAAGATCAAGCCCGTGGTCTTGTGCTGCCTGGTTATAACTATCTCGGACCCGG
20 AAACGGTCTCGATCGAGGAGAGCCTGTCAACAGGGCAGACGAGGTCGCGCGAGA
GCACGACATCTCGTACAACGAGCAGCTTGAGGCGGGAGACAACCCCTACCTCAA
GTACAACCACGCGGACGCCGAGTTTCAGGAGAAGCTCGCCGACGACACATCCTT
CGGGGGAAACCTCGGAAAGGCAGTCTTTCAGGCCAAGAAAAGGGTTCTCGAACC
TTTTGGCCTGGTTGAAGAGGGTGCTAAGACGGCCCCCTACCGGAAAGCGGATAGA
25 CGACCACTTTCCAAAAAGAAAGAAGGCTCGGACCGAAGAGGACTCCAAGCCTTC
CACCTCGTCAGACGCCGAAGCTGGACCCAGCGGATCCCAGCAGCTGCAAATCCC
AGCCCAACCAGCCTCAAGTTTGGGAGCTGATACAATGTCTGCGGGAGGTGGCGG
CCCATTGGGCGACAATAACCAAGGTGCCGATGGAGTGGGCAATGCCTCGGGAGA
TTGGCATTGCGATTCCACGTGGATGGGGGACAGAGTCGTCACCAAGTCCACCCG

AACCTGGGTGCTGCCCAGCTACAACAACCACCAGTACCGAGAGATCAAAAGCGG
CTCCGTCGACGGAAGCAACGCCAACGCGTTCTTTGGATACAGCACCCCCTGGGG
GTACTTTGACTTTAACCGCTTCCACAGCCACTGGAGCCCCCGAGACTGGCAAAGA
CTCATCAACAACACTACTGGGGCTTCAGACCCCGGTCCCTCAGAGTCAAAATCTTCA
5 ACATTCAAGTCAAAGAGGTACCGGTGCAGGACTCCACCACCACCATCGCCAACA
ACCTCACCTCCACCGTCCAAGTGTTTACGGACGACGACTACCAGCTGCCCTACGT
CGTCGGCAACGGGACCGAGGGATGCCTGCCGGCCTTCCCTCCGCAGGTCTTTACG
CTGCCGCAGTACGGTTACGCGACGCTGAACCGCGACAACACAGAAAATCCCACC
GAGAGGAGCAGCTTCTTCTGCCTAGAGTACTTTCCCAGCAAGATGCTGAGAACG
10 GGCAACAACCTTTGAGTTTACCTACAACCTTTGAGGAGGTGCCCTTCCACTCCAGCT
TCGCTCCCAGTCAGAACCTCTTCAAGCTGGCCAACCCGCTGGTGGACCAGTACTT
GTACCGCTTCGTGAGCACAATAACACTGGCGGAGTCCAGTTCAACAAGAACCT
GGCCGGGAGATACGCCAACACCTACAAAAACTGGTTCCCGGGGGCCCATGGGGCCG
AACCAGGGCTGGAACCTGGGCTCCGGGGTCAACCGCGCCAGTGTCAGCGCCTT
15 CGCCACGACCAATAGGATGGAGCTCGAGGGCGCGAGTTACCAGGTGCCCCCGCA
GCCGAACGGCATGACCAACAACCTCCAGGGCAGCAACACCTATGCCCTGGAGAA
CACTATGATCTTCAACAGCCAGCCGGCGAACCCGGGCACCACCGCCACGTACCTC
GAGGGCAACATGCTCATCACCAGCGAGAGCGAGACGCAGCCGGTGAACCGCGTG
GCGTACAACGTCGGCGGGCAGATGGCCACCAACAACCAGAGCTCCACCACTGCC
20 CCCGCGACCGGCACGTACAACCTCCAGGAAATCGTGCCCGGCAGCGTGTTGGATG
GAGAGGGACGTGTACCTCCAAGGACCCATCTGGGCCAAGATCCCAGAGACGGGG
GCGCACTTTACCCCTCTCCGGCCATGGGCGGATTTCGGACTCAAACACCCACCGC
CCATGATGCTCATCAAGAACACGCCTGTGCCCGGAAATATCACCAGCTTCTCGGA
CGTGCCCGTCAGCAGCTTCATCACCAGTACAGCACCGGGCAGGTCACCGTGGA
25 GATGGAGTGGGAGCTCAAGAAGGAAAACCTCCAAGAGGTGGAACCCAGAGATCC
AGTACACAAACAACACTACAACGACCCCCAGTTTGTGGACTTTGCCCCGGACAGCA
CCGGGGAATACAGAACCACCAGACCTATCGGAACGCGTTTCCTTACCCGACCCCT
TTAA

AAV6 (SEQ ID NO: 6):

ATGGCTGCCGATGGTTATCTTCCAGATTGGCTCGAGGACAACCTCTCTGAGGGCA
TTCGCGAGTGGTGGGACTTGAAACCTGGAGCCCCGAAACCCAAAGCCAACCAGC
AAAAGCAGGACGACGGCCGGGGTCTGGTGCTTCCTGGCTACAAGTACCTCGGAC
5 CCTTCAACGGACTCGACAAGGGGGAGCCCGTCAACGCGGCGGATGCAGCGGCCC
TCGAGCACGACAAGGCCTACGACCAGCAGCTCAAAGCGGGTGACAATCCGTACC
TGCGGTATAACCACGCCGACGCCGAGTTTCAGGAGCGTCTGCAAGAAGATACGT
CTTTTGGGGGCAACCTCGGGCGAGCAGTCTTCCAGGCCAAGAAGAGGGTTCTCG
AACCTTTTGGTCTGGTTGAGGAAGGTGCTAAGACGGCTCCTGGAAAGAAACGTC
10 CGGTAGAGCAGTCGCCACAAGAGCCAGACTCCTCCTCGGGCATTGGCAAGACAG
GCCAGCAGCCCGCTAAAAAGAGACTCAATTTTGGTCAGACTGGCGACTCAGAGT
CAGTCCCCGACCCACAACCTCTCGGAGAACCTCCAGCAACCCCCGCTGCTGTGGG
ACCTACTACAATGGCTTCAGGCGGTGGCGCACCAATGGCAGACAATAACGAAGG
CGCCGACGGAGTGGGTAAATGCCTCAGGAAATTGGCATTGCGATTCCACATGGCT
15 GGGCGACAGAGTCATCACCACCAGCACCCGAACATGGGCCTTGCCACCTATAA
CAACCACCTCTACAAGCAAATCTCCAGTGCTTCAACGGGGGCCAGCAACGACAA
CCACTACTTCGGCTACAGCACCCCCTGGGGGTATTTTGATTTCACAGATTCCAC
TGCCATTTCTCACCACGTGACTGGCAGCGACTCATCAACAACAATTGGGGATTCC
GGCCCAAGAGACTCAACTTCAAGCTCTTCAACATCCAAGTCAAGGAGGTCACGA
20 CGAATGATGGCGTCACGACCATCGCTAATAACCTTACCAGCACGGTTCAAGTCTT
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CTCCCTCCGTTCCCGGCGGACGTGTTTCATGATTCCGCAGTACGGCTACCTAACGC
TCAACAATGGCAGCCAGGCAGTGGGACGGTCATCCTTTTACTGCCTGGAATATTT
CCCATCGCAGATGCTGAGAACGGGCAATAACTTTACCTTCAGCTACACCTTCGAG
25 GACGTGCCTTTCCACAGCAGCTACGCGCACAGCCAGAGCCTGGACCGGCTGATG
AATCCTCTCATCGACCAGTACCTGTATTACCTGAACAGAACTCAGAATCAGTCCG
GAAGTGCCCAAAACAAGGACTTGCTGTTTAGCCGGGGGTCTCCAGCTGGCATGTC
TGTTACGCCCAAAAACCTGGCTACCTGGACCCTGTTACCGGCAGCAGCGCGTTTCT
AAAGTAAAAACAGACAACAACAACAGCAACTTTACCTGGACTGGTGCTTCAAAA

TATAACCTTAATGGGCGTGAATCTATAATCAACCCTGGCACTGCTATGGCCTCAC
 ACAAAGACGACAAAGACAAGTTCTTTCCCATGAGCGGTGTCATGATTTTTGGAAA
 GGAGAGCGCCGGAGCTTCAAACACTGCATTGGACAATGTCATGATCACAGACGA
 AGAGGAAATCAAAGCCACTAACCCCGTGGCCACCGAAAGATTTGGGACTGTGGC
 5 AGTCAATCTCCAGAGCAGCAGCACAGACCCTGCGACCGGAGATGTGCATGTTAT
 GGGAGCCTTACCTGGAATGGTGTGGCAAGACAGAGACGTATACCTGCAGGGTCC
 TATTTGGGCCAAAATTCCTCACACGGATGGACACTTTCACCCGTCTCCTCTCATG
 GGCGGCTTTGGACTTAAGCACCCGCCTCCTCAGATCCTCATCAAAAACACGCCTG
 TTCCTGCGAATCCTCCGGCAGAGTTTTCGGCTACAAAGTTTGCTTCATTCATCACC
 10 CAGTATTCCACAGGACAAGTGAGCGTGGAGATTGAATGGGAGCTGCAGAAAGAA
 AACAGCAAACGCTGGAATCCCGAAGTGCAGTATACATCTAACTTTGCCAAATCTG
 CCAACGTTGATTTCACTGTGGACAACAATGGACTTTATACTGAGCCTCGCCCCAT
 TGGCACACGTTTCCTCACCCGTCCCCTGTAA

15 *THERAPEUTIC USES*

Another aspect of the disclosure pertains to uses of the rAAV nucleic acid vectors and rAAV particles described herein for efficient transduction of cells, tissues, and/or organs of interest, and/or for use in gene therapy.

In some embodiments, the disclosure provides a method for transduction of cells,
 20 tissues, and/or organs of interest, comprising introducing into a cell, a composition
 comprising an effective amount of a rAAV particle described herein, such as an rAAV
 particle comprising a rAAV nucleic acid vector described herein.

