of the viral vector. Attempting to solve this problem by increasing the quantity of rAAV-reporter which is used leads to issues with creating a sufficiently robust assay signal (Z'-factor), in terms of distinguishing the resulting signal from the background. The Z-factor and Z'-factor are a widely-used parameter of assay quality in determining how well the signal is distinguished from the background and are discussed inter alia in Zhang et al. (Journal of Biomolecular Screening, Vol. 4, No. 2, 1999).

There is accordingly a need for assay methods which are capable of reliably measuring NAb titre without requiring a 24-hour incubation (or longer) of the rAAV-reporter with the target cells. It would be desirable to develop a robust assay to detect NAb titre levels which is capable of the rapid turnover of results within a day or alternatively overnight. There is a need for assay methods which are capable of reliably determining NAb titre even at low concentrations of antibody while still remaining robust (e.g. having a Z' of greater than 0.5).

**Summary**

The present disclosure relates to a luciferase-based transduction inhibition assay (TIA) with protocols that allow either same-day or next-day/overnight determination of a sample's inhibition titre (NAb titre). A crucial component of this method is a synthetic bright luciferase ("BrightLuc"), which can produce a robust luminescent signal at very low levels of expression. The present inventors have surprisingly found that the TIA provided herein is capable of detecting successful transduction after only 3 hours of transduction. In particular the present inventors have found that optimisation of various parameters (e.g. amount of vector used, cell numbers or MOI) can ensure a robust assay signal (Z' >0. 5) even after only 3 hours of transduction.

Accordingly in one aspect the present disclosure provides a method for determining neutralising antibody (NAb) titre to a viral vector comprising a capsid of interest in a sample from a subject, the method comprising a transduction inhibition assay (TIA) using a luciferase which comprises the following steps:

(a) incubating particles of a viral vector comprising the capsid of interest in (1) one or more reference solutions comprising the sample at varying dilutions; and (2) at least one control solution; wherein the viral vector of part (a) comprises a recombinant vector genome comprising a transgene encoding the luciferase;

(b) exposing each of the solutions from step (a) to a population of target cells which are susceptible to infection by the viral vector of interest;

(c) waiting for a set interval of time to allow transduction to occur;

(d) adding a substrate for the luciferase to the reference and control solutions and measuring the signal (RLU) obtained from the luciferase;

(e) comparing the signal (RLU) obtained from the luciferase in the at least one control solution with the signal (RLU) obtained from the luciferase in the reference solutions; and

(f) calculating the NAb titre;

wherein:

(i) the set interval of time in step (c) is less than 24 hours, optionally is 19 hours or less, optionally is 12 hours or less, optionally is 8 hours or less, optionally is 6 hours or less and optionally is 3 hours; and

(ii) the luciferase is a synthetic luciferase which provides enhanced luminescence relative to a firefly luciferase.

"NAb titre to a viral vector of interest" should be understood as referring to the NAb titre to the viral capsid. "Viral vector of interest" should be understood as referring to the viral vector comprising the capsid of interest.

A crucial component of this method is a synthetic bright luciferase ("BrightLuc"), which can produce a robust luminescent signal (Z'>0.5) at very low levels of expression. The present inventors have surprisingly found that the method of the disclosure provided herein is capable of detecting effective transduction, i.e. transduction resulting in gene expression, within only 3 hours of transduction.

Accordingly, a further aspect of the invention provides an AAV viral vector which comprises or encapsidates a recombinant vector genome comprising a transgene encoding a luciferase, wherein the luciferase is a synthetic luciferase which provides enhanced luminescence relative to a firefly luciferase.

The recombinant vector genome may be self-complementary. The recombinant vector genome may comprise a non-native promoter operably linked to the transgene encoding the luciferase. The promoter may be the CMV promoter. The AAV viral vector and/or the encoded luciferase may be as further defined herein.

The AAV viral vector may be provided together with a reagent which includes a substrate for the luciferase.

Accordingly, the present disclosure further provides a kit comprising an AAV viral vector of the disclosure which comprises or encapsidates a recombinant vector genome comprising a transgene encoding a luciferase, together with a reagent which includes a substrate for the luciferase. The kit may further comprise instructions for carrying out the method of the disclosure.

As set out in more detail below, a further advantage of the method of the disclosure is that it can be carried out on a suspension of target cells and does not require the target cells to be plated in advance. This permits target cells in suspension (including e.g. a suspension of thawed target cells) to be used directly in the method, without requiring a plating step in advance of the method.

Accordingly, the kit of the disclosure may further comprise a container comprising target cells. The target cells may be in suspension. The kit or the container may comprise insulating or cooling means.

It is preferred that the passage number of the target cells for use in the methods disclosed herein does not exceed 25.

