diseased, fragile retina. IVT delivery of AAV vectors may also be advantageous for more wide-spread 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

[0006a] In a first aspect, 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.

[0006b] In a second aspect, the present disclosure provides an antibody that specifically binds to the same epitope of AAV2.7m8 capsid as the antibody of the first aspect; optionally, wherein the epitope comprises the amino acid sequence LALGETTRPA (SEQ ID NO: 22) located at positions 588-597 of SEQ ID NO: 2.

[0006c] In a third aspect, the present disclosure provides an antibody that competes with an antibody of the first aspect 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.

[0006d] In a fourth aspect, the present disclosure provides an isolated polynucleotide or vector encoding the antibody of the first aspect.

[0006e] In a fifth aspect, the present disclosure provides an isolated host cell comprising the oligonucleotide or vector of fourth aspect.

[0006f] In a sixth aspect, the present disclosure provides a method of producing an antibody comprising culturing the host cell of the fifth aspect so that an antibody is produced.

[0006g] In a seventh aspect, the present disclosure provides a kit comprising: assay reagents; and an antibody of the first aspect.

[0006h] In a eighth aspect, the present disclosure 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 the antibody of the first aspect;

(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.

[0006i] In a ninth aspect, the present disclosure provides a method for detecting an AAV2.7m8 capsid protein, the method comprising: contacting the protein with an antibody of the first aspect and detecting the antibody bound to the protein.

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

[0014] In at least one example 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 example, 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 example, 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 example, 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 examples, the present disclosure also provides an isolated polynucleotide or a vector encoding an anti-AAV2.7m8 antibody of the present disclosure.

[0018] In another example, 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 example, 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 example, the present disclosure also provides a kit comprising: assay reagents; and an anti-AAV2.7m8 antibody of the present disclosure. In at least one example, 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 example, 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 example 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 example 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 example 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 example 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 example, 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 example, the AAV2.7m8 capsid protein is immobilized. In at least one example, the AAV2.7m8 capsid protein is in solution. In at least one example, the antibody comprises a detectable label. In at least one example, 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