In certain embodiments, the rAAV nucleic acid vectors and rAAV particles described
 herein are used for transduction of mammalian host cells, including for example, human,
 25 primate, murine, feline, canine, porcine, ovine, bovine, equine, epine, caprine and lupine host
 cells. In certain embodiments, the rAAV nucleic acid vectors and rAAV particles described
 herein are used for transduction of endothelial, epithelial, vascular, liver, lung, heart,
 pancreas, intestinal, kidney, muscle, bone, dendritic, cardiac, neural, blood, brain, fibroblast
 or cancer cells. In some embodiments, the rAAV particles comprising a modified AAV1

capsid described herein (*e.g.*, Y705F+Y731F+T492V) are used for transducing muscle (*e.g.*, mouse muscle). In some embodiments, the rAAV particles comprising a modified AAV5 capsid described herein (*e.g.*, Y436F+Y693F+Y719F) are used for transducing retinal cells (*e.g.*, mouse cells) or airway epithelial cells (*e.g.*, human cells). In some embodiments, the
5 rAAV particles comprising a modified AAV6 capsid described herein (*e.g.*, Y705F+Y731F+T492V) are used for transducing hematopoietic stem cells (*e.g.*, human cells), dendritic cells (*e.g.*, human cells), monocytes (*e.g.*, human cells), airway epithelial cells (*e.g.*, human cells), muscle (*e.g.*, mouse or dog muscle), liver (*e.g.*, mouse liver), pancreas (*e.g.*, mouse pancreas), or microglial cells (*e.g.*, mouse cells).

10 In one embodiment, cells, tissues, and/or organs of a subject are transduced using the rAAV particles described herein.

The term “subject,” as used herein, describes an organism, including mammals such as primates, to which treatment with the compositions according to the present disclosure can be provided. Mammalian species that can benefit from the disclosed methods of treatment
15 include, but are not limited to, humans; apes; chimpanzees; orangutans; monkeys; domesticated animals such as dogs and cats; livestock such as horses, cattle, pigs, sheep, goats, and chickens; and other animals such as mice, rats, guinea pigs, and hamsters.

In some embodiments, the disclosure provides a method for treatment of a disease, wherein the method comprises administering, to a subject in need of such treatment, an
20 effective amount of a composition comprising the rAAV particle described herein.

The term “treatment” or any grammatical variation thereof (*e.g.*, treat, treating, and treatment, etc.), as used herein, includes but is not limited to, alleviating a symptom of a disease or condition; and/or reducing, suppressing, inhibiting, lessening, ameliorating or affecting the progression, and/or severity of a disease or condition.

25 The term “effective amount,” as used herein, refers to an amount that is capable of treating a disease or condition or otherwise capable of producing an intended therapeutic effect (*e.g.*, transduction of a cell or tissue or organ).

The disclosure also provides for the use of a composition disclosed herein in the manufacture of a medicament for treating, preventing or ameliorating the symptoms of a

disease, disorder, dysfunction, injury or trauma, including, but not limited to, the treatment, prevention, and/or prophylaxis of a disease, disorder or dysfunction, and/or the amelioration of one or more symptoms of such a disease, disorder or dysfunction. Exemplary conditions for which rAAV viral based gene therapy may find particular utility include, but are not limited to, cancer, diabetes, autoimmune disease, kidney disease, cardiovascular disease, pancreatic disease, intestinal disease, liver disease, neurological disease, neuromuscular disorder, neuromotor deficit, neuroskeletal impairment, neurological disability, neurosensory dysfunction, stroke, alpha-1-antitrypsin (AAT) deficiency, Batten's disease, ischemia, an eating disorder, Alzheimer's disease, Huntington's disease, Parkinson's disease, skeletal disease and pulmonary disease.

In some embodiments, a composition comprising an rAAV particle comprising a modified AAV1 capsid protein as described herein (*e.g.*, a modified AAV1 capsid protein comprising replacement of tyrosine residues with non-tyrosine residues and a replacement of a threonine residue with a non-threonine residue at each of the positions corresponding to Y705, Y731, and T492 of the wild-type AAV1 capsid protein having the sequence of SEQ ID NO: 1) is used in a method of manufacturing a medicament for treating, preventing or ameliorating one or more symptoms of muscular dystrophy or alpha-1-antitrypsin deficiency.

In some embodiments, a composition comprising an rAAV particle comprising a modified AAV5 capsid protein as described herein (*e.g.*, a modified AAV5 capsid protein comprising replacement of tyrosine residues with non-tyrosine residues at each of the positions corresponding to Y436, Y693, and Y719 of a wild-type AAV5 capsid protein having the sequence of SEQ ID NO: 2) is used in a method of manufacturing a medicament for treating, preventing or ameliorating one or more symptoms of retinitis pigmentosa, age-related macular degeneration, or cystic fibrosis.

In some embodiments, a composition comprising an rAAV particle comprising a modified AAV6 capsid protein as described herein (*e.g.*, a modified AAV6 capsid protein comprising replacement of tyrosine residues with non-tyrosine residues and a replacement of a threonine residue with a non-threonine residue at each of the positions corresponding to Y705, Y731, and T492 of a wild-type AAV6 capsid protein having the sequence of SEQ ID

NO: 3) is used in a method of manufacturing a medicament for treating, preventing or ameliorating one or more symptoms of an immune disease that involves treatment with genetically-modified dendritic cells and/or macrophages, breast cancer, prostate cancer, pancreatic cancer, Alzheimer's disease, sickle cell disease, beta-thalassemia, or cardiovascular disease.

The disclosure also provides a method for treating or ameliorating the symptoms of such a disease, injury, disorder, or dysfunction in a mammal. Such methods generally involve at least the step of administering to a mammal in need thereof, one or more of the rAAV particles described herein, in an amount and for a time sufficient to treat or ameliorate the symptoms of such a disease, injury, disorder, or dysfunction in the mammal.

In some embodiments, a composition comprising an rAAV particle comprising a modified AAV1 capsid protein as described herein (*e.g.*, a modified AAV1 capsid protein comprising replacement of tyrosine residues with non-tyrosine residues and a replacement of a threonine residue with a non-threonine residue at each of the positions corresponding to Y705, Y731, and T492 of the wild-type AAV1 capsid protein having the sequence of SEQ ID NO: 1) is used in a method of treating, preventing or ameliorating one or more symptoms of muscular dystrophy or alpha-1-antitripsin deficiency.

In some embodiments, a composition comprising an rAAV particle comprising a modified AAV5 capsid protein as described herein (*e.g.*, a modified AAV5 capsid protein comprising replacement of tyrosine residues with non-tyrosine residues at each of the positions corresponding to Y436, Y693, and Y719 of a wild-type AAV5 capsid protein having the sequence of SEQ ID NO: 2) is used in a method of treating, preventing or ameliorating one or more symptoms of retinitis pigmentosa, age-related macular degeneration, or cystic fibrosis.

In some embodiments, a composition comprising an rAAV particle comprising a modified AAV6 capsid protein as described herein (*e.g.*, a modified AAV6 capsid protein comprising replacement of tyrosine residues with non-tyrosine residues and a replacement of a threonine residue with a non-threonine residue at each of the positions corresponding to Y705, Y731, and T492 of a wild-type AAV6 capsid protein having the sequence of SEQ ID

NO: 3) is used in a method of treating, preventing or ameliorating one or more symptoms of an immune disease that involves treatment with genetically-modified dendritic cells and/or macrophages, breast cancer, prostate cancer, pancreatic cancer, Alzheimer's disease, sickle cell disease, beta-thalassemia, or cardiovascular disease. In some embodiments, a composition comprising an rAAV particle comprising a modified AAV6 capsid protein as described herein is used in a method of targeting blood cells, blood stem cells, blood progenitor cells, dendritic cells, macrophages, and/or blood differentiated cells, or a combination thereof.

Such treatment regimens are particularly contemplated in human therapy, via administration of one or more compositions either intramuscularly, intravenously, subcutaneously, intrathecally, intraperitoneally, or by direct injection into an organ or a tissue of the subject under care, such as injection into the eye.

The disclosure also provides a method for providing to a mammal in need thereof, a therapeutically-effective amount of the rAAV compositions of the present disclosure, in an amount, and for a time effective to provide the patient with a therapeutically-effective amount of the desired therapeutic agent(s) encoded by one or more nucleic acid segments comprised within the rAAV vector. Preferably, the therapeutic agent is selected from the group consisting of a polypeptide, a peptide, an antibody, an antigen binding fragment, a ribozyme, a peptide nucleic acid, a siRNA, an RNAi, an antisense oligonucleotide and an antisense polynucleotide.

PHARMACEUTICAL COMPOSITIONS

The present disclosure also provides therapeutic or pharmaceutical compositions comprising the active ingredient, such as a rAAV particle described herein, in a form that can be combined with a therapeutically or pharmaceutically acceptable carrier. The rAAV particles may be prepared in a variety of compositions, and may also be formulated in appropriate pharmaceutical vehicles for administration to human or animal subjects.

The rAAV particles described herein and compositions comprising them provide new and useful therapeutics for the treatment, control, and amelioration of symptoms of a variety of disorders.

The disclosure also provides compositions comprising one or more of the disclosed
5 nucleic acid molecules, rAAV nucleic acid vectors, rAAV particles, or mammalian cells. As described herein, such compositions may further comprise a pharmaceutical excipient, buffer, or diluent, and may be formulated for administration to an animal, and particularly a human being. Such compositions may further optionally comprise a liposome, a lipid, a lipid complex, a microsphere, a microparticle, a nanosphere, or a nanoparticle, or may be
10 otherwise formulated for administration to the cells, tissues, organs, or body of a subject in need thereof. Such compositions may be formulated for use in a variety of therapies, such as for example, in the amelioration, prevention, and/or treatment of conditions such as peptide deficiency, polypeptide deficiency, peptide overexpression, polypeptide overexpression, including for example, conditions which result in diseases or disorders such as cancers,
15 tumors, or other malignant growths, neurological deficit dysfunction, autoimmune diseases, articular diseases, cardiac or pulmonary diseases, ischemia, stroke, cerebrovascular accidents, transient ischemic attacks (TIA); diabetes and/or other diseases of the pancreas; cardiocirculatory disease or dysfunction (including, *e.g.*, hypotension, hypertension, atherosclerosis, hypercholesterolemia, vascular damage or disease; neural diseases
20 (including, *e.g.*, Alzheimer's, Huntington's, Tay-Sach's and Parkinson's disease, memory loss, trauma, motor impairment, neuropathy, and related disorders); biliary, renal or hepatic disease or dysfunction; musculoskeletal or neuromuscular diseases (including, *e.g.*, arthritis, palsy, cystic fibrosis (CF), amyotrophic lateral sclerosis (ALS), multiple sclerosis (MS), muscular dystrophy (MD), and such like).