Firefly luciferase is well known in the field. A reference firefly luciferase is provided here as SEQ ID NO: 1. Accordingly the synthetic luciferase may have enhanced luminescence relative to a firefly luciferase having a sequence according to SEQ ID NO: 1.

Synthetic luciferases are known. Such "synthetic" luciferases are generally derived from naturally-occurring luciferases but are modified – often significantly – to optimise one or more of their properties so as to provide e.g. enhanced luminescence, greater stability, smaller size, and so on. Such modifications generally involve reducing the size, e.g. by removing one or more of the protein subunits; and/or modifying the amino acid sequence of the luciferase. Such modifications may include conservative or non-conservative substitution, addition or deletion.

The enhanced luminescence of the synthetic luciferase may be determined by measuring the luminescence signal (RLU) of the synthetic luciferase and its substrate and the luminescence signal (RLU) of the firefly luciferase and its luciferine substrate under the same conditions, which allows a comparison to be made. The signal (RLU) of the synthetic luciferase and its substrate may be greater by at least 5-fold, at least 10-fold, at least 50-fold, at least 100-fold, at least 150-fold or more than the signal (RLU) of the firefly and its luciferine substrate. It is preferred that the synthetic bright luciferase

for use with the method of the disclosure has a signal at least 80- to 100-fold or more than the above-defined firefly luciferase and its luciferine substrate.

Particularly preferred synthetic bright luciferases may be less than 50 kDa, less than 30 kDa, less than 25 kDa or less than 20 kDa. The synthetic bright luciferase may be ATP-independent.

The synthetic bright luciferase may use furimazine or coelenterazine as a substrate. The synthetic bright luciferase may use any known suitable derivatives or variants of furimazine or coelenterazine, or any other suitable known substrates. The choice of substrate will vary according to the synthetic bright luciferase chosen for the method and presents no difficulties to a person of skill in the relevant field.

The synthetic bright luciferase may comprise a sequence according to SEQ ID NO: 2 which has the commercial name "NanoLuc®" (Promega, US Patent No. 8,557,970). The synthetic bright luciferase may comprise a sequence having at least 90% or at least 95% identity with SEQ ID NO: 2. The synthetic bright luciferase may comprise a sequence which varies from SEQ ID NO: 2 by no more than one, no more than two, no more than three, no more than four or no more than five amino acids.

Alternatively, the synthetic bright luciferase may comprise a sequence according to SEQ ID NO: 3 which has the commercial name "TurboLuc®" (Thermo Scientific). The synthetic bright luciferase may comprise a sequence having at least 90% or at least 95% identity with SEQ ID NO: 3. The synthetic bright luciferase may comprise a sequence which varies from SEQ ID NO: 3 by no more than one, no more than two, no more than three, no more than four or no more than five amino acids.

Alternatively, the synthetic bright luciferase may comprise a sequence according to SEQ ID NO: 4 which has the commercial name "Lucia" (InvivoGen). The synthetic bright luciferase may comprise a sequence having at least 90% or at least 95% identity with SEQ ID NO: 4. The synthetic bright luciferase may comprise a sequence which varies from SEQ ID NO: 4 by no more than one, no more than two, no more than three, no more than four or no more than five amino acids.

Equivalents to the above synthetic bright luciferases are also encompassed within the scope of the method of the disclosure. Provided that the synthetic bright luciferase meets the test of having enhanced luminescence compared with a firefly luciferase as defined above, it is capable of being used in the method of the disclosure.

At least one control solution may comprise a negative control solution which lacks antibodies to the viral vector of interest. At least one control solution may comprise a first negative control solution which lacks antibodies to the viral vector of interest, and a second positive control solution which comprises a sufficient concentration of neutralising antibodies to maximally inhibit transduction of the viral vector of interest (as shown in Example 4 below, attaining complete 100% inhibition is not always practical or necessary). The positive control solution may be a solution of IVIG (in-vitro immunoglobulin). The IVIG solution will generally be at a sufficiently high concentration to ensure the maximal possible inhibition of the viral vector of interest. The IVIG may be at a concentration of at least 20 μg/ml, 30 μg/ml, 50 μg/ml or more. A concentration of at least 50 μg/ml is preferred.

Where at least one control solution comprises a positive control solution, the method may include a step of serially diluting the positive control solution and carrying out steps (a) to (d) above on the serial dilutions in order to establish the 50% inhibition level (EC50) of the positive control solution.

The sample from the patient will generally be a plasma sample.

The population of target cells may comprise at least 20,000 or 25,000 target cells. The population of target cells may comprise at least 50,000 target cells. The population of target cells may comprise at least 100,000 target cells, 150,000 target cells or more.

