

ANTI-AAV2.7M8 ANTIBODIES AND METHODS OF USE THEREOF**CROSS REFERENCE TO RELATED APPLICATION**

[0001] The present application claims priority of U.S. Provisional Application No. 63/591,622, filed October 19, 2023, the disclosure of which is hereby incorporated by reference herein in its entirety for all purposes.

FIELD

[0002] The present disclosure is directed to antibodies that specifically bind to the AAV2.7m8 capsid protein as antigen, and uses of these antibodies in assays for detecting and/or quantifying the presence of neutralizing antibodies during AAV2.7m8 gene therapy treatment, and/or detecting and/or quantifying the AAV2.7m8 capsid protein. The present disclosure also is directed to kits that include the anti-AAV2.7m8 antibodies of the present disclosure.

REFERENCE TO SEQUENCE LISTING

[0003] The official copy of the Sequence Listing is submitted concurrently with the specification as an ST26 formatted xml file of filename "17234-044WO1.xml", a creation date of October 16, 2024, and a size of 22,160 bytes. This Sequence Listing submitted concurrently is part of the specification and is incorporated in its entirety by reference herein.

BACKGROUND

[0004] Recombinant viruses show great promise and utility as vehicles to deliver therapeutic nucleic acids for gene therapy applications. A number of different recombinant viruses are used in these gene therapy applications based on a number of factors including the size of the nucleic acid to be delivered, the target cell or tissue to which to deliver the nucleic acid, the need for short or long term expression of the therapeutic nucleic acid, and integration of the therapeutic nucleic acid into the recipient's genome. Examples of viruses used in gene therapy applications include adeno-associated virus (AAV), adenovirus, lentivirus and herpes simplex virus (HSV).

[0005] AAV-mediated gene therapy administered sub-retinally (SR) can be effective for treatment of some ocular diseases. Available data suggests that neutralizing antibodies (nAbs) do not block transduction by AAV vectors following SR delivery due to ocular immune privilege. Intravitreal (IVT) administration is less invasive than SR and may be more beneficial for a

diseased, fragile retina. IVT delivery of AAV vectors may also be advantageous for more widespread retinal gene expression. Pre-existing nAbs, however, may negatively impact transduction and gene expression following IVT delivery. Detecting and quantifying nAbs prior to or after IVT administration is important for successful gene therapy treatment.

[0006] Accordingly, there is a need in the art for accurately detecting and quantifying nAbs associated with AAV gene therapy treatment.

SUMMARY

[0007] The present disclosure relates generally to the field of gene therapy using AAV vectors. In particular, the present disclosure is directed to antibodies that specifically bind to the AAV2.7m8 capsid protein used in gene therapy. The present disclosure is also directed to uses of these anti-AAV2.7m8 antibodies for detecting and/or quantifying the AAV2.7m8 capsid protein. Further, the disclosure is directed to the use of these anti-AAV2.7m8 antibodies in assays (and kits) for detecting and/or quantifying neutralizing antibodies (nAbs) that may be present in a subject that is administered or is a candidate to be administered a gene therapy treatment that includes the AAV2.7m8 capsid protein.

[0008] This summary is intended to introduce the subject matter of the present disclosure, but does not cover each and every embodiment, combination, or variation that is contemplated and described within the present disclosure. Further embodiments are contemplated and described by the disclosure of the detailed description, drawings, and claims.

[0009] In at least one embodiment, the present disclosure provides an antibody that specifically binds AAV2.7m8 capsid, wherein the antibody comprises a first light chain complementary determining region (CDR-L1) of SEQ ID NO: 5, a second light chain complementary determining region (CDR-L2) of SEQ ID NO: 6, a third light chain complementary determining region (CDR-L3) of SEQ ID NO: 7, a first heavy chain complementary determining region (CDR-H1) of SEQ ID NO: 13, a second heavy chain complementary determining region (CDR-H2) of SEQ ID NO: 14, and a third heavy chain complementary determining region (CDR-H3) of SEQ ID NO: 15.

[0010] In at least one embodiment of the antibody of the present disclosure, the antibody comprises a light chain variable domain (VL) amino acid sequence having at least 90% identity to a sequence selected from SEQ ID NO: 4; and/or a heavy chain variable domain (VH) amino acid sequence having at least 90% identity to a sequence selected from SEQ ID NO: 12.

[0011] In at least one embodiment of the antibody of the present disclosure, the antibody comprises: a light chain (LC) amino acid sequence having at least 90% identity to a sequence

selected from SEQ ID NO:20; and/or a heavy chain (HC) amino acid sequence having at least 90% identity to a sequence selected from SEQ ID NO: 21.

[0012] In at least one embodiment of the antibody of the present disclosure, the antibody is: (i) a monoclonal antibody; (ii) a chimeric antibody; (iii) a humanized antibody; (iv) a full length antibody of class IgG, optionally, wherein the class IgG antibody has an isotype selected from IgG1, IgG2, IgG3, and IgG4; (v) an antibody fragment, optionally selected from the group consisting of F(ab')₂, Fab', Fab, Fv, single domain antibody (VHH), and scFv; (vi) the antibody is a multi-specific antibody, optionally a bispecific antibody; and/or (vii) the antibody is a synthetic antibody, wherein the HVRs are grafted onto a scaffold or framework other than an immunoglobulin scaffold or framework; optionally, a scaffold selected from an alternative protein scaffold and an artificial polymer scaffold.

[0013] In at least one embodiment of the antibody of the present disclosure, the antibody is an immunoconjugate, optionally, wherein the immunoconjugate comprises a detectable moiety. In at least one embodiment the immunoconjugate comprises a detectable moiety selected from a radiolabel, a fluorescent label, and an indirect labeling moiety.

[0014] In at least one embodiment of the antibody of the present disclosure, the antibody is characterized by one or more of the following properties: (a) binds to AAV2.7m8 capsid protein with a binding affinity of 1×10^{-8} M or less, 1×10^{-9} M or less, 1×10^{-10} M or less, or 1×10^{-11} M or less; optionally, wherein the binding affinity is measured by equilibrium dissociation constant (K_D) to a AAV2.7m8 capsid polypeptide; or (b) binds to AAV2.7m8 capsid polypeptide of SEQ ID NO: 2 with a binding affinity that is increased by at least 2-fold, at least 3-fold, at least 5-fold, or at least 10-fold, relative to the binding affinity to AAV2 capsid protein; optionally, wherein the relative binding affinities are measured by equilibrium dissociation constant (K_D) to the AAV2.7m8 capsid polypeptide of SEQ ID NO: 2 and the AAV2 capsid polypeptide of SEQ ID NO: 1.

[0015] In at least one embodiment, the present disclosure provides an antibody that specifically binds to the same epitope of the AAV2.7m8 capsid protein as the antibody of the present disclosure; optionally, wherein the epitope comprises the amino acid sequence LALGETTRPA (SEQ ID NO: 22) located at positions 588-597 of SEQ ID NO: 2.

[0016] In at least one embodiment, the present disclosure provides an antibody that competes with an antibody of the present disclosure for binding to an epitope of AAV2.7m8 capsid protein and has a binding affinity of 1×10^{-9} M or less, 1×10^{-10} M or less, or 1×10^{-11} M or less; optionally, wherein the binding affinity is measured by equilibrium dissociation constant (K_D) to a AAV2.7m8 capsid polypeptide of SEQ ID NO: 2. In at least one embodiment, the

epitope comprises the amino acid sequence LALGETTRPA (SEQ ID NO: 22) located at positions 588-597 of SEQ ID NO: 2.

[0017] In other embodiments, the present disclosure also provides an isolated polynucleotide or a vector encoding an anti-AAV2.7m8 antibody of the present disclosure.

[0018] In another embodiment, the present disclosure provides an isolated host cell comprising a polynucleotide or vector encoding an anti-AAV2.7m8 antibody of the present disclosure.

[0019] In another embodiment, the present disclosure also provides a method of producing an anti-AAV2.7m8 antibody of the present disclosure comprising culturing a host cell a polynucleotide or vector encoding the anti-AAV2.7m8 antibody so that the antibody is produced.

[0020] In at least one embodiment, the present disclosure also provides a kit comprising: assay reagents; and an anti-AAV2.7m8 antibody of the present disclosure. In at least one embodiment, the kit assay reagents comprise: (a) an AAV2.7m8 vector that encodes a luciferase gene; (b) a luciferase substrate; and/or (c) cells from a transduction-permissive cell line; optionally, wherein the cells are from a 293T cell line.

[0021] In at least one embodiment, the present disclosure also provides a method for detecting neutralizing antibody activity against AAV2.7m8 in a human serum sample, the method comprising:

(a) providing: (i) a human serum sample; (ii) an assay solution comprising cells; (iii) a vector solution comprising an AAV2.7m8 vector that encodes a luciferase gene; (iv) a luciferase substrate solution; and (v) an AAV2.7m8 antibody solution comprising an antibody that specifically binds AAV2.7m8 capsid;

(b) combining an aliquot of the human serum sample, the assay solution, the vector solution, and the luciferase substrate solution to form a sample solution;

(c) combining an aliquot of the AAV2.7m8 antibody solution, the assay solution, the vector solution, and the luciferase substrate solution to form a positive control solution; and

(d) detecting luminescence signals from the sample solution and the positive control solution; whereby, the relative luminescence signal levels indicate presence and/or amount of neutralizing antibody activity in the human serum sample.