25 In some embodiments, the rAAV particles may be administered to a subject at concentrations ranging from 10^6 to 10^{14} particles/ml or 10^3 to 10^{13} particles/ml, or any values therebetween for either range, such as for example, about 10^6 , 10^7 , 10^8 , 10^9 , 10^{10} , 10^{11} , 10^{12} , 10^{13} , or 10^{14} particles/ml. In one embodiment, rAAV particles of higher than 10^{13} particles/ml are to be administered. In some embodiments, rAAV particles may be

administered to a subject at concentrations ranging from 10^6 to 10^{14} vector genomes(vgs)/ml or 10^3 to 10^{15} vgs/ml, or any values therebetween for either range, such as for example, about 10^6 , 10^7 , 10^8 , 10^9 , 10^{10} , 10^{11} , 10^{12} , 10^{13} , or 10^{14} vgs/ml. In one embodiment, rAAV particles of higher than 10^{13} vgs/ml are to be administered. The rAAV particles can be administered
5 as a single dose, or divided into two or more administrations as may be required to achieve therapy of the particular disease or disorder being treated. In some embodiments, 0.0001 ml to 10 mls are delivered to a subject.

In some embodiments of rAAV-based gene therapy regimens, a lower titer of infectious particles can be used when using the modified-capsid rAAV particles, than
10 compared to conventional gene therapy protocols.

In certain embodiments, the disclosure provides formulations of one or more rAAV-based compositions disclosed herein in pharmaceutically acceptable solutions for administration to a cell or an animal, either alone or in combination with one or more other modalities of therapy, and in particular, for therapy of human cells, tissues, and diseases
15 affecting man.

If desired, nucleic acid segments, RNA, DNA or PNA compositions that express one or more of therapeutic gene products may be administered in combination with other agents as well, such as, *e.g.*, proteins or polypeptides or various pharmaceutically-active agents, including one or more systemic or topical administrations of therapeutic polypeptides,
20 biologically active fragments, or variants thereof. In fact, there is virtually no limit to other components that may also be included, given that the additional agents do not cause a significant adverse effect upon contact with the target cells or host tissues. The rAAV particles may thus be delivered along with various other agents as required in the particular instance. Such compositions may be purified from host cells or other biological sources, or
25 alternatively may be chemically synthesized as described herein. Likewise, such compositions may further comprise substituted or derivatized RNA, DNA, siRNA, mRNA, tRNA, ribozyme, catalytic RNA molecules, or PNA compositions and such like.

Formulation of pharmaceutically-acceptable excipients and carrier solutions is well-known to those of skill in the art, as is the development of suitable dosing and treatment

regimens for using the particular compositions described herein in a variety of treatment regimens, including *e.g.*, oral, parenteral, intravenous, intranasal, intra-articular, intramuscular administration and formulation.

Typically, these formulations may contain at least about 0.1% of the active compound or more, although the percentage of the active ingredient(s) may, of course, be varied and may conveniently be between about 1 or 2% and about 70% or 80% or more of the weight or volume of the total formulation. Naturally, the amount of active compound(s) in each therapeutically-useful composition may be prepared in such a way that a suitable dosage will be obtained in any given unit dose of the compound. Factors such as solubility, bioavailability, biological half-life, route of administration, product shelf life, as well as other pharmacological considerations will be contemplated by one skilled in the art of preparing such pharmaceutical formulations, and as such, a variety of dosages and treatment regimens may be desirable.

In certain circumstances it will be desirable to deliver the rAAV particles in suitably formulated pharmaceutical compositions disclosed herein either subcutaneously, intraocularly, intravitreally, parenterally, subcutaneously, intravenously, intracerebro-ventricularly, intramuscularly, intrathecally, orally, intraperitoneally, by oral or nasal inhalation, or by direct injection to one or more cells, tissues, or organs by direct injection. The methods of administration may also include those modalities as described in U.S. Patents 5,543,158, 5,641,515 and/or 5,399,363 (each of which is specifically incorporated herein in its entirety by express reference thereto). Solutions of the active compounds as freebase or pharmacologically acceptable salts may be prepared in sterile water and may also suitably mixed with one or more surfactants, such as hydroxypropylcellulose. Dispersions may also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms of the rAAV particle compositions suitable for injectable use include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions (U.S. Patent

5,466,468, specifically incorporated herein in its entirety by express reference thereto). In all cases the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, saline, ethanol, polyol (*e.g.*, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and/or vegetable oils. Proper fluidity may be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants.

The term "carrier" refers to a diluent, adjuvant, excipient, or vehicle with which the compound is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum oil such as mineral oil, vegetable oil such as peanut oil, soybean oil, and sesame oil, animal oil, or oil of synthetic origin. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers.

The compositions of the present disclosure can be administered to the subject being treated by standard routes including, but not limited to, pulmonary, intranasal, oral, inhalation, parenteral such as intravenous, topical, transdermal, intradermal, transmucosal, intraperitoneal, intramuscular, intracapsular, intraorbital, intravitreal, intracardiac, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection.

For administration of an injectable aqueous solution, for example, the solution may be suitably buffered, if necessary, and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, intravitreal, subcutaneous and intraperitoneal administration. In this connection, a sterile aqueous medium that can be employed will be known to those of skill in the art in light of the present disclosure. For example, one dosage may be dissolved in 1 mL of isotonic NaCl solution and either added to 1000 mL of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). Some variation in dosage will necessarily

occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject. Moreover, for human administration, preparations should meet sterility, pyrogenicity, and the general safety and purity standards as required by, *e.g.*, FDA Office of Biologics standards.

5 Sterile injectable solutions are prepared by incorporating the rAAV particles in the required amount in the appropriate solvent with several of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In
10 the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

The rAAV particle compositions disclosed herein may also be formulated in a neutral
15 or salt form. Pharmaceutically-acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric
20 hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms such as injectable solutions, drug-release capsules, and the like.

25 The amount of rAAV particle compositions and time of administration of such compositions will be within the purview of the skilled artisan having benefit of the present teachings. It is likely, however, that the administration of therapeutically-effective amounts of the disclosed compositions may be achieved by a single administration, such as for example, a single injection of sufficient numbers of infectious particles to provide therapeutic benefit

to the patient undergoing such treatment. Alternatively, in some circumstances, it may be desirable to provide multiple, or successive administrations of the rAAV particle compositions, either over a relatively short, or a relatively prolonged period of time, as may be determined by the medical practitioner overseeing the administration of such compositions.

The composition may include rAAV particles, either alone, or in combination with one or more additional active ingredients, which may be obtained from natural or recombinant sources or chemically synthesized.

EXPRESSION VECTORS

The present disclosure contemplates a variety of AAV-based expression systems, and nucleic acid vectors. In one embodiment the preferred AAV expression vectors comprise at least a first nucleic acid segment that encodes a therapeutic peptide, protein, or polypeptide. In another embodiment, the preferred AAV expression vectors disclosed herein comprise at least a first nucleic acid segment that encodes an antisense molecule. In another embodiment, a promoter is operatively linked to a sequence region that encodes a functional mRNA, a tRNA, a ribozyme or an antisense RNA.

The choice of which expression vector and ultimately to which promoter a polypeptide coding region is operatively linked depends directly on the functional properties desired, *e.g.*, the location and timing of protein expression, and the host cell to be transformed. These are well known limitations inherent in the art of constructing recombinant DNA molecules. However, a vector useful in practicing the present disclosure is capable of directing the expression of the functional RNA to which it is operatively linked.

A variety of methods have been developed to operatively link DNA to vectors via complementary cohesive termini or blunt ends. For instance, complementary homopolymer tracts can be added to the DNA segment to be inserted and to the vector DNA. The vector and DNA segment are then joined by hydrogen bonding between the complementary homopolymeric tails to form recombinant DNA molecules.

To express a therapeutic agent in accordance with the present disclosure one may prepare a rAAV particle comprising a rAAV nucleic acid vector that comprises a therapeutic agent-encoding nucleic acid segment under the control of one or more promoters. To bring a sequence "under the control of" a promoter, one positions the 5' end of the transcription initiation site of the transcriptional reading frame generally between about 1 and about 50 nucleotides "downstream" of (i.e., 3' of) the chosen promoter. The "upstream" promoter stimulates transcription of the DNA and promotes expression of the encoded polypeptide. This is the meaning of "recombinant expression" in this context. Particularly preferred recombinant vector constructs are those that comprise a rAAV nucleic acid vector. Such vectors are described in detail herein.

When the use of such nucleic acid vectors is contemplated for introduction of one or more exogenous proteins, polypeptides, peptides, ribozymes, and/or antisense oligonucleotides, to a particular cell transfected with the nucleic acid vector, one may employ the rAAV nucleic acid vectors disclosed herein by genetically modifying the vectors to further comprise at least a first exogenous polynucleotide operably positioned downstream and under the control of at least a first heterologous promoter that expresses the polynucleotide in a cell comprising the vector to produce the encoded peptide, protein, polypeptide, ribozyme, siRNA, RNAi or antisense oligonucleotide. Such constructs may employ heterologous promoters that are constitutive, inducible, or even cell-specific promoters. Exemplary such promoters include, but are not limited to, viral, mammalian, and avian promoters, including for example a CMV promoter, a beta-actin promoter, a hybrid CMV promoter, a hybrid beta-actin promoter, an EF1 promoter, a U1a promoter, a U1b promoter, a Tet-inducible promoter, a VP16-LexA promoter, and such like.

The nucleic acid vectors or expression systems may also further comprise one or more enhancers, regulatory elements, transcriptional elements, to alter or effect transcription of the heterologous gene cloned in the rAAV vectors. For example, the rAAV vectors of the present disclosure may further comprise at least a first CMV enhancer, a synthetic enhancer, or a cell- or tissue-specific enhancer. The exogenous polynucleotide may also further comprise one or more intron sequences.