The target cells may be any mammalian cells which can be efficiently transduced by the viral vector being tested. In particular, the target cells may be HEK-293, HEK-293T,CHO, BHK, MDCK, 10T1/2, WEHI cells, COS, BSC 1, BSC 40, BMT 10, VERO, WI38, MRC5, A549, HT1080, 293, B-50, 3T3, NIH3T3, HepG2, Saos-2, Huh7, HER, HEK, HEL, or HeLa cells. The target cells may be HEK293 cells, which may be HEK293T cells. The skilled person will readily be able to select a cell type for use with the method of the disclosure, based on the particular viral vector of interest being tested.

The viral particles referred to herein may be adeno-associated virus (AAV) viral particles. The AAV particles may have a capsid of or deriving from naturally-occurring AAV serotypes AAV1, AAV2, AAV3, AAV3B, AAV4, AAV5, AAV6, AAV7, AAV8, AAV9, AAV 10, AAV11, AAV 12, or a mixture thereof. AAV5 is of interest because it does not effectively transduce the cells conventionally used in a TIA and thus the method of the present disclosure provides a way to use TIA with AAV5 while still obtaining a robust signal. Accordingly in one example the AAV particles have a capsid of or deriving from AAV5.

Alternatively, the AAV particles may have a capsid which is non-naturally occurring/synthetic/engineered. Such capsids may be AAV3B-derived, and in particular may have a capsid comprising any one of SEQ ID NOs: 31, 46, 47, 54 or 56 from WO 2013/029030 (which correspond to SEQ ID NOs: 6, 7, 8, 9 or 10 of the present application, respectively), and in particular the capsid LK03 (SEQ ID NO: 31 from WO 2013/029030 (SEQ ID NO: 6 of the present application)). The viral particles may have a capsid defined by SEQ ID NO: 5. The capsid may have at least 95%, 96%, 97%, 98% or 99% identity to the above capsids.

The viral particles may be lentiviral particles.

Step (a) of the method may comprise incubating the particles of the viral vector of interest at a concentration of between $1.7x10^7$ and $1.7x10^5$ vg/ml. An exemplified concentration is $8.3x10^6$ vg/ml.

The viral particles and target cells may be present at a ratio of vg:cells (multiplicity of infection (MOI)) which may be 250:1 or less, 200:1 or less, 100:1 or less, 50:1 or less, 25:1 or less, 10:1 or less, or 1:1 or less.

In particular the present inventors have found that optimisation of various parameters (e.g. amount of vector used, cell numbers or MOI) can ensure that the method of the disclosure gives rise to a robust assay signal (Z' >0.5) even after only 3 hours of transduction.

Accordingly, the method of the disclosure may have a calculated Z' value of more than 0.5, 0.6 or more, 0.7 or more, or 0.75 or more.

The incubation step (a) may be for any length of time which allows any neutralising antibody present in the sample to bind to and neutralise the viral particles. This may be 1 hour, for 2 hours, for 3 hours, or more.

The sample diluent may comprise or may be healthy human plasma. Alternatively, it may comprise fetal bovine serum (FBS) which may be IgG-depleted FBS. The sample diluent may further comprise DMEM, which may be phenol-red free DMEM.

A dilution of the sample and/or the positive control may comprise a serial dilution. The serial dilution may comprise a 2-fold dilution factor. The serial dilution may comprise one or more of 1:2, 1:4, 1:8,

1:16, 1:32, 1:64, 1:128, 1:256, 1:1024, 1:2048, 1:4096 and so on; and/or may comprise 1 or more of 1:10, 1:50, 1:100, 1:200, 1:400, 1:800, 1:1600, 1:3200 and so on. The serial dilution may comprise between 5 and 15 dilutions. The serial dilution may comprise between 8 and 11 dilutions. The number of dilutions used is open to the skilled person.

In contrast with known methods, the method of the disclosure does not require the use of target cells which have been plated in advance. The method of the disclosure does not require the target cells to be immobilised or otherwise adhered to an assay plate. The present inventors have surprisingly found that a TIA method wherein the transduction is carried out on target cells which are in suspension is highly effective. Without being bound by any theory, the present inventors speculate that carrying out the transduction step on target cells in suspension may have a positive effect on the sensitivity of the assay.

Accordingly, step (b) and step (c) of the method of the disclosure may be carried out on a population of target cells in suspension. Step (b) may therefore be defined as a step of exposing each of the solutions from step (a) to a population of target cells in suspension, which target cells are susceptible to infection by the viral vector of interest.

Step (b) may include the step of providing the population of target cells on an assay plate and adding the solutions thereto. The assay plate may be a multi-well assay plate. An assay plate may be used irrespective of whether the target cells are in suspension.

An advantage of not requiring cells to be plated in advance of the assay is a reduction in complexity, as well as a reduction in time taken for the overall assay. Thus, the present disclosure provides an assay where the overall time taken, as well as the transduction time, is greatly reduced.

Accordingly, the method of the disclosure may therefore be carried out from start to finish within a period of 24 hours or less.