[0022] In at least one embodiment of the method, the antibody that specifically binds AAV2.7m8 capsid is an anti-AAV2.7m8 antibody of the present disclosure.

[0023] In at least one embodiment of the method for detecting neutralizing antibody activity against AAV2.7m8 in a human serum sample, wherein step (c) comprises combining at least 2, 3, 4, 5, 6, 7, 8, 9, or more different aliquots with different dilutions of the AAV2.7m8 antibody

solution to form at least 2, 3, 4, 5, 6, 7, 8, 9, or more positive control solutions having different concentrations of the AAV2.7m8 antibody for use in preparing a response curve.

[0024] In at least one embodiment of the method for detecting neutralizing antibody activity against AAV2.7m8 in a human serum sample, the method further comprises steps of combining an aliquot of the assay solution, and the luciferase substrate solution to form a negative control solution; and detecting luminescence signals from the negative control solution.

[0025] In at least one embodiment of the method for detecting neutralizing antibody activity against AAV2.7m8 in a human serum sample, the cells pre-exposed to an AAV2.7m8 vector that encodes a luciferase gene are from a transduction-permissive cell line; optionally, wherein the cell line is 293T.

[0026] In at least one embodiment, the present disclosure also provides a method for detecting an AAV2.7m8 capsid protein, the method comprising: contacting the protein with an anti-AAV2.7m8 antibody of the present disclosure and detecting the antibody bound to the protein. In at least one embodiment, the AAV2.7m8 capsid protein is immobilized. In at least one embodiment, the AAV2.7m8 capsid protein is in solution. In at least one embodiment, the antibody comprises a detectable label. In at least one embodiment, the detectable label selected from a radiolabel, a fluorescent label, and an indirect labeling moiety.

BRIEF DESCRIPTION OF THE DRAWINGS

[0027] A better understanding of the novel features and advantages of the present disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and the accompanying drawings (also "Figure" and "FIG." herein), of which:

[0028] FIGS. 1A, 1B, 1C, 1D, 1E, 1F, 1G, 1H, and 1I depict Octet BLI plots of the nine rabbit anti-AAV2.7m8 antibodies (clones 1-8, and 11) prepared and assayed for AAV2.7m8 affinity as described in Example 1. The plots labeled "Antibody 1" corresponds to the rabbit anti-AAV2.7m8 from "clone 1", etc. "Antibody 6" corresponds to the "clone 6," the anti-AAV2.7m8 with the highest affinity that was sequenced and used in the nAb assay of Example 2.

DETAILED DESCRIPTION

[0029] For the descriptions herein and the appended claims, the singular forms "a", and "an" include plural referents unless the context clearly indicates otherwise. Thus, for example, reference to "a protein" includes more than one protein, and reference to "a compound" refers to more than one compound. It is further noted that the claims may be drafted to exclude any

optional element. As such, this statement is intended to serve as antecedent basis for use of such exclusive terminology as “solely,” “only” and the like in connection with the recitation of claim elements, or use of a “negative” limitation. The use of “comprise,” “comprises,” “comprising” “include,” “includes,” and “including” are interchangeable and not intended to be limiting. It is to be further understood that where descriptions of various embodiments use the term “comprising,” those skilled in the art would understand that in some specific instances, an embodiment can be alternatively described using language “consisting essentially of” or “consisting of.”

[0030] Where a range of numeric values is provided, unless the context clearly dictates otherwise, it is understood that each intervening integer of the value, and each tenth of each intervening integer of the value, unless the context clearly dictates otherwise, between the upper and lower limit of that range, and any other stated or intervening value in that stated range, is encompassed within the invention. The upper and lower limits of these smaller ranges may independently be included in the smaller ranges, and are also encompassed within the invention, subject to any specifically excluded limit in the stated range.

[0031] Numeric ranges are inclusive of the numbers defining the range. The term about is used herein to mean plus or minus ten percent (10%) of a value. For example, “about 100” refers to any number between 90 and 110. The term “about” a range refers to that range minus 10% of its lowest value and plus 10% of its greatest value. Reference to “about” a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter per se.

[0032] A scientific format of exponential notation may be used in the present disclosure where part of a number is replaced with E+n, in which E (exponent) multiplies the preceding number by 10 to the nth power. For example, a 2-decimal scientific format displays 12345678901 as 1.23E10, which is 1.23 times 10 to the 10th power, and may be written alternatively as 1.23×10^{10} . Similarly, 1.23E-10 may be written alternatively as 1.23×10^{-10} .

[0033] Generally, the nomenclature used herein and the techniques and procedures described herein include those that are well understood and commonly employed by those of ordinary skill in the art, such as the common techniques and methodologies described in e.g., Green and Sambrook, *Molecular Cloning: A Laboratory Manual* (Fourth Edition), Vols. 1-3, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y., 2012 (hereinafter “Sambrook”); and *Current Protocols in Molecular Biology*, F. M. Ausubel et al., eds., originally published in 1987 in book form by Greene Publishing Associates, Inc. and John Wiley & Sons, Inc., and regularly supplemented through 2011, and now available in journal format online as *Current Protocols in*

Molecular Biology, Vols. 00 - 130, (1987-2020), published by Wiley & Sons, Inc. in the Wiley Online Library (hereinafter "Ausubel").

[0034] Unless otherwise indicated, nucleic acids are written left to right in 5' to 3' orientation; amino acid sequences are written left to right in amino to carboxy orientation, respectively.

[0035] 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 the present invention pertains. It is to be understood that the terminology used herein is for describing particular embodiments only and is not intended to be limiting. For purposes of interpreting this disclosure, the following description of terms will apply and, where appropriate, a term used in the singular form will also include the plural form and vice versa.

[0036] The headings provided herein are not limitations of the various aspects or embodiments of the invention which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification as a whole.

[0037] The terminology used herein is for the purpose of describing particular examples only and is not intended to be limiting. As used herein, the singular forms "a", "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. Furthermore, to the extent that the terms "including", "includes", "having", "has", "with", or variants thereof are used in either the detailed description and/or the claims, such terms are intended to be inclusive in a manner similar to the term "comprising". The term "comprising" as used herein is synonymous with "including" or "containing," and is inclusive or open-ended.

[0038] The term "subject", "patient", or "individual" refers to a mammal including but not limited to, primates, such as humans and non-human primates ("NHPs"), e.g., African green monkeys and rhesus monkeys, mammalian sport animals, mammalian farm animals, mammalian pets and rodents. In some embodiments, the subject is a human.

[0039] Biological samples useful in the methods of the present disclosure include any sample obtained from a subject contains the biological molecules (e.g., RNA or DNA) relevant to the method for measuring the expression levels of the gene signature that identifies the risk of inflammatory response. Accordingly, it is contemplated that biological samples can comprise blood, intravitreal fluid, cerebrospinal fluid, peritoneal fluid, pleural fluid, bronchoalveolar lavage fluid, sputum, nasal brushings, throat swabs, urine, amniotic fluid, plasma, serum, saliva, semen, bone marrow, tissue or fine needle biopsy samples, stool, skin, or cells therefrom. In another embodiment, the biological sample comprises blood. The biological sample can be obtained from the subject at any point relevant to the particular method, such as prior to any

rAAV treatment (e.g., screening potential candidates), or any time during or after rAAV treatment (e.g., monitoring patients). It is also contemplated that the biological samples can include tissue sections, such as frozen sections for immuno-histological analysis.

[0040] The terms “treat,” “treating,” “treatment,” and other grammatical equivalents as used herein, refer to alleviating, abating or ameliorating a disease or disorder, or symptoms of a disease or disorder, preventing additional symptoms of the disease or disorder, ameliorating or preventing the underlying causes of symptoms, inhibiting a disease or disorder, e.g., arresting the development of a disease or disorder, relieving a disease or disorder, causing regression of a disease or disorder, or stopping the symptoms of a disease or disorder, and are intended to include prophylaxis and prevention. The terms further include achieving a therapeutic benefit and/or a prophylactic benefit, such as the benefit achieved with the eradication or amelioration of one or more of the physiological symptoms associated with a disease or disorder such that an improvement is observed in the subject, notwithstanding that, the subject may still be afflicted with a disease or disorder.

[0041] The terms “administer,” “administering,” “administration,” and the like, as used herein, can refer to the methods that are used to enable delivery of therapeutics or pharmaceutical compositions to the desired site of biological action. These methods include intravitreal or subretinal injection to an eye.