THERAPEUTIC KITS

The disclosure also encompasses kits comprising one or more of the rAAV particles, nucleic acid vectors, proteins, host cells, and/or compositions described herein together with one or more pharmaceutically-acceptable excipients, carriers, diluents, adjuvants, and/or other components, as may be employed in the formulation of particular delivery formulations, and in the preparation of therapeutic agents for administration to a subject, and in particular, to a human. In particular, such kits may comprise one or more of the disclosed rAAV particle compositions in combination with instructions for using the composition in the treatment of such disorders in a subject, and may typically further include containers prepared for convenient commercial packaging.

Therapeutic kits may also be prepared that comprise at least one of the compositions disclosed herein and instructions for using the composition as a therapeutic agent. The container means for such kits may typically comprise at least one vial, test tube, flask, bottle, syringe or other container means, into which the disclosed rAAV particle composition(s) may be placed, and preferably suitably aliquoted. Where a second therapeutic polypeptide composition is also provided, the kit may also contain a second distinct container means into which this second composition may be placed. Alternatively, the plurality of therapeutic biologically active compositions may be prepared in a single pharmaceutical composition, and may be packaged in a single container means, such as a vial, flask, syringe, bottle, or other suitable single container means. The kits of the present disclosure will also typically include a means for containing the vial(s) in close confinement for commercial sale, such as, *e.g.*, injection or blow-molded plastic containers into which the desired vial(s) are retained.

AAV CAPSID PROTEINS

Supramolecular assembly of approximately 60 individual capsid protein subunits into a non-enveloped, T-1 icosahedral lattice capable of protecting the AAV DNA genome is a critical step in the life-cycle of the helper-dependent human parvovirus, adeno-associated virus (AAV), such as AAV2. The mature 20-nm diameter AAV2 particle is composed of

three structural proteins designated VP1, VP2, and VP3 (molecular masses of 87, 73, and 62 kDa respectively) in a ratio of 1:1:18. Based on its symmetry and these molecular weight estimates, of the 60 capsid proteins comprising the particle, three are VP1 proteins, three are VP2 proteins, and fifty-four are VP3 proteins.

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BIOLOGICAL FUNCTIONAL EQUIVALENTS

Also provided herein are modifications to the structure of the AAV capsid proteins as described herein to obtain functional rAAV particles that possess desirable characteristics, particularly with respect to improved delivery of therapeutic gene constructs to selected mammalian cell, tissues, and organs for the treatment, prevention, and prophylaxis of various diseases and disorders, as well as means for the amelioration of symptoms of such diseases, and to facilitate the expression of exogenous therapeutic and/or prophylactic polypeptides of interest via rAAV vector-mediated gene therapy. As mentioned above, one of the key aspects of the disclosure is the introduction of modifications into specific capsid protein sequences to produce modified vectors with improved properties for effecting gene therapy in mammalian systems.

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For example, certain amino acids may be substituted for other amino acids in a protein structure without appreciable loss of interactive binding capacity with structures such as, for example, antigen-binding regions of antibodies or binding sites on substrate molecules. Since it is the interactive capacity and nature of a protein that defines that protein's biological functional activity, certain amino acid sequence substitutions can be made in a protein sequence, and, of course, its underlying DNA coding sequence, and nevertheless obtain a protein with like properties. It is thus contemplated herein that various changes may be made in the polynucleotide or polypeptide sequences disclosed herein, without appreciable loss of their biological utility or activity.

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In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art (Kyte and Doolittle, 1982, incorporated herein by reference). It is accepted that the relative hydropathic character of the

amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like. Each amino acid has been assigned a hydropathic index on the basis of their hydrophobicity and charge characteristics (Kyte and Doolittle, 1982), these are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophan (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

EXEMPLARY DEFINITIONS

In accordance with the present disclosure, polynucleotides, nucleic acid segments, nucleic acid sequences, and the like, include, but are not limited to, DNAs (including and not limited to genomic DNAs), genes, peptide nucleic acids (PNAs), RNAs (including, but not limited to, rRNAs, mRNAs, miRNAs and tRNAs), nucleosides, and suitable nucleic acid segments either obtained from natural sources, chemically synthesized, modified, or otherwise prepared or synthesized in whole or in part by the hand of man.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure pertains. Although any methods and compositions similar or equivalent to those described herein can be used in the practice or testing of the present disclosure, the preferred methods and compositions are described herein. For purposes of the present disclosure, the following terms are defined below:

The term “promoter,” as used herein refers to a region or regions of a nucleic acid sequence that regulates transcription.

The term “regulatory element,” as used herein, refers to a region or regions of a nucleic acid sequence that regulates transcription. Exemplary regulatory elements include, but are not limited to, enhancers, post-transcriptional elements, transcriptional control sequences, and such like.

The term “vector,” as used herein, refers to a nucleic acid molecule (typically comprised of DNA) capable of replication in a host cell and/or to which another nucleic acid segment can be operatively linked so as to bring about replication of the attached segment. A plasmid, cosmid, or a viral genome is an exemplary vector.

5 The term “substantially corresponds to,” “substantially homologous,” or “substantial identity,” as used herein, denote a characteristic of a nucleic acid or an amino acid sequence, wherein a selected nucleic acid or amino acid sequence has at least about 70 or about 75 percent sequence identity as compared to a selected reference nucleic acid or amino acid sequence. More typically, the selected sequence and the reference sequence will have at least
10 about 76, 77, 78, 79, 80, 81, 82, 83, 84 or even 85 percent sequence identity, and more preferably at least about 86, 87, 88, 89, 90, 91, 92, 93, 94, or 95 percent sequence identity. More preferably still, highly homologous sequences often share greater than at least about 96, 97, 98, or 99 percent sequence identity between the selected sequence and the reference sequence to which it was compared.

15 The percentage of sequence identity may be calculated over the entire length of the sequences to be compared, or may be calculated by excluding small deletions or additions which total less than about 25 percent or so of the chosen reference sequence. The reference sequence may be a subset of a larger sequence, such as a portion of a gene or flanking sequence, or a repetitive portion of a chromosome. However, in the case of sequence
20 homology of two or more polynucleotide sequences, the reference sequence will typically comprise at least about 18-25 nucleotides, more typically at least about 26 to 35 nucleotides, and even more typically at least about 40, 50, 60, 70, 80, 90, or even 100 or so nucleotides.

Desirably, which highly homologous fragments are desired, the extent of percent identity between the two sequences will be at least about 80%, preferably at least about 85%,
25 and more preferably about 90% or 95% or higher, as readily determined by one or more of the sequence comparison algorithms well-known to those of skill in the art, such as *e.g.*, the FASTA program analysis described by Pearson and Lipman (Proc. Natl. Acad. Sci. USA, 85(8):2444-8, April 1988).

The term "operably linked" or "operatively linked," as used herein, refers to that the nucleic acid sequences being linked are typically contiguous, or substantially contiguous, and, where necessary to join two protein coding regions, contiguous and in reading frame. However, since enhancers generally function when separated from the promoter by several
5 kilobases and intronic sequences may be of variable lengths, some polynucleotide elements may be operably linked but not contiguous.

Without further elaboration, it is believed that one skilled in the art can, based on the above description, utilize the present disclosure to its fullest extent. The following specific embodiments are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. All publications cited herein are incorporated by reference for the purposes or subject matter referenced herein.

EXAMPLES

Example 1. AAV1, AAV5, and AAV6 capsid mutants have enhanced transgene expression levels

Specific AAV1, AAV5, AAV6 capsid mutants were tested for transduction efficiency. The mutants tested are shown in the table below. Exemplary tissues/organs for use with each serotype are also shown.

Serotypes	Mutations	Exemplary Tissues/Organs
AAV1	Y705F+Y731F+T492V	Muscle (mouse)
AAV5	Y436F+Y693F+Y719F	Retina (mouse) Airway epithelial cells (human)
AAV6	Y705F+Y731F+T492V	Hematopoietic stem cells, dendritic cells, monocytes, airway epithelial cells (human) Muscle

		(mouse, dog) Microglial cells, pancreas, liver (mouse)
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Transduction efficiency of WT and various capsid-modified AAV1 serotype vectors in a murine muscle cell line *in vitro* [C2C12] and in murine muscles *in vivo*

Wild-type (WT) and mutant AAV1 vectors carrying a nucleic acid encoding GFP or Alpha-1 antitrypsin (AAT) were produced using standard methods and efficiency of each vector was evaluated *in vitro* and *in vivo*.

For *in vitro* analysis, EGFP expression levels was measured at 48 h post-infection following transduction at an MOI (multiplicity of infection) of 1×10^3 vgs/cell using C2C12 cells. EGFP transgene expression was assessed as the total area of green fluorescence (pixel²) per visual field (mean $\pm$ SD).

For *in vivo* analysis, AAT expression levels were measured in muscle by ELISA following intramuscular injection of WT (AAV1) and mutant Y705-731F+T492V mutant AAV1 vectors (AAV1m) in C57BL6/J mice.

The transduction efficiency of the AAV1 Y705F+Y731F+T492V vector was significantly higher than that of the wild-type vector both *in vitro* in C2C12 muscle cells (FIG. 1A) and *in vivo* in mouse muscle (FIG. 1B).

Imaging of major organs for GFP expression following intraperitoneal injection of various mutant scAAV6 vectors in mice *in vivo*

WT and mutant AAV6 vectors carrying a nucleic acid encoding mCherry were produced using standard methods and efficiency of each vector was evaluated *in vivo*.

mCherry expression analysis of different mice organs was performed following i.p. injection of 10^{11} vgs of WT and mutant AAV6 vectors. Organs were harvested two weeks post-injection and analyzed using a UVP BioImaging System.

The transduction efficiency of the AAV6 Y705F+Y731F+T492V was significantly

higher than the wild-type vector in the liver and pancreas (FIG. 2).

Transduction efficiency of WT and various mutant scAAV6 vectors in mouse pancreas *in vivo*

5 WT and mutant AAV6 vectors carrying a nucleic acid encoding EGFP were produced using standard methods and efficiency of each vector was evaluated *in vivo*.

Mice pancreas were harvested two weeks post-injection of 10^{11} vgs of WT and mutant-AAV6 vectors and analyzed for EGFP expression. DAPI and anti-insulin antibodies were used for nuclear and cytoplasmic staining.

10 The transduction efficiency of the AAV6 Y705F+Y731F+T492V was significantly higher than the wild-type vector in pancreas, both at the edge of the organ and in the interior (FIG. 3).