Step (d) may include a step of lysing the cells to release the synthetic luciferase into the solution, prior to adding the substrate. Alternatively, a substrate may be used which can penetrate the cells without the need for lysing them beforehand. As another alternative, the synthetic luciferase may be capable of being secreted from the target cells into the solution.

The NAb titre may be determined or quantified in a variety of ways.

The NAb titre may be calculated in step (f) above as the dilution of the reference solution at which the signal (RLU) obtained is 50% of the signal (RLU) obtained in the control solution. This may be calculated as a simple visual comparison between the dilutions of the reference solution(s) and the control solution(s).

The 50% inhibition may be compared relative to the negative control solution.

In step (f) of the method of the disclosure, the NAb titre may be calculated by using a nonlinear regression model to fit the reference solution data (i.e. the RLU values obtained from the reference solutions) to a curve and obtain a precise half-maximal value at which 50% neutralisation occurs. The non-linear regression model may be applied to the luminescent signal (RLU) from each sample dilution. The non-linear regression model may be applied to the luminescent signal (RLU) from each sample dilution after normalising with positive (maximal inhibition) and/or negative (0% inhibition) controls. NAb titre may be calculated by fitting a four-parameter variable-slope model to the luminescent signal from each sample dilution after normalising with both positive (maximal inhibition) and negative (0% inhibition) controls.

The NAb titre may be calculated as the interpolated titre at 50% transduction inhibition (TI50).

The method of the disclosure is sensitive enough to distinguish between negative NAb samples and positive NAb samples with greater accuracy than ELISA. In particular, the method of the disclosure permits a population of patients to be rapidly and reliably stratified in a short period of time to determine their eligibility or suitability for gene therapy, based on their NAb titres to the viral vector which will be administered in the therapy.

Accordingly, another aspect of the disclosure provides a method of determining whether a patient is eligible for gene therapy using a viral vector comprising the capsid of interest, the method comprising determining the NAb titre (i.e. the titre of NAbs specific for the capsid of interest in the gene therapy viral vector) of the patient to said viral vector using the method defined above and comparing it with a determined or pre-determined threshold value wherein if the NAb titre is at or below the threshold value, the patient is eligible for gene therapy using the viral vector.

The threshold value at which a NAb titre is deemed to be acceptable, such that the patient is accordingly eligible for gene therapy, is something that a practitioner of skill in the art is able to determine without undue burden, for example based on clinical experience with vector particles possessing the same or similar particles.

Where a patient is defined as having a NAb titre that indicates that they are not currently eligible for gene therapy, one or more conventional methods known in the art may be employed to remove/deplete the NAb from their plasma, or at least to reduce the NAb titre to the threshold level or below, which will make the patient eligible for gene therapy. Techniques such as apheresis/plasmapheresis are clinical techniques which can be used in the clinic to remove/deplete pathogenic immunoglobulins. These techniques can reduce the NAb titres to AAV by 2-3-fold after each administration. However, several rounds of plasmapheresis may be required since some immunoglobulins have much longer half-life than others. Immunoglobulin G (IgG) levels in particular are characterised by a "rebound" phenomenon and can return to 40% of pre-apheresis levels within 48 h without concomitant immunosuppressive therapy.

The method of plasmapheresis referred to herein may be double filtration plasmapheresis (DFPP).

Accordingly another aspect of the disclosure provides a method of monitoring the progress of depletion of immunoglobulin which is specific for a viral vector comprising a capsid of interest, such as plasmapheresis or targeted depletion of immunoglobulin, in a patient wherein the method comprises the steps of:

(a) determining the patient NAb titre to a viral vector comprising a capsid of interest within a set interval of time following a round of immunoglobulin depletion by carrying out the method as described herein on a sample from the patient;

(b) determining whether the NAb titre following immunoglobulin depletion is below a predetermined threshold value for eligibility of gene therapy;

and optionally

(c) determining, based on the NAb titre following immunoglobulin depletion, whether a further round of immunoglobulin depletion is appropriate; and optionally comparing the NAb titre following immunoglobulin depletion with an initial NAb titre to determine whether the immunoglobulin depletion has had any significant effect.

In a related aspect, the disclosure provides a use of an AAV viral vector in the manufacture of a medicament for treating a genetic disorder, comprising:

(a) a method of immunoglobulin depletion to be performed on a patient;

(b) the progress of depletion of immunoglobulin to be monitored using the method as described herein; and

(c) the AAV viral vector to be administered once the immunoglobulin is sufficiently depleted,

wherein the AAV viral vector comprises a transgene that encodes a polypeptide implicated in the genetic disorder, the AAV viral vector comprises a capsid, and the patient has antibodies to the capsid.

The set interval of time will be governed *inter alia* by the length of time required for successful transduction in the method of the disclosure. The set interval of time may be 24 hours or less, 19 hours or less, 12 hours or less, 9 hours or less or 6 hours or less.