[0042] The term “pharmaceutical composition,” or simply “composition” as used herein, can refer to a biologically active compound, optionally mixed with at least one pharmaceutically acceptable chemical component, such as, though not limited to carriers, stabilizers, diluents, dispersing agents, suspending agents, thickening agents, excipients and the like.

[0043] The terms “effective amount,” “therapeutically effective amount” or “pharmaceutically effective amount” as used herein, can refer to a sufficient amount of at least one pharmaceutical composition or compound being administered which will relieve to some extent one or more signs or symptoms of the ocular disease, ocular disorder or ocular condition being treated. In some embodiment, the effective amount of a pharmaceutical composition may be administered to a subject in need thereof as a unit dose (as described in further detail elsewhere herein).

[0044] A “vector” as used herein refers to a macromolecule or association of macromolecules that comprises or associates with a polynucleotide and which can be used to mediate delivery of the polynucleotide to a cell. Illustrative vectors include, for example, plasmids, viral vectors, liposomes, and other gene delivery vehicles.

[0045] By “neutralizing antibody” or “nAb” is meant an antibody which recognizes a specific antigen and inhibits the effect(s) of the antigen in the host (e.g., a human). As used herein, the

antibody can be a single antibody or a plurality of antibodies. In certain embodiments, a neutralizing antibody can inhibit the effect(s) of the antigen of at least 1, 2, 3, 4, 5, 6, 7, 8, 9 or more difference strains of AAV. For example, the neutralizing antibody can inhibit infectivity of (e.g., cell entry), or gene expression directed by, an AAV. Neutralizing antibodies that bind a virus, e.g., an AAV can, for example, exert a substantial deleterious effect on infectivity by, transduction by, gene expression directed by, or another property or activity of the virus.

[0046] As used herein, “biological species” is any molecule that is able to form a specific interaction with any other biological molecule.

[0047] As used herein, “nucleic acid” can be DNA, RNA, singled stranded double stranded, or certain aptamers and any variant thereof.

[0048] As used herein, “protein” is any molecule in which its units are linked together by peptide bonds, typically linking amino acids together to form the protein.

[0049] As used herein, “carrier” is any molecule that is able to deliver a therapeutic or diagnostic molecule into a biological system, such as in a gene delivery system.

[0050] As used herein, a “payload” molecule refers to a therapeutically or diagnostically active molecule that is administered to a mammal, preferably human beings, and delivered to the mammal while carried inside a carrier molecule and is later released inside the body.

[0051] AAV particles, variants, and the AAV2.7m8 capsid protein

[0052] “AAV,” as used herein, refers to an adeno-associated virus, and may be used to refer to the virus itself or derivatives thereof. The term covers all subtypes and both naturally occurring and recombinant forms, except where required otherwise. The term “AAV” includes AAV type 1 (AAV-1), AAV type 2 (AAV-2), AAV type 3 (AAV-3), AAV type 4 (AAV-4), AAV type 5 (AAV-5), AAV type 6 (AAV-6), AAV type 7 (AAV-7), AAV type 8 (AAV-8), avian AAV, bovine AAV, canine AAV, equine AAV, primate AAV, non-primate AAV, and ovine AAV. “Primate AAV” refers to AAV that infect primates, “non-primate AAV” refers to AAV that infect non-primate mammals, “bovine AAV” refers to AAV that infect bovine mammals, etc.

[0053] Genomic sequences of various serotypes of AAV are well known in the art, as well as the sequences of the native terminal repeats (TRs), Rep proteins, and capsid subunits. For example, such sequences can be found in the literature or in public databases such as GenBank. See, e.g., GenBank Accession Numbers NC_002077 (AAV-1), AF063497 (AAV-1), NC_001401 (AAV-2), AF043303 (AAV-2), NC_001729 (AAV-3), NC-001829 (AAV-4), U89790 (AAV-4), NC_006152 (AAV-5), AF028704 and AAB95450 (AAV-6), AF513851 (AAV-7), AF513852 (AAV-8), and NC_006261 (AAV-8), the disclosures of which are incorporated by reference herein for teaching AAV nucleic acid and amino acid sequences. See also, e.g.,

Srivistava et al. (1983) J. Virology 45:555; Chiorini et al. (1998) J. Virology 71:6823; Chiorini et al. (1999) J. Virology 73:1309; Bantel-Schaal et al. (1999) J. Virology 73:939; Xiao et al. (1999) J. Virology 73:3994; Muramatsu et al. (1996) Virology 221:208; Shade et al. (1986) J. Virol. 58:921; Gao et al. (2002) Proc. Nat. Acad. Sci. USA 99:11854; Moris et al. (2004) Virology 33:375-383; international patent publications WO 00/28061, WO 99/61601, WO 98/11244; and U.S. Pat. No. 6,156,303.

[0054] Polynucleotide sequences that encode any of the AAV capsid proteins may be readily generated based on the amino acid sequence and the known genetic code, including codon-optimized sequences.

[0055] An "AAV variant" or "AAV mutant" as used herein refers to a viral particle composed of: a) a variant AAV capsid protein, where the variant AAV capsid protein comprises at least one amino acid difference (e.g., amino acid substitution, amino acid insertion, amino acid deletion) relative to a corresponding parental AAV capsid protein, where the AAV capsid protein does not correspond to the amino acid sequence present of a naturally occurring AAV capsid protein; and, optionally, b) a heterologous nucleic acid comprising a nucleotide sequence encoding a heterologous gene product, wherein the variant AAV capsid protein confers increased binding to heparan or a heparan sulfate proteoglycan as compared to the binding by an AAV virion comprising the corresponding parental AAV capsid protein. In certain embodiments, the variant capsid protein confers: a) increased infectivity of a retinal cell compared to the infectivity of the retinal cell by an AAV virion comprising the corresponding parental AAV capsid protein; b) altered cellular tropism as compared to the tropism of an AAV virion comprising the corresponding parental AAV capsid protein; and/or c) an increased ability to bind and/or cross the ILM as compared to an AAV virion comprising the corresponding parental AAV capsid protein.

[0056] In some embodiments, an AAV variant can have a variant AAV capsid protein, e.g., the capsid protein of the AAV can include an insertion within a capsid protein. Exemplary insertions can include, VP1, of AAV type 1 (AAV-1), AAV type 2 (AAV-2), AAV type 3 (AAV-3), AAV type 4 (AAV-4), AAV type 5 (AAV-5), AAV type 6 (AAV-6), AAV type 7 (AAV-7), AAV type 8 (AAV-8), AAV type 9 (AAV-9), AAV type 10 (AAV-10), AAV rh.10, avian AAV, bovine AAV, canine AAV, equine AAV, primate AAV, non-primate AAV, bovine AAV, AAV2/7m8, AAVShH10, AAV2.5T, AAV2.5T/7m8, AAV9/7m8, and AAV5/7m8.

[0057] The variant, AAV2.5T capsid proteins and virions are described in U.S. Pat. No. 9,233,131, in which the VP1-encoding amino acid sequences of AAV2.5T is provided as SEQ ID NO:42 and FIGS. 10A-B of U.S. Pat. No. 9,233,131.

[0058] The AAV2.7m8 capsid protein is described in U.S. Pat. No. 9,193,956.

[0059] Polypeptide sequences of the wild-type AAV2 capsid protein, and the variant AAV2.7m8 capsid protein are provided in Table 1 (below) and the accompanying Sequence Listing.

[0060] TABLE 1: AAV2 and AAV2.7m8 Capsid Polypeptide Sequences

Name	AA Sequence	SEQ ID NO:
Wildtype AAV2	MAADGYLPDWLEDLTLSEGIQWWKLKPGPPPPKPAERHKDDSRGLVLPGY KYLGFENGLDKGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADAEF QERLKEDTSFGGNLGRAVFQAKKRVLEPLGLVEEPVKTPAGKKRPVEHSP VEPDSSSGTGKAGQQPARKRLNFGQTGDADSVDPQPLGQPPAAPSGLGT NTMATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTTSTRTWALP TYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDENRFHCHFSRDPWQRLI NNNWGFRPKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQL PYVLGSAHQGCLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPS QMLRTGNNTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTNT PSGTTTQSRQLQFSQAGASDIRDQSRNWLPGPCYRQQRVSKTSADNNNSEY SWTGATKYHLNGRDSLVPNPGPAMASHKDDEEKFFPQSGVLI FGKQGSEKT NVDIEKVMITDEEEIRTNPVATEQYGSVSTNLQRGNRQAATADVNTQGV LPGMVWQDRDVYLQGP IWAKI PHTDGHFHPSPLMGGFGLKHPPPQILIKN TPVPANPSTTFSAAKFASFITQYSTGQVSVEIEWELQKENS KRWNPEIQY TSYNKSVNVDFTVDTNGVYSEPRPIGTRYLTRNL	1
AAV2.7m8	MAADGYLPDWLEDLTLSEGIQWWKLKPGPPPPKPAERHKDDSRGLVLPGY KYLGFENGLDKGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADAEF QERLKEDTSFGGNLGRAVFQAKKRVLEPLGLVEEPVKTPAGKKRPVEHSP VEPDSSSGTGKAGQQPARKRLNFGQTGDADSVDPQPLGQPPAAPSGLGT NTMATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTTSTRTWALP TYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDENRFHCHFSRDPWQRLI NNNWGFRPKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQL PYVLGSAHQGCLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPS QMLRTGNNTFSYTFEDVPFHSSYAHSQSLDRLMNPLIDQYLYYLSRTNT PSGTTTQSRQLQFSQAGASDIRDQSRNWLPGPCYRQQRVSKTSADNNNSEY SWTGATKYHLNGRDSLVPNPGPAMASHKDDEEKFFPQSGVLI FGKQGSEKT NVDIEKVMITDEEEIRTNPVATEQYGSVSTNLQRGN <u>LALGETTRPAR</u> QA ATADVNTQGVLPGMVWQDRDVYLQGP IWAKI PHTDGHFHPSPLMGGFGLK HPPPQILIKNTVPANPSTTFSAAKFASFITQYSTGQVSVEIEWELQKEN SKRWNPEIQYTSYNKSVNVDFTVDTNGVYSEPRPIGTRYLTRNL	2
191205-18-PAC (Loop) polypeptide	CRGNLALGETTRPAR	3