Transduction efficiency of WT and mutant scAAV6 vectors in primary murine glial cells *in vivo*

15 WT and mutant AAV6 vectors carrying a nucleic acid encoding EGFP were produced using standard methods and efficiency of each vector was evaluated *in vivo*.

Mice brains were harvested two weeks post-injection of 1×10^{10} vgs of WT and mutant-AAV6 vectors expressing EGFP. Primary cells were cultured and the EGFP
20 expression level was analyzed by fluorescence microscopy. Anti-CD11B antibodies were used to identify glial cells.

The transduction efficiency of the AAV6 Y705F+Y731F+T492V was significantly higher than the wild-type vector in glial cells (FIG. 4).

Transduction efficiency of Y705+731F+T492V mutant scAAV6 vectors in microglial cells in injected brains in mice *in vivo*

25 WT and mutant AAV6 vectors carrying a nucleic acid encoding EGFP were produced using standard methods and efficiency of each vector was evaluated *in vivo*.

Mice brains were harvested two weeks post-injection of 1×10^{10} vgs of WT and

mutant-AAV6 vectors expressing EGFP. Microglia/macrophage-specific marker anti-IBA antibodies were used to identify EGFP positive glial cells.

The transduction efficiency of the AAV6 Y705F+Y731F+T492V was significantly higher than the wild-type vector in glial cells in brain tissue (FIG. 5).

Transduction efficiency of Y705+731F+T492V scAAV6 vectors in neurons and astrocytes in injected brains in mice *in vivo*

Mice brains were harvested two weeks post injection of 1×10^{10} vgs of WT and mutant-AAV6 vectors expressing EGFP. Neurons and astrocytes were identified by cell-specific morphology. Microglia/macrophage-specific marker anti-IBA antibodies were used.

The AAV6 Y705F+Y731F+T492V was able to transduce neuronal and astrocyte cells in brain tissue (FIG. 6).

Transduction efficiency of WT and various mutant AAV serotype vectors in human airway epithelial cells *in vitro*

Human normal airway epithelial [4011] cells were infected with WT and mutant AAV1, 2, 5, 6 vectors expressing EGFP at an MOI 2×10^3 vg/ cell. EGFP expression analysis was performed 48 hours post-infection by fluorescent microscopy. Transgene expression was assessed as the total area of green fluorescence (pixel²) per visual field (mean $\pm$ SD).

The transduction efficiency of the AAV6 Y705F+Y731F+T492V was significantly higher than the wild-type vector of several serotypes (AAV1, AAV2, AAV5, and AAV6) in human airway epithelial cells (FIG. 7). Double and triple mutants of AAV5 (such as AAV5 triple mutant (Y436F+Y693F+Y719F)), were found to have improved properties compared to the wild-type AAV5 vector (data not shown).

Transduction efficiency of WT and various mutant AAV2 and AAV6 serotype vectors in primary human monocyte-derived dendritic cells *in vitro*

WT and mutant AAV2 and AAV6 vectors carrying a nucleic acid encoding EGFP were produced using standard methods and efficiency of each vector was evaluated *in vitro*.

Leukapheresis-derived peripheral blood mononuclear cells (PBMCs) were differentiated to dendritic cells (DCs) in the presence of recombinant human IL-4 (500 U/mL) and GM-CSF (800 U/mL). EGFP expression analysis was performed at 48 hours post-infection following transduction at an MOI of 2×10^3 vgs/cell. Transgene expression was assessed as the total area of green fluorescence (pixel²) per visual field (mean $\pm$ SD).

The transduction efficiency of the AAV6 Y705F+Y731F+T492V was significantly higher than the wild-type vector of several serotypes (AAV2 and AAV6) in primary human monocyte-derived dendritic cells (FIG. 8A and 8B).

OTHER EMBODIMENTS

All of the features disclosed in this specification may be combined in any combination. Each feature disclosed in this specification may be replaced by an alternative feature serving the same, equivalent, or similar purpose. Thus, unless expressly stated otherwise, each feature disclosed is only an example of a generic series of equivalent or similar features.

From the above description, one skilled in the art can easily ascertain the essential characteristics of the present disclosure, and without departing from the spirit and scope thereof, can make various changes and modifications of the disclosure to adapt it to various usages and conditions. Thus, other embodiments are also within the claims.

EQUIVALENTS

While several inventive embodiments have been described and illustrated herein, those of ordinary skill in the art will readily envision a variety of other means and/or structures for performing the function and/or obtaining the results and/or one or more of the advantages described herein, and each of such variations and/or modifications is deemed to be within the scope of the inventive embodiments described herein. More generally, those skilled in the art will readily appreciate that all parameters, dimensions, materials, and configurations described herein are meant to be exemplary and that the actual parameters,

dimensions, materials, and/or configurations will depend upon the specific application or applications for which the inventive teachings is/are used. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific inventive embodiments described herein. It is, therefore, to be understood that the foregoing embodiments are presented by way of example only and that, within the scope of the appended claims and equivalents thereto, inventive embodiments may be practiced otherwise than as specifically described and claimed. Inventive embodiments of the present disclosure are directed to each individual feature, system, article, material, kit, and/or method described herein. In addition, any combination of two or more such features, systems, articles, materials, kits, and/or methods, if such features, systems, articles, materials, kits, and/or methods are not mutually inconsistent, is included within the inventive scope of the present disclosure.

All definitions, as defined and used herein, should be understood to control over dictionary definitions, definitions in documents incorporated by reference, and/or ordinary meanings of the defined terms.

All references, patents and patent applications disclosed herein are incorporated by reference with respect to the subject matter for which each is cited, which in some cases may encompass the entirety of the document.

The indefinite articles “a” and “an,” as used herein in the specification and in the claims, unless clearly indicated to the contrary, should be understood to mean “at least one.”

The phrase “and/or,” as used herein in the specification and in the claims, should be understood to mean “either or both” of the elements so conjoined, i.e., elements that are conjunctively present in some cases and disjunctively present in other cases. Multiple elements listed with “and/or” should be construed in the same fashion, i.e., “one or more” of the elements so conjoined. Other elements may optionally be present other than the elements specifically identified by the “and/or” clause, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, a reference to “A and/or B”, when used in conjunction with open-ended language such as “comprising” can refer, in one embodiment, to A only (optionally including elements other than B); in another embodiment,

to B only (optionally including elements other than A); in yet another embodiment, to both A and B (optionally including other elements); etc.

As used herein in the specification and in the claims, “or” should be understood to have the same meaning as “and/or” as defined above. For example, when separating items in a list, “or” or “and/or” shall be interpreted as being inclusive, i.e., the inclusion of at least one, but also including more than one, of a number or list of elements, and, optionally, additional unlisted items. Only terms clearly indicated to the contrary, such as “only one of” or “exactly one of,” or, when used in the claims, “consisting of,” will refer to the inclusion of exactly one element of a number or list of elements. In general, the term “or” as used herein shall only be interpreted as indicating exclusive alternatives (i.e. “one or the other but not both”) when preceded by terms of exclusivity, such as “either,” “one of,” “only one of,” or “exactly one of.” “Consisting essentially of,” when used in the claims, shall have its ordinary meaning as used in the field of patent law.

As used herein in the specification and in the claims, the phrase “at least one,” in reference to a list of one or more elements, should be understood to mean at least one element selected from any one or more of the elements in the list of elements, but not necessarily including at least one of each and every element specifically listed within the list of elements and not excluding any combinations of elements in the list of elements. This definition also allows that elements may optionally be present other than the elements specifically identified within the list of elements to which the phrase “at least one” refers, whether related or unrelated to those elements specifically identified. Thus, as a non-limiting example, “at least one of A and B” (or, equivalently, “at least one of A or B,” or, equivalently “at least one of A and/or B”) can refer, in one embodiment, to at least one, optionally including more than one, A, with no B present (and optionally including elements other than B); in another embodiment, to at least one, optionally including more than one, B, with no A present (and optionally including elements other than A); in yet another embodiment, to at least one, optionally including more than one, A, and at least one, optionally including more than one, B (and optionally including other elements); etc.

It should also be understood that, unless clearly indicated to the contrary, in any methods claimed herein that include more than one step or act, the order of the steps or acts of the method is not necessarily limited to the order in which the steps or acts of the method are recited.

5 In the claims, as well as in the specification above, all transitional phrases such as “comprising,” “including,” “carrying,” “having,” “containing,” “involving,” “holding,” “composed of,” and the like are to be understood to be open-ended, i.e., to mean including but not limited to. Only the transitional phrases “consisting of” and “consisting essentially of” shall be closed or semi-closed transitional phrases, respectively, as set forth in the United
10 States Patent Office Manual of Patent Examining Procedures, Section 2111.03.

Claims

What is claimed is:

1. A modified adeno-associated virus (AAV) capsid protein, wherein a VP3 region of the modified AAV capsid protein comprises a replacement of tyrosine residues with non-tyrosine residues and/or a replacement of a threonine residue with a non-threonine residue at positions corresponding to:

Y705, Y731, and T492 of a wild-type AAV1 capsid protein having the sequence of SEQ ID NO: 1,

Y436, Y693, and Y719 of a wild-type AAV5 capsid protein having the sequence of SEQ ID NO: 2, or

Y705, Y731, and T492 of a wild-type AAV6 capsid protein having the sequence of SEQ ID NO: 3.

2. The modified AAV capsid protein of claim 1, wherein the modified AAV capsid protein is a modified AAV1 capsid protein and the modified AAV1 capsid protein comprises replacement of tyrosine residues with non-tyrosine residues and a replacement of a threonine residue with a non-threonine residue at each of the positions corresponding to Y705, Y731, and T492 of the wild-type AAV1 capsid protein having the sequence of SEQ ID NO: 1.

3. The modified AAV capsid protein of claim 1, wherein the modified AAV capsid protein is a modified AAV5 capsid protein and the modified AAV5 capsid protein comprises replacement of tyrosine residues with non-tyrosine residues at each of the positions corresponding to Y436, Y693, and Y719 of a wild-type AAV5 capsid protein having the sequence of SEQ ID NO: 2.

4. The modified AAV capsid protein of claim 1, wherein the modified AAV capsid protein is a modified AAV6 capsid protein and the modified AAV6 capsid protein comprises replacement of tyrosine residues with non-tyrosine residues and a replacement of a threonine

residue with a non-threonine residue at each of the positions corresponding to Y705, Y731, and T492 of a wild-type AAV6 capsid protein having the sequence of SEQ ID NO: 3.