The method of immunoglobulin depletion may be any such method which is known in the art, such as apheresis/plasmapheresis or targeted depletion of immunoglobulin. Targeted depletion of immunoglobulin may include methods relying on an affinity matrix or a binding moiety which is specific for immunoglobulin or specific for IgG or antibodies having specificity for the viral vector of interest.

The method of immunodepletion may specifically target immunoglobulin. The method of immunodepletion may use an extracorporeal device which binds IgG. Alternatively, the method of immunodepletion may comprise the administration of an enzyme (such as a IgG cysteine protease or IgG endoglycosidases) which digest human IgG. The method of immunodepletion may comprise a method of administering to the subject an agent which reduces Fc receptor binding of serum IgG molecules.

The agent or enzyme may be an IgG cysteine protease from a *Streptococcus* bacterium such as *Streptococcus pyogenes*, or an IgG endoglycosidase from a *Streptococcus* bacterium, such as *Streptococcus pyogenes*, *Streptococcus equi* or *Streptococcus zooepidemicus*, or from *Corynebacterium pseudotuberculosis*, *Enterococcus faecalis*, or *Elizabethkingia meningoseptica*. The agent or enzyme may have a sequence according to SEQ ID NO: 11 or SEQ ID NO: 12, or a fragment or variant thereof which has IgG cysteine protease activity.

This method of monitoring may be carried out with reference to an initial NAb titre which is obtained prior to any immunoglobulin depletion (for example, if the method of the disclosure is first used to determine whether the patient is eligible for gene therapy, their initial NAb titre will be known). “Initial NAb titre” is therefore to be understood as meaning the NAb titre of the patient pre-depletion and

thus refers to pre-depletion levels of NAb in the patient. Accordingly, step (c) of the method of monitoring may include a step of comparing the NAb titre following immunoglobulin depletion with an initial NAb titre to determine whether the immunoglobulin depletion has had any effect.

The method of monitoring may be carried out following one or more further rounds of immunoglobulin depletion on the patient. Each additional round of immunoglobulin depletion may be performed on the same or consecutive days.

Thus, the method of monitoring may additionally include the following steps:

(d) determining the patient NAb titre to a viral vector comprising a capsid of interest within a set interval of time following a further round of immunoglobulin depletion by carrying out the method of the disclosure on a sample from the patient;

(e) determining whether the NAb titre following said further round of immunoglobulin depletion is below a predetermined threshold value for eligibility of gene therapy;

and optionally

(f) determining, based on the NAb titre following immunoglobulin depletion, whether a further round of immunoglobulin depletion is appropriate.

The step of determining whether yet a further round of immunoglobulin depletion is appropriate may include a step of comparing the NAb titre following the previous rounds of immunoglobulin depletion with one another, and optionally with the initial NAb titre, in order to determine whether the immunoglobulin depletion is having the intended effect.

It will be well within the capability of the person of skill in the art to determine whether a further round of immunoglobulin depletion is appropriate. If for example there is very little difference between the initial NAb titre and the NAb titres following more than one round of immunoglobulin depletion, it might be deemed inappropriate to subject the patient to yet a further round of immunoglobulin depletion. Alternatively, if the NAb titre following more than one round of immunoglobulin depletion is very close to the determined or pre-determined threshold value which would render the patient eligible for gene therapy, it may be deemed appropriate to subject the patient to yet a further round of immunoglobulin depletion. Generally, if NAb levels are still above threshold, another round of immunoglobulin depletion is appropriate, unless the previous round has not had a significant effect.

Thus, the method of monitoring may additionally include the following steps:

(g) determining the NAb levels in the patient within a set interval of time following the previous round of immunoglobulin depletion by carrying out the method of the disclosure on a sample from the patient;

(h) determining whether the NAb titre following the previous round of immunoglobulin depletion is below a determined or pre-determined threshold value for eligibility of gene therapy;

and optionally

(i) determining whether a further round of immunoglobulin depletion is appropriate.

It will be understood that immunoglobulin levels rebound following depletion, such that it is generally desirable to carry out the method of monitoring, and all required rounds of immunoglobulin depletion, with a period of no more than four days, preferably within three days, and most preferably within 48 hours or less. Accordingly all of steps (a) to (c), (a) to (f) or (a) to (i) may be carried out within a period of 4 days or less, within 72 hours or less, within 48 hours or less or within 24 hours.

It is preferred that the further round(s) of immunoglobulin depletion of step (c), step (f) and step (i) is/are carried out within 48 hours or less of the previous round of immunoglobulin depletion, and preferably the day after the previous round of immunoglobulin depletion.

Each further round of immunoglobulin depletion may be carried out within 24 hours of the previous round of immunoglobulin depletion.