[0061] As shown in bolded underline in SEQ ID NO: 2, the AAV2.7m8 variant capsid protein includes the 10 amino acid “7m8” insert of LALGETTRPA (SEQ ID NO: 22) between amino acids 587 and 588 of the wildtype AAV2 genome.

[0062] Anti-AAV neutralizing antibodies

[0063] As noted elsewhere herein, gene therapy mediated by AAV can be an effective for treatment for ocular diseases. Pre-existing antibodies capable of binding to and neutralizing AAV vectors used in such gene therapeutics can negatively impact transduction and gene expression following IVT delivery. Detecting and quantifying nAbs prior to or after IVT administration is important for successful gene therapy treatment.

[0064] Anti-AAV2.7m8 antibodies

[0065] Antibodies that specifically bind to therapeutic AAV capsid proteins can be used in assays for the detection of AAV neutralizing antibodies (nAbs). Specifically, these anti-AAV antibodies can be used as positive controls in assays for detecting and quantifying the levels of pre-existing nAbs in patient samples. In at least one embodiment, the present disclosure is directed to anti-AAV2.7m8 antibodies that bind the variant AAV2.7m8 capsid protein. Such antibodies that bind specifically and with high affinity to the AAV2.7m8 capsid protein can be produced and screened using a variety of techniques known in the art for the production of antibodies. The present disclosure provides an exemplary anti-AAV2.7m8 antibody ("clone 6") that was generated in rabbits using the 191205-18-PAC (Loop) polypeptide of SEQ ID NO: 3 derived from the AAV2.7m8 polypeptide sequence of SEQ ID NO: 2. The generation and screening of this anti-AAV2.7m8 antibody is described in Example 1, and the relevant amino acid sequences of the antibody CDR, Framework, VH and VL domains are provide in Table 2 and the accompanying Sequence Listing.

[0066] TABLE 2: Exemplary "clone 6" anti-AAV2.7m8 antibody sequences

Region	AA Sequence	SEQ ID NO:
VL	MDTRAPTQLLGLLLWLPGATFAQVLTQTPSPVSAALGGTVTINCQSSQSVA RDNRLAWFQQKPGQPPKLLIYRASTLASGVPSRFRKSGSGTQFTLTISGVQC DDAATYYCQGGHSAGDGRAFGGGTEVVVK	4
CDR-L1	QSSQSVAARDNRLA	5
CDR-L2	RASTLAS	6
CDR-L3	QGGHSAGDGRA	7
FR-L1	AQVLTQTPSPVSAALGGTVTINC	8
FR-L2	WFQQKPGQPPKLLIY	9
FR-L3	GVPSRFRKSGSGTQFTLTISGVQCDDAATYYC	10
FR-L4	FGGGTEVVVK	11
VH	METGLRWLLLVAVLKGVQCQSVEESRGGLIKPTDTLTCTVSGFSLSDYGV NWRQAPNGLEWIGIISNGGRYYASWAKSRATITRNTNENTVTLKMTSLT AADTATYFCAREDLYVASDIWGPGLTVTVSS	12
CDR-H1	DYGVN	13
CDR-H2	IISNGGRYYASWAKS	14
CDR-H3	EDLYVASDI	15
FR-H1	SVEESRGGLIKPTDTLTCTVSGFSL	16
FR-H2	WVRQAPNGLEWIG	17

FR-H3	RATITRNTNENTVTLKMTSLTAADTATYFCAR	18
FR-H4	WGPGTLTVVSS	19
LC	MDTRAPTQLLGLLLLLWLPGATFAQVLTQTPSPVSAALGGTVTINCQSSQSVA RDNRLAWFQQKPGQPPKLLIYRASTLASGVPSRFKSGSGTQFTLTISGVQC DDAATYYCQGGHSAGDGRAFGGGTEVVVKG	20
HC	METGLRWLLLVAVLKGVQCQSVEESRGGLIKPTDTLTLTCTVSGFSLSDYGV NWRQAPGNGLEWIGIISNGGRYYASWAKSRATITRNTNENTVTLKMTSLT AADTATYFCAREDLYVASDIWGPGTLTVVSS	21

[0067] As shown in the Examples, the “clone 6” anti-AAV2.7m8 antibody exhibits high binding specificity and affinity for the AAV2.7m8 capsid protein of SEQ ID NO: 2. As described further below, this high specificity and affinity makes this antibody particularly useful in a number of assays directed to detecting and quantifying the AAV2.7m8 capsid protein.

[0068] Generally, the CDR sequences of an antibody define the binding and affinity characteristics of an antibody with its specific antigen. The present disclosure contemplates a range of anti-AAV2.7m8 antibodies that include the 6 CDR sequences shown in Table 2. Thus, the present disclosure contemplates a range of antibodies that specifically bind AAV2.7m8 capsid with affinity and specificity of “clone 6”, wherein the antibodies comprise a first light chain complementary determining region (CDR-L1) of SEQ ID NO: 5, a second light chain complementary determining region (CDR-L2) of SEQ ID NO: 6, a third light chain complementary determining region (CDR-L3) of SEQ ID NO: 7, a first heavy chain complementary determining region (CDR-H1) of SEQ ID NO: 13, a second heavy chain complementary determining region (CDR-H2) of SEQ ID NO: 14, and a third heavy chain complementary determining region (CDR-H3) of SEQ ID NO: 15.

[0069] Additionally, the present disclosure contemplates anti-AAV2.7m8 antibodies, wherein the antibody comprises a light chain variable domain (VL) amino acid sequence having at least 90% identity to a sequence selected from SEQ ID NO: 4; and/or a heavy chain variable domain (VH) amino acid sequence having at least 90% identity to a sequence selected from SEQ ID NO: 12.

[0070] In another embodiment the present disclosure contemplates anti-AAV2.7m8 antibodies comprising a light chain (LC) amino acid sequence having at least 90% identity to a sequence selected from SEQ ID NO:20; and/or a heavy chain (HC) amino acid sequence having at least 90% identity to a sequence selected from SEQ ID NO: 21.

[0071] The exemplary “clone 6” anti-AAV2.7m8 antibody was generated in a rabbit and includes rabbit framework and immunoglobulin fragments, however, one of ordinary skill will recognize that an equivalent functional anti-AAV2.7m8 antibody can be prepared in other antibody frameworks and formats. Accordingly, in at least one embodiment the present

disclosure also contemplates that the anti-AAV2.7m8 antibody can be in any of the following antibody formats: (i) a monoclonal antibody; (ii) a chimeric antibody; (iii) a humanized antibody; (iv) a full length antibody of class IgG, optionally, wherein the class IgG antibody has an isotype selected from IgG1, IgG2, IgG3, and IgG4; (v) an antibody fragment, optionally selected from the group consisting of F(ab')₂, Fab', Fab, Fv, single domain antibody (VHH), and scFv; (vi) the antibody is a multi-specific antibody, optionally a bispecific antibody; and/or (vii) the antibody is a synthetic antibody, wherein the HVRs are grafted onto a scaffold or framework other than an immunoglobulin scaffold or framework; optionally, a scaffold selected from an alternative protein scaffold and an artificial polymer scaffold.

[0072] Similarly, one of ordinary skill will recognize that the exemplary "clone 6" anti-AAV2.7m8 antibody of the present disclosure can be modified via standard antibody and/or polypeptide conjugation techniques well-known in the art. Accordingly, in at least one embodiment, the present disclosure contemplates that the anti-AAV2.7m8 antibody can be an immunoconjugate, for example, a conjugate with a detectable moiety such as a radiolabel, a fluorescent label, and an indirect labeling moiety. The modification with a detectable moiety can allow the anti-AAV2.7m8 antibody to be used in a range of assays for detection and quantification of AAV2.7m8 capsid protein.