5 5. The modified capsid protein of any one of claims 1 to 4, wherein the non-tyrosine residues are phenylalanine and the non-threonine residue is valine.

6. A nucleic acid molecule encoding a modified capsid proteins of any one of claims 1 to 5.

10 7. The nucleic acid molecule of claim 6, wherein the nucleic acid molecule is a plasmid.

8. A recombinant AAV (rAAV) particle comprising a modified capsid protein of any one of claims 1 to 5.

15 9. A composition comprising a rAAV particle of claim 8 and a pharmaceutically-acceptable carrier.

10. A method, comprising:
 contacting a host cell with an rAAV particle comprising the modified AAV1 capsid
20 protein of claim 2 or claim 5 or a composition comprising the rAAV particle and a
 pharmaceutically-acceptable carrier.

11. The method of claim 10, wherein the host cell is a muscle cell.

25 12. A method, comprising:
 contacting a host cell with an rAAV particle comprising the modified AAV5 capsid
 protein of claim 3 or claim 5 or a composition comprising the rAAV particle and a
 pharmaceutically-acceptable carrier.

13. The method of claim 12, wherein the host cell is a retinal or airway epithelial cell.

14. A method, comprising:

contacting a host cell with an rAAV particle comprising the modified AAV6 capsid
5 protein of claim 4 or claim 5 or a composition comprising the rAAV particle and a
pharmaceutically-acceptable carrier.

15. The method of claim 14, wherein the host cell is a hematopoietic stem cell, a dendritic
cell, a monocyte, an airway epithelial cell, a muscle cell or a microglial cell.

10 16. A kit comprising any of the modified capsid proteins of claim 1 to 5, or the rAAV
particle of claim 8, or the composition of claim 9.