As demonstrated in the Examples below, “consecutive” cycles of plasmapheresis (meaning that each cycle is delivered the day after the previous cycle) are capable of reducing the patient’s initial NAb titre to a level that is appropriate for clinical trial eligibility and/or for gene therapy. In particular, five cycles of plasmapheresis over five consecutive days are predicted to reduce a patient’s initial NAb titre by up to 94%.

Accordingly the method may comprise:

(a) two rounds of immunoglobulin depletion over the course of two days;

(b) three rounds of immunoglobulin depletion over the course of three days;

(c) four rounds of immunoglobulin depletion over the course of four days; or

(d) five rounds of immunoglobulin depletion over the course of five days.

Accordingly it will be well understood that the method of the disclosure, which permits an accurate measurement of NAb titre to be obtained in less than 24 hours, provides a clear advantage over currently known methods which require incubation/transduction periods of 24 hours or more before the result can be obtained.

**Detailed Description**

*General Definitions*

Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by a person skilled in the art to which this invention belongs.

In general, the term “comprising” is intended to mean including but not limited to.

In some embodiments of the invention, the word “comprising” is replaced with the phrase “consisting of” or the phrase “consisting essentially of”. The term “consisting of” is intended to be limiting.

As used herein, "gene therapy" is the insertion of nucleic acid sequences (e.g., genes) into an individual's cells and/or tissues to treat a disease, such as hereditary diseases where a defective mutant allele is replaced or supplemented with a functional one. Acquired diseases such as blood clotting disorders can be treated by gene therapy.

"Adeno-associated viruses" (AAV), from the parvovirus family, are small viruses with a genome of single stranded DNA. These viruses are useful as gene therapy vectors. Because AAV are not associated with pathogenic disease in humans, AAV vectors are able to deliver therapeutic proteins and agents to human patients without causing substantial AAV pathogenesis.

“Lentiviruses” are a genus of retrovirus which can integrate a significant amount of viral cDNA into the DNA of the host cell and can efficiently infect nondividing cells, making them an efficient means of gene delivery. Lentiviruses can also be used to stably over-express certain genes, thus allowing researchers to examine the effect of increased gene expression in a model system. Another common

**CLAIMS**

1. A method for determining neutralising antibody (NAb) titre to a viral vector comprising a capsid of interest in a sample from a subject, the method comprising a transduction inhibition assay (TIA) using a luciferase which includes the following steps:

(a) incubating particles of a viral vector comprising the capsid of interest in (1) one or more reference solutions comprising the sample at varying dilutions, and (2) at least one control solution, wherein the viral vector of part (a) comprises a recombinant vector genome comprising a transgene encoding the luciferase;

(b) exposing each of the solutions from step (a) to a population of target cells which are susceptible to infection by the viral vector of interest;

(c) waiting for a set interval of time to allow transduction to occur;

(d) adding a substrate for the luciferase to the reference and control solutions and measuring the signal (RLU) obtained from the luciferase;

(e) comparing the signal (RLU) obtained from the luciferase in the at least one control solution with the signal (RLU) obtained from the luciferase in the reference solutions; and

(f) calculating the NAb titre;

wherein:

(i) the set interval of time in step (c) is 6 hours or less; and

(ii) the luciferase is a synthetic luciferase which provides enhanced luminescence relative to a firefly luciferase.

2. The method for determining NAb titre according to claim 1, wherein the set interval in step (c) is 3 hours.

3. The method for determining NAb titre according to any one of the preceding claims wherein the synthetic luciferase has enhanced luminescence relative to a firefly luciferase having a sequence according to SEQ ID NO: 1.

4. The method for determining NAb titre according to any one of the preceding claims wherein enhanced luminescence of the synthetic luciferase is determined by measuring the luminescence signal (RLU) of the synthetic luciferase and its substrate and the luminescence signal (RLU) of the firefly luciferase and its luciferine substrate under the same conditions.

5. The method for determining NAb titre according to claim 4, wherein the signal (RLU) of the synthetic luciferase and its substrate is greater by at least 5-fold, at least 10-fold, at least 50-fold, at least 100-fold, at least 150-fold or more than the signal (RLU) of the firefly and its luciferine substrate.

6. The method for determining NAb titre according to any one of the preceding claims wherein the synthetic bright luciferase comprises:

(a) a sequence according to SEQ ID NO: 2;

(b) a sequence having at least 90% or at least 95% identity with SEQ ID NO: 2;

(c) a sequence which varies from SEQ ID NO: 2 by no more than one, no more than two, no more than three, no more than four or no more than five amino acids;

(d) a sequence according to SEQ ID NO: 3;

(e) a sequence having at least 90% or at least 95% identity with SEQ ID NO: 3; or

(f) a sequence which varies from SEQ ID NO: 3 by no more than one, no more than two, no more than three, no more than four or no more than five amino acids;

(g) a sequence according to SEQ ID NO: 4;

(h) a sequence having at least 90% or at least 95% identity with SEQ ID NO: 4; or

(i) a sequence which varies from SEQ ID NO: 4 by no more than one, no more than two, no more than three, no more than four or no more than five amino acids.