[0073] The exemplary "clone 6" anti-AAV2.7m8 antibody of Table 2 has been defined in terms of its CDR structure. However, because it was generated and screened to specifically bind 7m8 sequence of SEQ ID NO: 2, the present disclosure contemplates that other anti-AAV2.7m8 antibodies that specifically bind to this same epitope can be prepared and used in assays for specifically detecting and quantifying the AAV2.7m8 capsid protein. Thus, in at least one embodiment, the present disclosure provides an antibody that can compete with the "clone 6" anti-AAV2.7m8 antibody for binding to human AAV2.7m8 capsid polypeptide of SEQ ID NO: 2 (e.g., has a binding affinity of 1×10^{-9} M or less, 1×10^{-10} M or less, or 1×10^{-11} M or less), or more specifically, that binds to the epitope comprising the amino acid sequence LALGETTRPA (SEQ ID NO: 22) located at positions 588-597 of SEQ ID NO: 2.

[0074] The exemplary "clone 6" anti-AAV2.7m8 antibody is characterized in the Examples as having high affinity for the AAV2.7m8 capsid protein of SEQ ID NO: 2. The present disclosure contemplates a range of anti-AAV2.7m8 antibodies having functional characteristics similar to those exhibited by "clone 6" antibody. For example, an anti-AAV2.7m8 antibody characterized by one or more of the following properties: (a) binds to AAV2.7m8 capsid protein with a binding affinity of 1×10^{-8} M or less, 1×10^{-9} M or less, 1×10^{-10} M or less, or 1×10^{-11} M or less; optionally, wherein the binding affinity is measured by equilibrium dissociation constant

(K_D) to a AAV2.7m8 capsid protein; or (b) binds to AAV2.7m8 capsid of SEQ ID NO: 2 with a binding affinity that is increased by at least 2-fold, at least 3-fold, at least 5-fold, or at least 10-fold, relative to the binding affinity to AAV2 capsid protein; optionally, wherein the relative binding affinities are measured by equilibrium dissociation constant (K_D) to the AAV2.7m8 capsid polypeptide of SEQ ID NO: 2 and the AAV2 capsid polypeptide of SEQ ID NO: 1.

[0075] Uses of anti-AAV2.7m8 antibodies

[0076] As noted elsewhere herein, the antibodies of the present disclosure that specifically bind to AAV2.7m8 capsid protein with high affinity can be used in assays for detecting and/or quantifying the presence of AAV2.7m8 neutralizing antibodies in samples. For example, the anti-AAV2.7m8 antibodies can be used as a positive control in a method for detecting neutralizing antibody (nAbs) activity against AAV2.7m8 in a human serum sample. Generally, such an nAb assay method comprises the steps of:

(a) providing: (i) a human serum sample; (ii) an assay solution comprising cells; (iii) a vector solution comprising an AAV2.7m8 vector that encodes a luciferase gene; (iv) a luciferase substrate solution; and (v) an antibody solution comprising an anti-AAV2.7m8 antibody that specifically binds AAV2.7m8 capsid;

(b) combining an aliquot of the human serum sample, the assay solution, the vector solution, and the luciferase substrate solution to form a sample solution;

(c) combining an aliquot of the AAV2.7m8 antibody solution, the assay solution, the vector solution, and the luciferase substrate solution to form a positive control solution; and

(d) detecting luminescence signals from the sample solution and the positive control solution; whereby, the relative luminescence signal levels indicate presence and/or amount of neutralizing antibody activity in the human serum sample. It is contemplated that step (c) can include combining at least 2, 3, 4, 5, 6, 7, 8, 9, or more different aliquots with different dilutions of the AAV2.7m8 antibody solution to form at least 2, 3, 4, 5, 6, 7, 8, 9, or more positive control solutions having different concentrations of the AAV2.7m8 antibody for use in preparing a response curve. It is also contemplated that the assay method can include further steps. For example, a step of combining an aliquot of the assay solution, and the luciferase substrate solution to form a negative control solution; and detecting luminescence signals from the negative control solution.

[0077] Additionally, it is contemplated that in the assay method, the cells can be pre-exposed to an AAV2.7m8 vector that encodes a luciferase gene, are from a transduction-permissive cell line; optionally, wherein the cell line is 293T.

[0078] Additionally, it is contemplated that anti-AAV2.7m8 antibodies can be used in other assays and diagnostic uses for the detection and quantification of the AAV2.7m8 capsid protein.

[0079] In at least one embodiment, the present disclosure also provides a method for detecting an AAV2.7m8 capsid protein, the method comprising: contacting the protein with an anti-AAV2.7m8 antibody of the present disclosure and detecting the antibody bound to the protein. In at least one embodiment, the AAV2.7m8 capsid protein is immobilized. In at least one embodiment, the AAV2.7m8 capsid protein is in solution. In at least one embodiment, the antibody comprises a detectable label. In at least one embodiment, the detectable label selected from a radiolabel, a fluorescent label, and an indirect labeling moiety.

[0080] Further, the disclosure is directed to the use of these anti-AAV2.7m8 antibodies in assays (and kits) for detecting and/or quantifying neutralizing antibodies (nAbs) that may be present in a subject that is administered or is a candidate to be administered a gene therapy treatment that includes the AAV2.7m8 capsid protein. It is contemplated that an anti-AAV2.7m8 antibody as disclosed herein can be included in a kit with reagents useful in an assay with the antibody for the detection and/or quantification of the AAV2.7m8 capsid protein in a sample, such as a blood sample from a patient. For example, the assay reagents included in the kit with the antibody can include but are not limited to: (a) an AAV2.7m8 vector that encodes a luciferase gene; (b) a luciferase substrate; and/or (c) cells from a transduction-permissive cell line; optionally, wherein the cells are from a 293T cell line. The kit also may include instructions on how to use the kit to determine relative amounts of the payload and the carrier in a biological sample.

[0081] The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims.

EXAMPLES

[0082] Various features and embodiments of the disclosure are illustrated in the following representative examples, which are intended to be illustrative, and not limiting. Those skilled in the art will readily appreciate that the specific examples are only illustrative of the invention as described more fully in the claims which follow thereafter. Every embodiment and feature described in the application should be understood to be interchangeable and combinable with every embodiment contained within.

[0083] **Example 1: Preparation of an Anti-AAV2.7m8 Antibody**

[0084] This example illustrates the preparation of rabbit anti-AAV2.7m8 antibodies of the present disclosure, and a comparative BLI affinity study of the binding of these antibodies for the target antigen AAV2.7m8 capsid protein relative to affinity for the wild-type AAV2 capsid protein.

[0085] ***Materials and methods***

[0086] Polypeptide sequences of AAV2, and AAV2.7m8, used to generate and screen antibodies are provided in Table 1 (above) and the accompanying Sequence Listing.

[0087] A. Antibody generation, screening, and cloning

[0088] The 14 aa loop polypeptide distinct for AAV2.7m8 was modified with an N-terminal Cys to provide the 191205-18-PAC (Loop) polypeptide CRGNLALGETTRPAR (SEQ ID NO: 3). This polypeptide was conjugated to keyhole limpet hemocyanin (KLH) carrier protein. Rabbits were immunized with this KLH-peptide conjugate at least 4 times and bled to provide 20-25 mL of serum per animal. The collected serum was enriched for B-cells that specifically bind AAV2.7m8 using an affinity column modified with the specific AAV2.7m8 loop polypeptide of SEQ ID NO: 3.

[0089] ELISA was carried out on the affinity purified rabbit serum samples and confirmed the presence of antibodies exhibiting monospecific binding affinity for AAV2.7m8 (SEQ ID NO: 2). Paired heavy and light chains from the enriched B cells identified in ELISA were cloned and used to transfect HEK293 cells. A screening assay of supernatants from the transfected HEK293 cells were used to identify a total of 9 rabbit antibodies (clones 1-8, and 11) with high affinity for AAV2.7m8.

[0090] B. BLI affinity assay of anti-AAV2.7m8 antibodies

[0091] The binding affinity of the anti-AAV2.7m8 antibodies was determined using standard Octet Bio-Layer Interferometry (BLI) binding analysis. Briefly, (1) the nine rabbit anti-AAV2.7m8 antibodies (clones 1-8, & 11) were immobilized to protein A biosensors; (2) each immobilized antibody biosensor tip was dipped into an analyte well containing buffer and AAV.7m8-luciferase vector (34.4nM) or AAV2-CAG-luciferase vector (17.2nM); (3) control biosensor tips with no immobilized antibody were also dipped into the analyte well to test for non-specific binding.

[0092] The BLI signals from analyte association and dissociation from the immobilized antibody were analyzed using the standard Octet data software and analysis. First, a reference subtraction is performed on all recorded traces using the control well (biosensor that underwent same steps as experimental wells but no analyte for the association step) and then all the traces

are aligned to the beginning of the association step. The entirety of the association and dissociation steps are used to fit a 1:1 binding model for the nine rabbit anti-AAV2.7m8 antibodies.