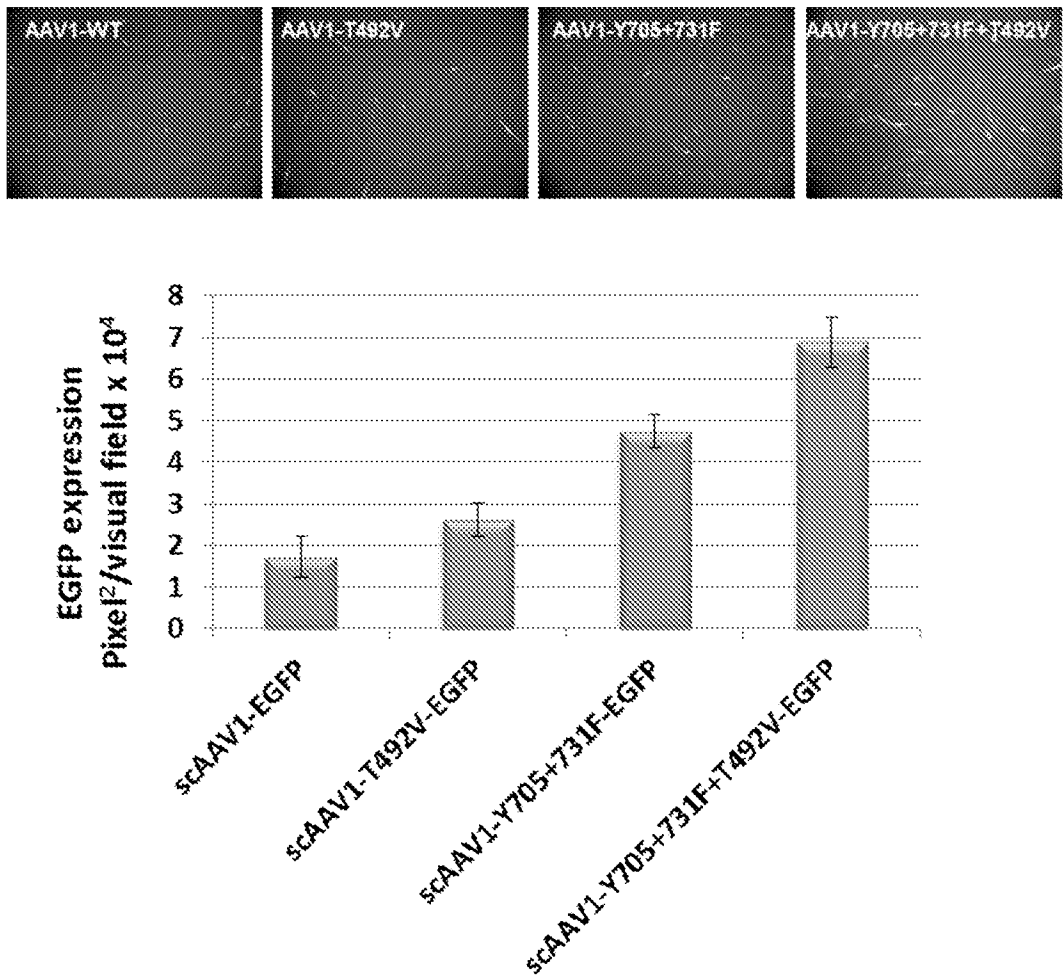


FIG. 1A

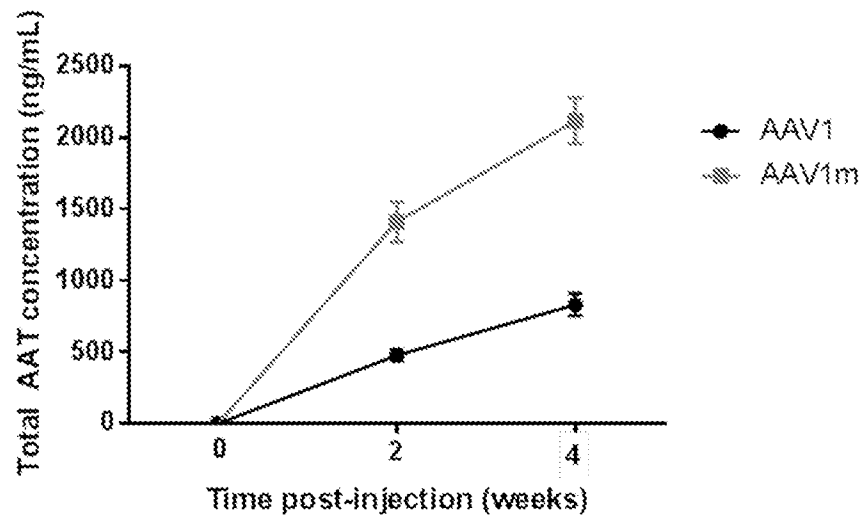


FIG. 1B

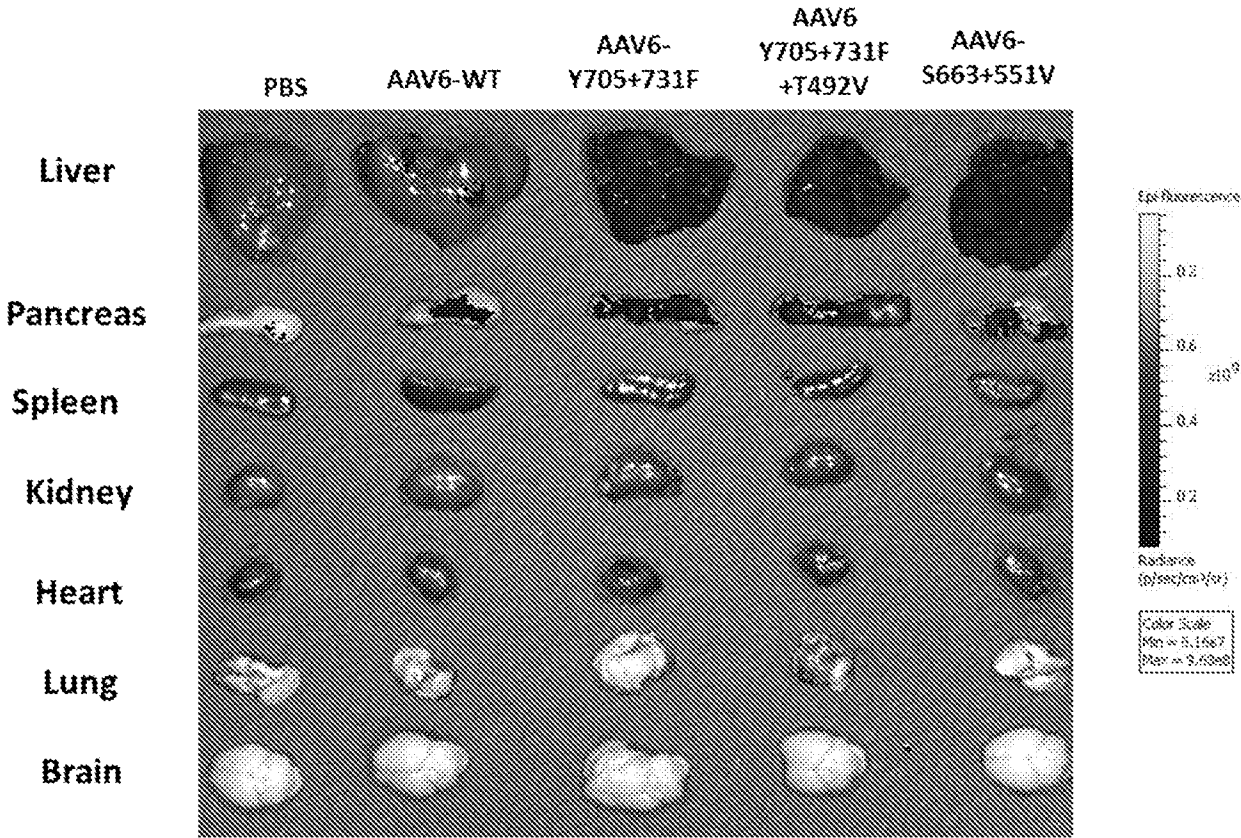


FIG. 2

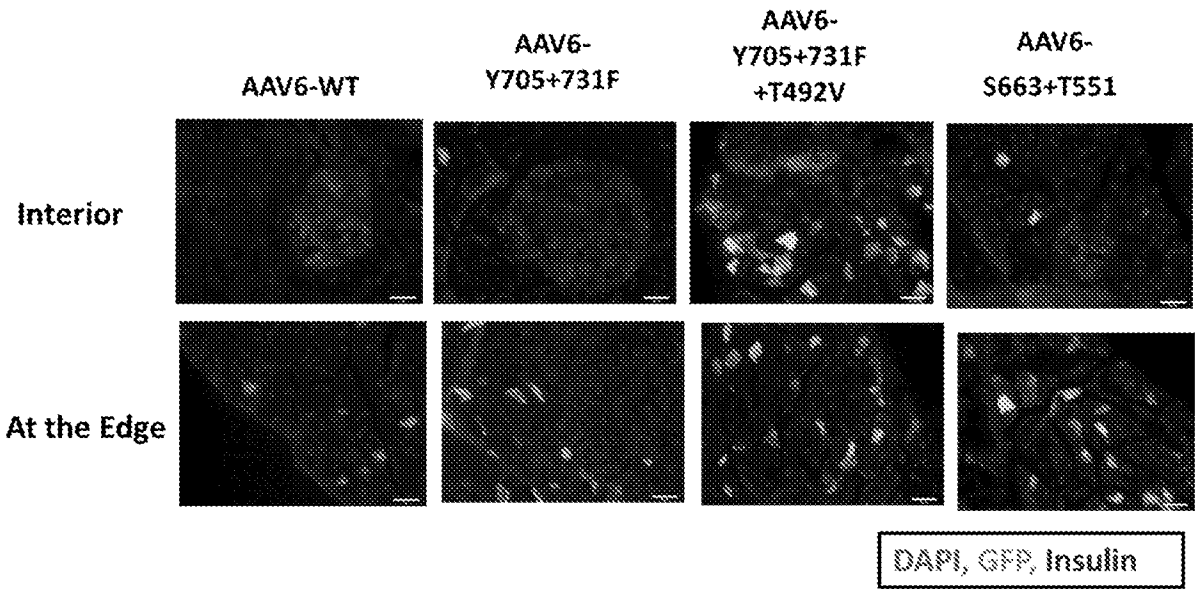


FIG. 3

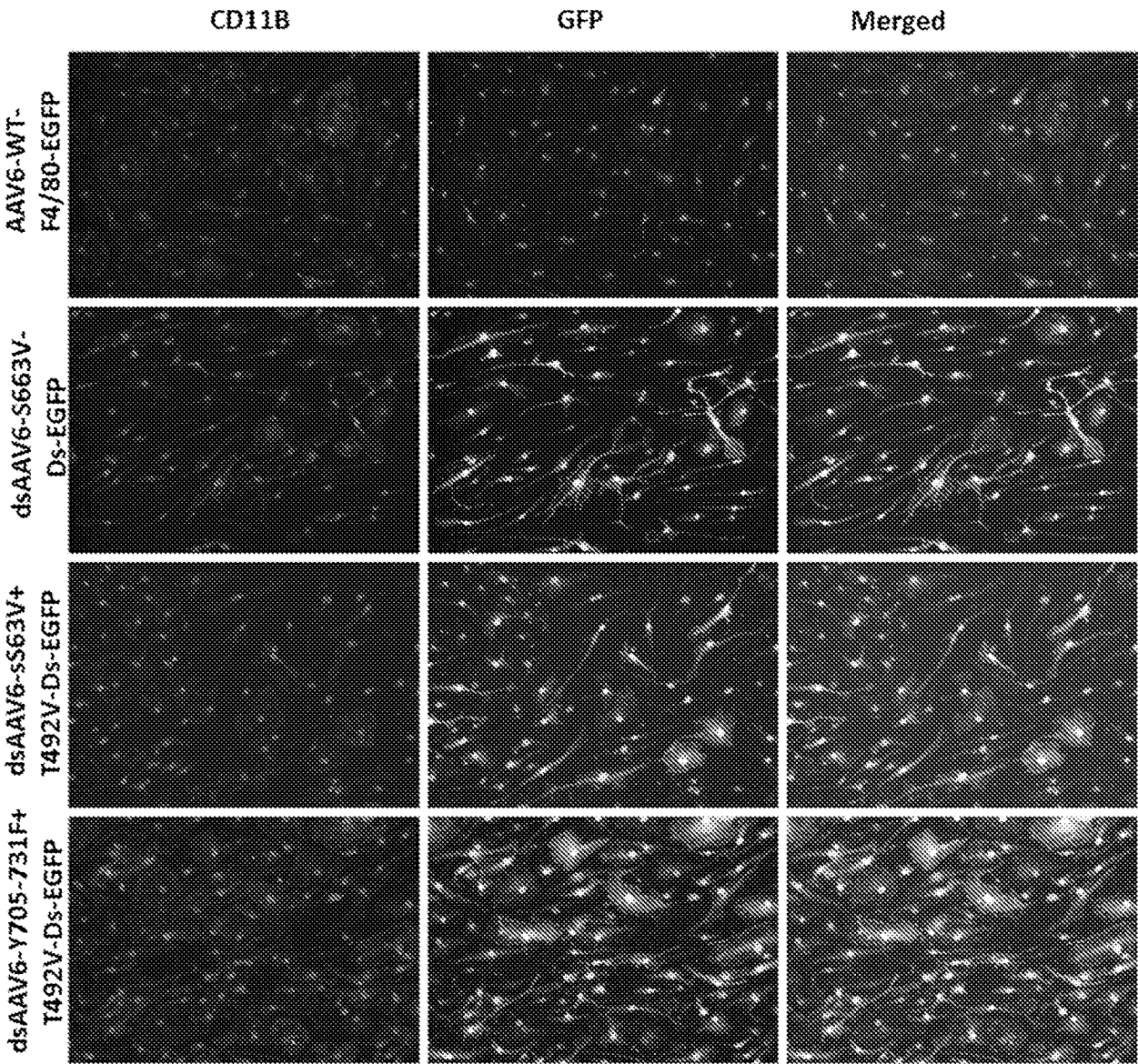


FIG. 4

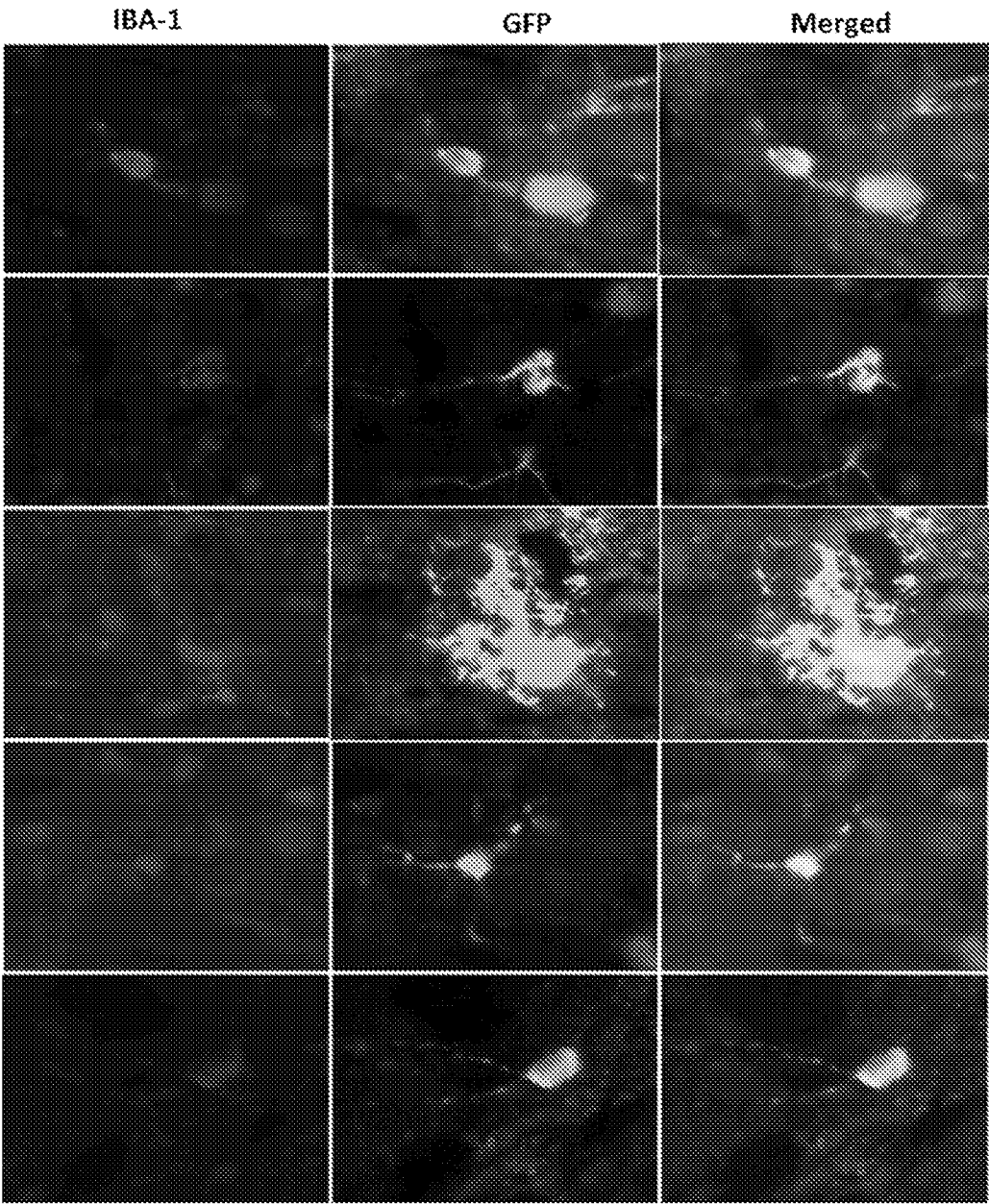


FIG. 5

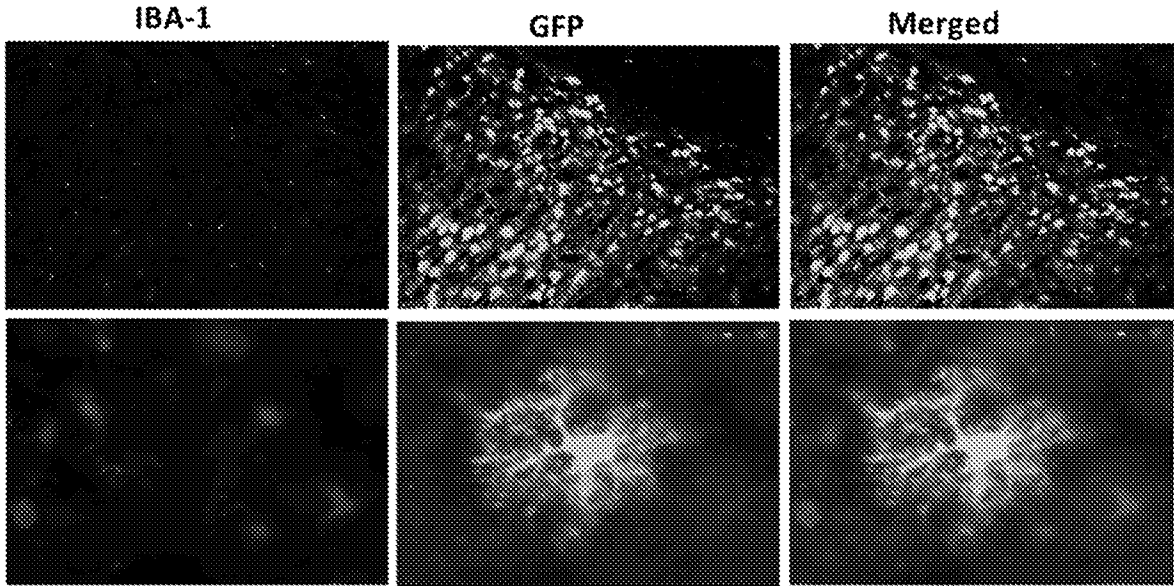


FIG. 6

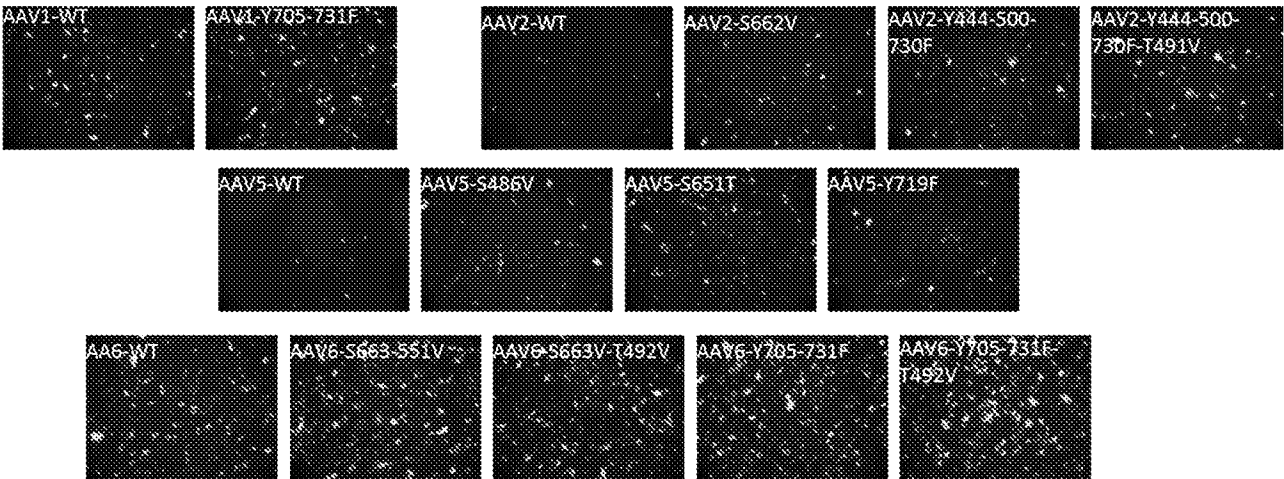


FIG. 7

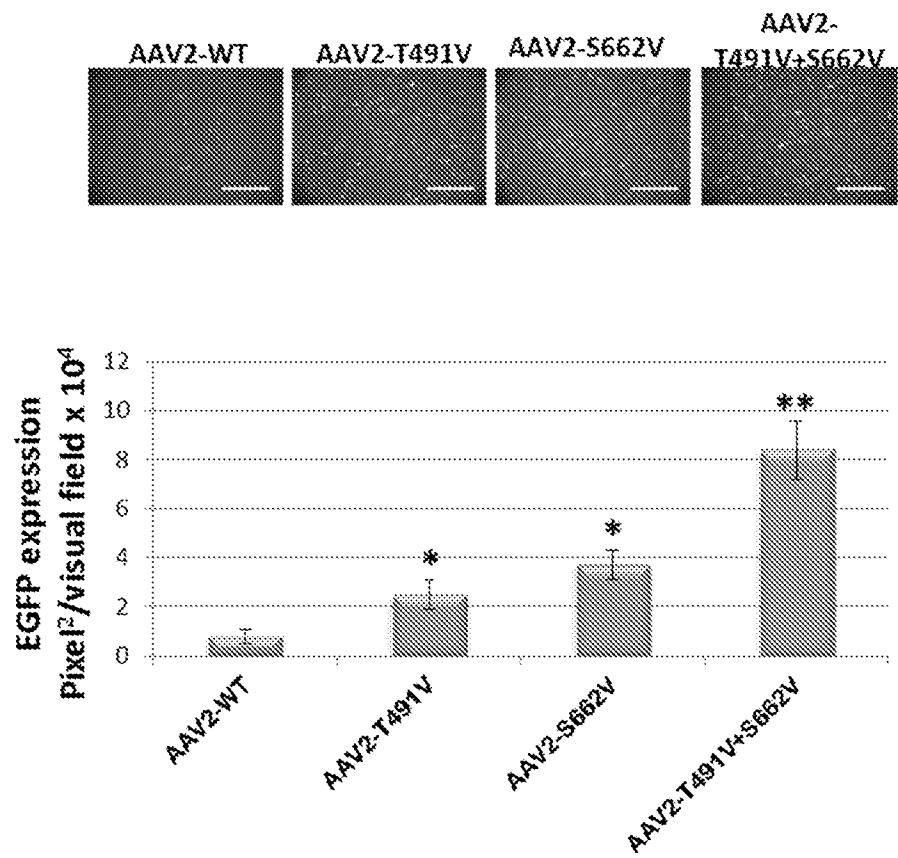


FIG. 8A

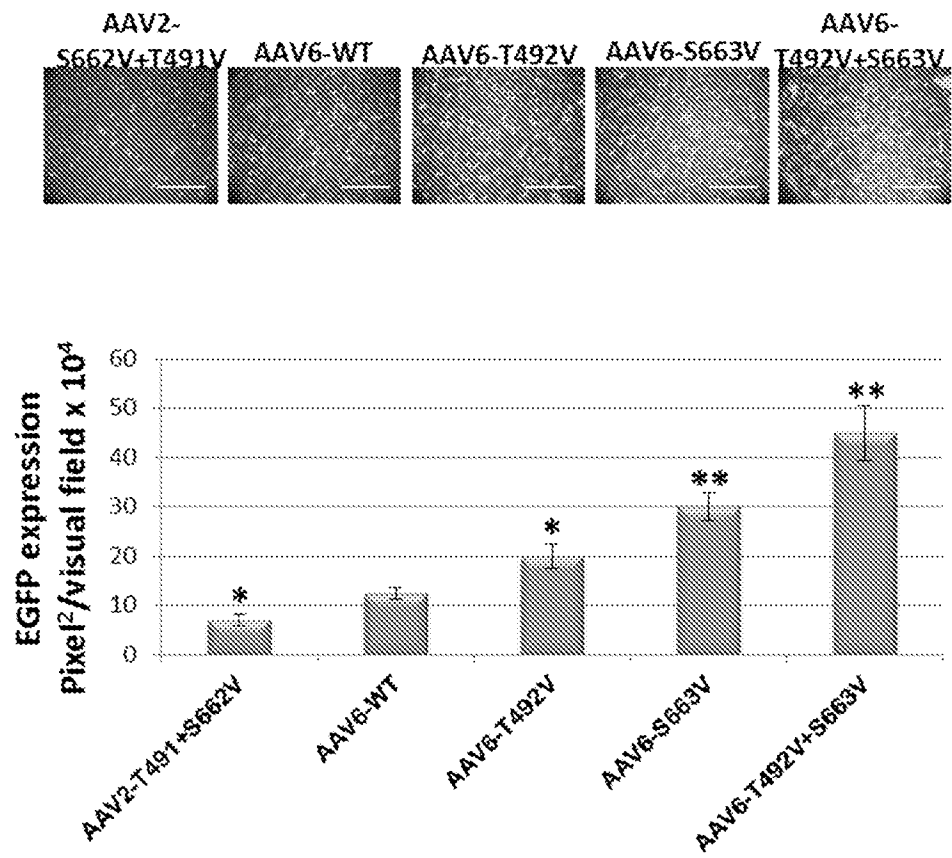


FIG. 8B

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/16422

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - C07K 14/005, C12N 15/861 (2016.01)

CPC - C12N 2750/14111, C12N 2750/14122

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC(8) - C07K 14/005, C12N 15/861 (2016.01)

CPC - C12N 2750/14111, C12N 2750/14122

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

CPC - C12N 15/86, C07K 14/005

(keyword limited; terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

PatBase, Google Patents, Google Scholar

Search terms: adeno-associated virus, adeno associated virus, AAV, rAAV, AAV1, AAV5, AAV6, serotype 1, serotype 5, serotype 6, tyrosine, Tyr, Y, threonine, Thr, T, mutant, substitute, replace, Y705, Tyr705, Tyr 705, position 705, Y731, Tyr731, Tyr 731, position 731

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2014/0341852 A1 (UNIVERSITY OF FLORIDA RESEARCH FOUNDATION, INC.) 20	1, 4, 5/(1,4)
---	November 2014 (20.11.2014) para [0017], [0021], [0022], [0460], [0517]; Table 6; Fig 33-B	-----
Y		2, 3, 5/(2,3)
Y	US 2003/0138772 A1 (GAO et al.) 24 July 2003 (24.07.2003) para [0013]; SEQ ID NOs: 64, 114	2, 3, 5/(2,3)

☐ Further documents are listed in the continuation of Box C.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

18 April 2016

Date of mailing of the international search report

05 MAY 2016

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents

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Facsimile No. 571-273-8300

Authorized officer:

Lee W. Young

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/16422

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 6-16
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.