7. The method for determining NAb titre according to any one of the preceding claims, wherein:

(a) at least one control solution comprises a negative control solution which lacks antibodies to the viral vector of interest; or

(b) at least one control solution comprises a first negative control solution which lacks antibodies to the viral vector of interest, and a second positive control solution which

comprises a sufficient concentration of neutralising antibodies to maximally inhibit transduction of the viral vector.

8. The method for determining NAb titre according to claim 7(b), wherein the positive control solution comprises IVIG (in-vitro immunoglobulin); optionally wherein the IVIG has a concentration of at least 20 μg/ml, 30 μg/ml, 50 μg/ml or more.

9. The method for determining NAb titre according to any one of the preceding claims wherein the sample from the patient is a plasma sample.

10. The method for determining NAb titre according to any one of the preceding claims wherein the sample diluent comprises IgG-depleted fetal bovine serum; optionally wherein the sample diluent further comprises DMEM.

11. The method for determining NAb titre according to any one of the preceding claims wherein the population of target cells comprises at least 20,000, at least 25,000, at least 50,000, at least 100,000, at least 150,000, or more than 150,000 target cells.

12. The method for determining NAb titre according to any one of the preceding claims wherein the target cells are HEK293 or HEK293T cells.

13. The method for determining NAb titre according to any one of the preceding claims wherein the viral particles are adeno-associated virus (AAV) viral particles.

14. The method for determining NAb titre according to claim 13 wherein the viral vector comprises:

(a) a capsid of or deriving from naturally occurring AAV serotypes AAV 3 and/or AAV3B;

(b) a capsid having at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity to SEQ ID NO: 5;

(c) a capsid having at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity to SEQ ID NO: 6;

(d) a capsid having at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity to SEQ ID NO: 7;

(e) a capsid having at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity to SEQ ID NO: 8;

(f) a capsid having at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity to SEQ ID NO: 9;

(g) a capsid having at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity to SEQ ID NO: 10; or

(h) a capsid of or deriving from naturally occurring serotype AAV5.

15. The method for determining NAb titre according to any one of the preceding claims, wherein the NAb titre is calculated or quantified using a nonlinear regression model to fit the reference solution data to a curve and obtain a precise half-maximal value at which 50% neutralisation occurs.

16. An AAV vector which encapsidates or comprises a recombinant vector genome comprising a transgene encoding a luciferase, wherein the luciferase is a synthetic luciferase which provides enhanced luminescence relative to a firefly luciferase.

17. The AAV vector of claim 16, or the method of any one of claims 1 to 15, wherein the recombinant vector genome is self-complementary.

18. The AAV vector of claim 16 or claim 17, wherein the recombinant vector genome comprises a transgene encoding a synthetic bright luciferase which comprises or consists of:

(a) a sequence according to SEQ ID NO: 2;

(b) a sequence having at least 90% or at least 95% identity with SEQ ID NO: 2;

(c) a sequence which varies from SEQ ID NO: 2 by no more than one, no more than two, no more than three, no more than four or no more than five amino acids;

(d) a sequence according to SEQ ID NO: 3;

(e) a sequence having at least 90% or at least 95% identity with SEQ ID NO: 3;

(f) a sequence which varies from SEQ ID NO: 3 by no more than one, no more than two, no more than three, no more than four or no more than five amino acids;

(g) a sequence according to SEQ ID NO: 4;

(h) a sequence having at least 90% or at least 95% identity with SEQ ID NO: 4; or

(i) a sequence which varies from SEQ ID NO: 4 by no more than one, no more than two, no more than three, no more than four or no more than five amino acids.

19. The AAV vector of any one of claims 16 to 18, wherein the viral particles comprise:

(a) a capsid of or deriving from naturally occurring AAV serotypes AAV 3 and/or AAV3B;

(b) a capsid having at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity to SEQ ID NO: 5;

(c) a capsid having at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity to SEQ ID NO: 6;

(d) a capsid having at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity to SEQ ID NO: 7;

(e) a capsid having at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity to SEQ ID NO: 8;

(f) a capsid having at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity to SEQ ID NO: 9; or

(g) a capsid having at least 95%, at least 96%, at least 97%, at least 98% or at least 99% identity to SEQ ID NO: 10.

20. A method of determining whether a patient is eligible for gene therapy using a viral vector comprising a capsid of interest, the method comprising determining the NAb titre of the patient to said viral vector using the method of any one of claims 1 to 15 and comparing it with a pre-determined threshold value, wherein if the Nab titre is at or below the threshold value, the patient is eligible for gene therapy using the viral vector.