[0093] The AAV.7m8 target analyte exhibited no non-specific binding to the control biosensor tips with no immobilized antibody. The commercially obtained “AAV2-CAG-luciferase” vector target analyte exhibited non-specific binding to the control biosensor tips, likely due to un-disclosed impurities in the commercial formulation.

[0094] Results

[0095] The AAV2.7m8-luciferase vector target analyte bound to six of the nine rabbit anti-AAV2.7m8 antibodies that were tested. None of the nine rabbit anti-AAV2.7m8 antibodies bound to the AAV2-CAG-luciferase vector target analyte. As shown by the BLI signal traces shown in **FIGS. 1A-1I**, the anti-AAV2.7m8 antibodies of clone 1 (**FIG. 1A**), clone 6 (**FIG. 1H**), and clone 7 (**FIG. 1C**) exhibited the highest specific binding affinity for the AAV.7m8 target analyte.

[0096] Based on its high affinity for AAV2.7m8 target analyte measured in BLI, the “clone 6” anti-AAV2.7m8 antibody was selected for sequencing. The CDR, FR, and VL and VH domain sequences of “clone 6” are provided in Table 2.

[0097] Example 2: Anti-AAV2.7m8 Neutralizing Antibody Assay (nAb)

[0098] This example illustrates an assay method using the exemplary “clone 6” anti-AAV2.7m8 antibody of Example 1, and for comparison, the “IVIG” antibody pool which binds the wild-type AAV2 capsid protein antigen, to detect and/or quantify the levels of AAV2 capsid neutralizing antibodies in a biological sample.

[0099] Briefly, in the assay method, a solution of transduction-permissive 293T cells is exposed to an AAV2.7m8 vector that encodes the firefly luciferase gene. Successful transduction of the viral encoded luciferase gene yields an increase in a luminescent signal when the luciferase substrate is added to the solution. Addition of a human serum sample containing neutralizing antibodies (nAbs) with the capacity to neutralize the AAV2.7m8 vector in this solution results in reduced AAV2.7m8 transduction and reduced luminescence. Serial dilutions of the exemplary antibody (“clone 6” or “IVIG”) added to solutions of 293T cells exposed to AAV2.7m8 virus similarly reduce luminescence and thereby act as a positive control. The presence and amount of nAbs is determined by monitoring the decrease in luminescent signal relative level to the level of signal obtained from cells exposed to an AAV.7m8-luciferase virus that has been pre-incubated with a non-neutralizing serum.

[0100] *Materials and methods*

[0101] The “clone 6” anti-AAV2.7m8 antibody is serially diluted and then combined with luciferase vector (AAV.7m8-luciferase). The antibody and vector mixtures are incubated for 1 hour at 37°C., 5% CO₂, then added to pre-seeded HEK 293 cells at an MOI of 2.5E5- 5E5 vg/cell. The plates of transduced cells are incubated at 37°C., 5% CO₂ for 18-24 hours. Post incubation, plates are cooled to room temperature and 100 µL of One-Glo (Promega) reagent is added to each well. The luminescence signal is read in a SpectraMax M3 instrument (Molecular Devices) and % inhibition is calculated and graphed using a 4-PL fit on the SoftMax Pro software (Version 7.1.2).

[0102] A detailed list of materials and description of the assay protocol is as follows:

[0103] A. Reagents and Materials

[0104] “Clone 6” anti-AAV2.7m8 antibody positive surrogate control.

[0105] 293T complete growth medium: cIMDM (IMDM w/GlutaMax+10% hi FBS)

[0106] 293 transductions medium: TM (OptiMEM w/GlutaMax + 1% FBS)

[0107] AAV2.7m8.CMV.Luc vector (Lot # 18-293-005).

[0108] AAV2-CBA-Luciferase (Vector Biolabs Lot# 109128-200807).

[0109] PBS sterile, Ca⁺⁺, Mg⁺⁺ free, Gibco: 10010-23, Lot # 2085516.

[0110] Hi Fetal Bovine Serum (FBS), Gibco: 10438-026. Lot # 2086963.

[0111] Trypsin-EDTA 0.05% 1X, Gibco: 25300-054. Lot # 2085644.

[0112] One-Glo, Promega, E6130, Lot # 0000275700.

[0113] IMDM with GlutaMax, Gibco: 31980-030, Lot # 2150863.

[0114] OptiMEM w/ GlutaMax, Gibco: 51985-034, Lot # 2053509).

[0115] Reagent reservoirs, Costar 4870.

[0116] 96-well flat-bottomed tissue culture plate, white microwell, Thermo Scientific: 136102.

[0117] 96-well u-bottomed polypropylene plates, Greiner Bio-one REF 650261.

[0118] Molecular Devices SpectraMax M3.

[0119] SoftMax Pro software.

[0120] B. Assay Protocol

[0121] Grow 293T cells for at least four passages post-thaw.

[0122] Day 1: Plating Cells

[0123] 1. Warm fresh media, trypsin and transduction media (TM).

[0124] 2. Aspirate medium from flask.

[0125] 3. Rinse cell with 10 mL of PBS and aspirate from flask

[0126] 4. Add 2.0 mL of 0.5% Trypsin-EDTA, tap flask periodically for 3-5 minutes until cells not adhered.

[0127] 5. When cells are no longer adhered, add 8.0 mL of fresh media, mix and transfer to 15 mL centrifuge tube.

[0128] 6. Spin at 130 X G at R.T. for 10 minutes.

[0129] 7. Carefully Aspirate supernatant.

[0130] 8. Resuspend pellet in 5.0 mL of fresh media by pipetting up and down with serological pipet at least six times.

[0131] 9. Add 0.5 mL of cells to 19.5 mL of fresh media in new flask and incubate for next passage.

[0132] 10. Add slightly more than 100 μ L of resuspended cells to an Eppendorf tube.

[0133] 11. Add exactly 100 μ L from first Eppendorf tube to a second Eppendorf tube.

[0134] 12. Add exactly 100 μ L of trypan blue to the second Eppendorf tube and mix well without lysing.

[0135] 13. Fill both chambers of disposable hemocytometer with stained cells.

[0136] 14. Count cells on Countess II FL according to following analysis and quantification template:

[0137] Live cell number: Chamber A ____X10⁶ + Chamber B ____X10⁶.

[0138] Mean # live cells = ____X 10⁶ cells/mL;

[0139] Viability: Live A = ____% Dead A = ____; Live B = ____% Dead B = ____%

[0140] Dilution (5 plates);

[0141] (i) Desired concentration: 1.875E5 cells/mL;

[0142] (ii) Total vol. needed: ____ (# plates) X 96 = ____ (# wells) X 0.080mL = ____mL + 20% = ____mL;

[0143] (iii) Vol. of live cells: ____mL X 1.875 X 10⁵cells/mL = _____cells ÷ mean live cell number = ____mL

[0144] (iv) Volume of TM: ____mL (Total vol. needed) - ____mL (Vol. of live cells = _____mL.

[0145] 15. Add 80 μ L of diluted cells to all wells of 96-well white tissue culture plates and incubate O.N. Cells per well: 1.5E4 cells/well.

[0146] **Day 2: Preparation of Standard and AAV Vector; and transduction of 293T cells**

[0147] 1. Prepare "clone 6" mAb 3-12, for rows A-D; (ii) Add 135 μ L of TM to well A2; (iii) Mix 15 μ L of "clone 6" mAb to well A2; (iv) Add 150 μ L of 7m8 supernatants to well B2-D2; (v) Serial dilution of 75 μ L from wells A2-A11, B2-B11, C2-C11 and D2-D11.

[0148] 2. Prepare 2.5 mL of diluted vector AAV2.7m8-CMV-LUC, Lot#ADVM19-293-012, 9.53E12 vg/mL (MOI is 2.5E5 vg/cell), as follows: (i) $1.5E4 \text{ cells/well} \times 2.5E5 \text{ vg/cell} = 3.75E9 \text{ vg/well} = 3.75E9 \text{ vg}/40 \mu\text{L} = 9.38E10 \text{ vg/mL}$; (ii) Dilute stock vector: $9.38E10 \text{ vg/mL} / 9.53E12 \text{ vg/mL} = 9.84 \mu\text{L stock} + 2.490 \text{ mL of TM}$.

[0149] 3. Prepare 2.5 mL of diluted vector AAV2-CBA-LUC, Lot#, 1.0E13 vg/mL (MOI is 5.33E5 vg/cell), as follows: (i) $1.5E4 \text{ cells/well} \times 5.33E5 \text{ vg/cell} = 8E9 \text{ vg/well} = 8E9 \text{ vg}/40 \mu\text{L} = 2E11 \text{ vg/mL}$; (ii) Dilute stock vector: $2E11 \text{ vg/mL} / 1.0E13 \text{ vg/mL} = 20 \mu\text{L stock} + 2.480 \text{ mL of TM}$.

[0150] 4. Pre-bind "clone 6" mAb as follows: (i) Add 30 μL of diluted vector to all wells of new U-bottom pre-bind plate; (ii) Add 30 μL of diluted mAb and samples from dilution plate to pre-bind plate; (iii) Add sterile lid and incubate at 37°C., 5% CO₂ for 1 hour.

[0151] 5. Perform transduction as follows: (i) Add 20 μL from pre-bind plate to cell plate in duplicate; (ii) Seal plate with porous seal and incubate at 37°C., 5% CO₂ for 24 hours.

[0152] Day 3: Read and Data Analysis

[0153] 1. Thaw One-Glo at R.T. Protect from light.

[0154] 2. Take plates out of incubator and allow to equilibrate to R.T. for 30 minutes.

[0155] 3. Add 100 μL to each well to columns 1-12.

[0156] 4. Shake plate for 2½ minutes at 200 RPM.

[0157] 5. Read plates on SpectraMax using SoftMax Pro within 30 minutes.

[0158] 6. Analyze on SoftMax Pro Software

[0159] Results

[0160] Comparative results for "clone 6" and "IVIG" obtained in the neutralizing antibody assay are shown below in Table 3.

[0161] TABLE 3

Antibody Concentration (mg/mL)	Mean % Luciferase Inhibition
"Clone 6" (Anti-AAV2.7m8)	
0.071	99.873
0.035	99.932
0.018	99.861
0.009	98.635
0.004	84.85
0.002	81.879
0.001	52.888
0.001	20.297
0	1.891
0	-1.462

“IVIG” (anti-AAV2)	
10	99.886
5	99.964
2.5	99.912
1.25	99.724
0.625	94.62
0.312	64.13
0.156	29.931
0.078	11.81
0.039	-2.245
0.02	1.534

[0162] As shown by these results, the luciferase inhibition exhibited by the “clone 6” antibody was significantly greater than that exhibited by the “IVIG” antibody. The EC₅₀ value determined “clone 6” (9.9×10^{-4}) indicated a 200-fold increase in binding specificity in the assay relative to the EC₅₀ value measured for the “IVIG” antibody (2.0×10^{-1}). This increased binding specificity reflects the fact that the “clone 6” antibody was generated for specific binding to the AAV2.7m8 capsid protein rather than the AAV2 capsid protein. The “clone 6” antibody, thus, can provide a much more sensitive (e.g., 200-fold) and accurate assay for the detection of antibodies capable of neutralizing the AAV2.7m8 vector in samples from patients.

[0163] Notwithstanding the appended claims, the disclosure set forth herein is also defined by the following embodiments, which may be beneficial alone or in combination, with one or more of the other embodiments disclosed herein. Without limiting the foregoing description, certain non-limiting embodiments of the disclosure numbered as below are provided, wherein each of the individually numbered embodiments may be used or combined with any of the preceding or following embodiments. Thus, this is intended to provide support for all such combinations and is not necessarily limited to specific combinations explicitly provided below:

[0164] Embodiment 1: An antibody that specifically binds AAV2.7m8 capsid, wherein the antibody comprises a first light chain complementary determining region (CDR-L1) of SEQ ID NO: 5, a second light chain complementary determining region (CDR-L2) of SEQ ID NO: 6, a third light chain complementary determining region (CDR-L3) of SEQ ID NO: 7, a first heavy chain complementary determining region (CDR-H1) of SEQ ID NO: 13, a second heavy chain complementary determining region (CDR-H2) of SEQ ID NO: 14, and a third heavy chain complementary determining region (CDR-H3) of SEQ ID NO: 15.

[0165] Embodiment 2: The antibody of Embodiment 1, wherein the antibody comprises a light chain variable domain (VL) amino acid sequence having at least 90% identity to a sequence selected from SEQ ID NO: 4; and/or a heavy chain variable domain (VH) amino acid sequence having at least 90% identity to a sequence selected from SEQ ID NO: 12.

[0166] Embodiment 3: The antibody of any one of Embodiments 1-2, wherein the antibody comprises: a light chain (LC) amino acid sequence having at least 90% identity to a sequence selected from SEQ ID NO:20; and/or a heavy chain (HC) amino acid sequence having at least 90% identity to a sequence selected from SEQ ID NO: 21.

[0167] Embodiment 4: The antibody of any one of Embodiments 1-3, wherein the antibody is: (i) a monoclonal antibody; (ii) a chimeric antibody; (iii) a humanized antibody; (iv) a full length antibody of class IgG, optionally, wherein the class IgG antibody has an isotype selected from IgG1, IgG2, IgG3, and IgG4; (v) an antibody fragment, optionally selected from the group consisting of F(ab')₂, Fab', Fab, Fv, single domain antibody (VHH), and scFv; (vi) the antibody is a multi-specific antibody, optionally a bispecific antibody; and/or (vii) the antibody is a synthetic antibody, wherein the HVRs are grafted onto a scaffold or framework other than an immunoglobulin scaffold or framework; optionally, a scaffold selected from an alternative protein scaffold and an artificial polymer scaffold.

[0168] Embodiment 5: The antibody of any one of Embodiments 1-4, wherein the antibody is an immunoconjugate, optionally, wherein the immunoconjugate comprises a detectable label.

[0169] Embodiment 6: The antibody of Embodiment 5, wherein the immunoconjugate comprises a detectable label selected from a radiolabel, a fluorescent label, and an indirect labeling moiety.

[0170] Embodiment 7: The antibody of any one of Embodiments 1-6, wherein the antibody is characterized by one or more of the following properties:

- (a) binds to AAV2.7m8 capsid with a binding affinity of 1×10^{-8} M or less, 1×10^{-9} M or less, 1×10^{-10} M or less, or 1×10^{-11} M or less; optionally, wherein the binding affinity is measured by equilibrium dissociation constant (K_D) to a AAV2.7m8 capsid polypeptide of SEQ ID NO: 2; and
- (b) binds to AAV2.7m8 capsid with a binding affinity that is increased by at least 2-fold, at least 3-fold, at least 5-fold, or at least 10-fold, relative to the binding affinity to AAV2 capsid; optionally, wherein the relative binding affinities are measured by equilibrium dissociation constant (K_D) to the AAV2.7m8 capsid polypeptide of SEQ ID NO: 2 and the AAV2 capsid polypeptide of SEQ ID NO: 1

[0171] Embodiment 8: An antibody that specifically binds to the same epitope of AAV2.7m8 capsid as the antibody of any one of Embodiments 1-7; optionally, wherein the epitope comprises the amino acid sequence LALGETTRPA (SEQ ID NO: 22) located at positions 588-597 of SEQ ID NO: 2.

[0172] Embodiment 9: An antibody that competes with an antibody of any one of Embodiments 1-8 for binding to an epitope of AAV2.7m8 capsid and has a binding affinity of 1×10^{-9} M or less, 1×10^{-10} M or less, or 1×10^{-11} M or less; optionally, wherein the binding affinity is measured by equilibrium dissociation constant (K_D) to a AAV2.7m8 capsid polypeptide of SEQ ID NO: 2

[0173] Embodiment 10: The antibody of Embodiment 9, wherein the epitope comprises the amino acid sequence LALGETTRPA (SEQ ID NO: 22) located at positions 588-597 of SEQ ID NO: 2.

[0174] Embodiment 11: An isolated polynucleotide or vector encoding the antibody of any one of Embodiments 1-10.

[0175] Embodiment 12: An isolated host cell comprising the oligonucleotide or vector of Embodiment 11.

[0176] Embodiment 13: A method of producing an antibody comprising culturing the host cell of Embodiment 12 so that an antibody is produced.

[0177] Embodiment 14: A kit comprising: assay reagents; and an antibody of any one of Embodiments 1-10.

[0178] Embodiment 15: The kit of Embodiment 14, wherein the assay reagents comprise: (a) an AAV2.7m8 vector that encodes a luciferase gene; (b) a luciferase substrate; and/or (c) cells from a transduction-permissive cell line; optionally, wherein the cells are from a 293T cell line.

[0179] Embodiment 16: A method for detecting neutralizing antibody activity against AAV2.7m8 in a human serum sample, the method comprising:

(a) providing: (i) a human serum sample; (ii) an assay solution comprising cells; (iii) a vector solution comprising an AAV2.7m8 vector that encodes a luciferase gene; (iv) a luciferase substrate solution; and (v) an AAV2.7m8 antibody solution comprising an antibody that specifically binds AAV2.7m8 capsid;

(b) combining an aliquot of the human serum sample, the assay solution, the vector solution, and the luciferase substrate solution to form a sample solution;

(c) combining an aliquot of the AAV2.7m8 antibody solution, the assay solution, the vector solution, and the luciferase substrate solution to form a positive control solution; and

(d) detecting luminescence signals from the sample solution and the positive control

solution; whereby, the relative luminescence signal levels indicate presence and/or amount of neutralizing antibody activity in the human serum sample.

[0180] Embodiment 17: The method of Embodiment 16, wherein the antibody that specifically binds AAV2.7m8 capsid is an antibody of any one of Embodiments 1-10.