21. A method of monitoring the progress of depletion of immunoglobulin which is specific for a viral vector comprising a capsid of interest, such as plasmapheresis or targeted depletion of immunoglobulin, in a patient wherein the method comprises the steps of:

(a) determining the patient NAb titre to a viral vector comprising a capsid of interest within a set interval of time following a round of immunoglobulin depletion by carrying out the method of any one of claims 1 to 15 on a sample from the patient;

(b) determining whether the NAb titre following immunoglobulin depletion is below a predetermined threshold value for eligibility of gene therapy;

and optionally

(c) determining, based on the NAb titre following immunoglobulin depletion, whether a further round of immunoglobulin depletion is appropriate; and optionally comparing the NAb titre following immunoglobulin depletion with an initial NAb titre to determine whether the immunoglobulin depletion has had any significant effect.

22. The method of claim 21, wherein the method additionally comprises:

(d) determining the patient NAb titre to a viral vector comprising a capsid of interest within a set interval of time following a further round of immunoglobulin depletion by carrying out the method of any one of claims 1 to 15 on a sample from the patient;

(e) determining whether the NAb titre following said further round of immunoglobulin depletion is below a pre-determined threshold value for eligibility of gene therapy;

and optionally

(f) determining, based on the NAb titre following immunoglobulin depletion, whether yet a further round of immunoglobulin depletion is appropriate; and optionally comparing the NAb titre following the previous rounds of immunoglobulin depletion with one another, and optionally with an initial NAb titre, in order to determine whether the immunoglobulin depletion has had any significant effect.

23. The method of claim 21 or claim 22, wherein the method additionally comprises:

(g) determining the patient NAb titre to a viral vector comprising a capsid of interest within a set interval of time following a further round of immunoglobulin depletion by carrying out the method of any one of claims 1 to 15 on a sample from the patient;

(h) determining whether the NAb titre following said further round of immunoglobulin depletion is below a pre-determined threshold value for eligibility of gene therapy;

and optionally

(i) determining whether yet a further round of immunoglobulin depletion is appropriate.

24. The method of any one of claims 21 to 23, wherein the further round(s) of immunoglobulin depletion of step (c), step (f) and step (i) is/are carried out within 48 hours or less of the previous round of immunoglobulin depletion; optionally wherein the further round of immunoglobulin depletion of step (c), step (f) and step (i) is carried out the day after the previous round of immunoglobulin depletion; and optionally wherein the further round of immunoglobulin depletion is carried out within 24 hours of the previous round of immunoglobulin depletion.

25. The method of any one of claims 21 to 24, wherein the method comprises:

(a) two rounds of immunoglobulin depletion over the course of two days;

(b) three rounds of immunoglobulin depletion over the course of three days;

(c) four rounds of immunoglobulin depletion over the course of four days; or

(d) five rounds of immunoglobulin depletion over the course of five days.

26. The method of any one of claims 21 to 25, wherein the immunoglobulin depletion method is plasmapheresis; and optionally wherein the immunoglobulin depletion method is double filtration plasmapheresis (DFPP).

27. The method of any one of claims 21 to 25, wherein the immunoglobulin depletion method is:

(a) a method of immunodepletion which specifically targets immunoglobulin;

(b) a method of immunodepletion which uses an extracorporeal device which binds IgG; or

(c) a method of immunodepletion which comprises that an agent or enzyme (such as a IgG cysteine protease or IgG endoglycosidases) which digests human IgG is to be administered, optionally wherein the method comprises that an agent or enzyme which reduces Fc receptor binding of serum IgG molecules is to be administered to the subject; optionally wherein the agent or enzyme is an IgG cysteine protease from a *Streptococcus* bacterium such as *Streptococcus pyogenes*, or an IgG endoglycosidase from a *Streptococcus* bacterium, such as *Streptococcus pyogenes*, *Streptococcus equi* or *Streptococcus zooepidemicus*, or from *Corynebacterium pseudotuberculosis*, *Enterococcus faecalis*, or *Elizabethkingia meningoseptica*; and optionally wherein the agent or enzyme comprises a

sequence according to SEQ ID NO: 11 or SEQ ID NO: 12, or a fragment or variant thereof which has IgG cysteine protease activity.

28. Use of an AAV viral vector in the manufacture of a medicament for treating a genetic disorder, comprising:

(a) a method of immunoglobulin depletion to be performed on a patient;

(b) the progress of depletion of immunoglobulin to be monitored using the method of any one of claims 21 to 26; and

(c) the AAV viral vector to be administered once the immunoglobulin is sufficiently depleted,

wherein the AAV viral vector comprises a transgene that encodes a polypeptide implicated in the genetic disorder, the AAV viral vector comprises a capsid, and the patient has antibodies to the capsid.