[0181] Embodiment 18: The method of any one of Embodiments 16-17, wherein step (c) comprises combining at least 2, 3, 4, 5, 6, 7, 8, 9, or more different aliquots with different dilutions of the AAV2.7m8 antibody solution to form at least 2, 3, 4, 5, 6, 7, 8, 9, or more positive control solutions having different concentrations of the AAV2.7m8 antibody for use in preparing a response curve.

[0182] Embodiment 19: The method of any one of Embodiments 16-18, wherein the method further comprises steps of combining an aliquot of the assay solution, and the luciferase substrate solution to form a negative control solution; and detecting luminescence signals from the negative control solution.

[0183] Embodiment 20: The method of any one of Embodiments 16-19, wherein the cells pre-exposed to an AAV2.7m8 vector that encodes a luciferase gene are from a transduction-permissive cell line; optionally, wherein the cell line is 293T.

[0184] Embodiment 21: A method for detecting an AAV2.7m8 capsid protein, the method comprising: contacting the protein with an antibody of any one of Embodiments 1-10 and detecting the antibody bound to the protein.

[0185] Embodiment 22: The method of Embodiment 21, wherein the AAV2.7m8 capsid protein is immobilized.

[0186] Embodiment 23: The method of Embodiment 21, wherein the AAV2.7m8 capsid protein is in solution.

[0187] Embodiment 24: The method of any one of Embodiments 21-23, wherein the antibody comprises a detectable label.

[0188] Embodiment 25: The method of Embodiment 24, wherein the detectable label selected from a radiolabel, a fluorescent label, and an indirect labeling moiety.

[0189] While the foregoing disclosure of the present invention has been described in some detail by way of example and illustration for purposes of clarity and understanding, this disclosure including the examples, descriptions, and embodiments described herein are for illustrative purposes, are intended to be exemplary, and should not be construed as limiting the present disclosure. It will be clear to one skilled in the art that various modifications or changes to the examples, descriptions, and embodiments described herein can be made and are to be

included within the spirit and purview of this disclosure and the appended claims. Further, one of skill in the art will recognize a number of equivalent methods and procedure to those described herein. All such equivalents are to be understood to be within the scope of the present disclosure and are covered by the appended claims.

CLAIMS

What is claimed is:

1. An antibody that specifically binds AAV2.7m8 capsid, wherein the antibody comprises a first light chain complementary determining region (CDR-L1) of SEQ ID NO: 5, a second light chain complementary determining region (CDR-L2) of SEQ ID NO: 6, a third light chain complementary determining region (CDR-L3) of SEQ ID NO: 7, a first heavy chain complementary determining region (CDR-H1) of SEQ ID NO: 13, a second heavy chain complementary determining region (CDR-H2) of SEQ ID NO: 14, and a third heavy chain complementary determining region (CDR-H3) of SEQ ID NO: 15.
2. The antibody of claim 1, wherein the antibody comprises a light chain variable domain (VL) amino acid sequence having at least 90% identity to a sequence selected from SEQ ID NO: 4; and/or a heavy chain variable domain (VH) amino acid sequence having at least 90% identity to a sequence selected from SEQ ID NO: 12.
3. The antibody of claim 1, wherein the antibody comprises: a light chain (LC) amino acid sequence having at least 90% identity to a sequence selected from SEQ ID NO:20; and/or a heavy chain (HC) amino acid sequence having at least 90% identity to a sequence selected from SEQ ID NO: 21.
4. The antibody of claim 1, wherein the antibody is: (i) a monoclonal antibody; (ii) a chimeric antibody; (iii) a humanized antibody; (iv) a full length antibody of class IgG, optionally, wherein the class IgG antibody has an isotype selected from IgG1, IgG2, IgG3, and IgG4; (v) an antibody fragment, optionally selected from the group consisting of F(ab')₂, Fab', Fab, Fv, single domain antibody (VHH), and scFv; (vi) the antibody is a multi-specific antibody, optionally a bispecific antibody; and/or (vii) the antibody is a synthetic antibody, wherein the HVRs are grafted onto a scaffold or framework other than an immunoglobulin scaffold or framework; optionally, a scaffold selected from an alternative protein scaffold and an artificial polymer scaffold.
5. The antibody of claim 1, wherein the antibody is an immunoconjugate, optionally, wherein the immunoconjugate comprises a detectable label.

6. The antibody of claim 5, wherein the immunoconjugate comprises a detectable label selected from a radiolabel, a fluorescent label, and an indirect labeling moiety.
7. The antibody of claim 1, wherein the antibody is characterized by one or more of the following properties:
 - (c) binds to AAV2.7m8 capsid with a binding affinity of 1×10^{-8} M or less, 1×10^{-9} M or less, 1×10^{-10} M or less, or 1×10^{-11} M or less; optionally, wherein the binding affinity is measured by equilibrium dissociation constant (K_D) to a AAV2.7m8 capsid polypeptide of SEQ ID NO: 2; and
 - (d) binds to AAV2.7m8 capsid with a binding affinity that is increased by at least 2-fold, at least 3-fold, at least 5-fold, or at least 10-fold, relative to the binding affinity to AAV2 capsid; optionally, wherein the relative binding affinities are measured by equilibrium dissociation constant (K_D) to the AAV2.7m8 capsid polypeptide of SEQ ID NO: 2 and the AAV2 capsid polypeptide of SEQ ID NO: 1
8. An antibody that specifically binds to the same epitope of AAV2.7m8 capsid as the antibody of claim 1; optionally, wherein the epitope comprises the amino acid sequence LALGETTRPA (SEQ ID NO: 22) located at positions 588-597 of SEQ ID NO: 2.
9. An antibody that competes with an antibody of claim 1 for binding to an epitope of AAV2.7m8 capsid and has a binding affinity of 1×10^{-9} M or less, 1×10^{-10} M or less, or 1×10^{-11} M or less; optionally, wherein the binding affinity is measured by equilibrium dissociation constant (K_D) to a AAV2.7m8 capsid polypeptide of SEQ ID NO: 2
10. The antibody of claim 9, wherein the epitope comprises the amino acid sequence LALGETTRPA (SEQ ID NO: 22) located at positions 588-597 of SEQ ID NO: 2.
11. An isolated polynucleotide or vector encoding the antibody of claim 1.
12. An isolated host cell comprising the oligonucleotide or vector of claim 11.
13. A method of producing an antibody comprising culturing the host cell of claim 12 so that an antibody is produced.
14. A kit comprising: assay reagents; and an antibody of claim 1.

15. The kit of claim 14, wherein the assay reagents comprise: (a) an AAV2.7m8 vector that encodes a luciferase gene; (b) a luciferase substrate; and/or (c) cells from a transduction-permissive cell line; optionally, wherein the cells are from a 293T cell line.
16. A method for detecting neutralizing antibody activity against AAV2.7m8 in a human serum sample, the method comprising:
 - (a) providing: (i) a human serum sample; (ii) an assay solution comprising cells; (iii) a vector solution comprising an AAV2.7m8 vector that encodes a luciferase gene; (iv) a luciferase substrate solution; and (v) an AAV2.7m8 antibody solution comprising an antibody that specifically binds AAV2.7m8 capsid;
 - (b) combining an aliquot of the human serum sample, the assay solution, the vector solution, and the luciferase substrate solution to form a sample solution;
 - (c) combining an aliquot of the AAV2.7m8 antibody solution, the assay solution, the vector solution, and the luciferase substrate solution to form a positive control solution; and
 - (d) detecting luminescence signals from the sample solution and the positive control solution; whereby, the relative luminescence signal levels indicate presence and/or amount of neutralizing antibody activity in the human serum sample.
17. The method of claim 16, wherein the antibody that specifically binds AAV2.7m8 capsid is an antibody of claim 1.
18. The method of claim 16, wherein step (c) comprises combining at least 2, 3, 4, 5, 6, 7, 8, 9, or more different aliquots with different dilutions of the AAV2.7m8 antibody solution to form at least 2, 3, 4, 5, 6, 7, 8, 9, or more positive control solutions having different concentrations of the AAV2.7m8 antibody for use in preparing a response curve.
19. The method of claims 16, wherein the method further comprises steps of combining an aliquot of the assay solution, and the luciferase substrate solution to form a negative control solution; and detecting luminescence signals from the negative control solution.
20. The method of claim 16, wherein the cells pre-exposed to an AAV2.7m8 vector that encodes a luciferase gene are from a transduction-permissive cell line; optionally, wherein the cell line is 293T.

21. A method for detecting an AAV2.7m8 capsid protein, the method comprising:
contacting the protein with an antibody of claim 1 and detecting the antibody bound to the protein.
22. The method of claim 21, wherein the AAV2.7m8 capsid protein is immobilized.
23. The method of claim 21, wherein the AAV2.7m8 capsid protein is in solution.
24. The method of claim 21, wherein the antibody comprises a detectable label.
25. The method of claim 24, wherein the detectable label selected from a radiolabel, a fluorescent label, and an indirect labeling moiety.