

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
5 (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;
 - 10 (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;
 - 15 (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
 - 20 (ii) the luciferase is a synthetic luciferase which provides enhanced luminescence relative to a firefly luciferase.
- 25 2. The method for determining NAb titre according to claim 1, wherein the set interval in step (c) is 6 hours or less; and optionally 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
30 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:

- 10 (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;
- 15 (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
- 20 (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:

- 25 (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.

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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.
- 5 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.
- 10 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;
 - 15 (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;
 - 20 (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
 - 25 (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;
 - 30 (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
- 5 (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;
- 20 (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
- 25 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 24 on a sample from the patient;
- 30 (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

- 5 (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:

- 10 (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 the invention 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
- 15 (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 set interval of time is 24 hours or less, 19 hours or less, 12 hours or less, 9 hours or less or 6 hours or less.

20 25. The method of any one of claims 21 to 24, 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.

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26. The method of any one of claims 21 to 25, 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
- 30 (d) five rounds of immunoglobulin depletion over the course of five days.

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

28. The method of any one of claims 21 to 26, 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
- 5 (c) a method of immunodepletion which comprises the administration of an agent or enzyme (such as a IgG cysteine protease or IgG endoglycosidases) which digests human IgG, optionally wherein the method comprises administering to the subject an agent or enzyme which reduces Fc receptor binding of serum IgG molecules; optionally wherein the agent or enzyme is an IgG cysteine protease from a *Streptococcus* bacterium such as *Streptococcus pyogenes*,
10 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.

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29. An AAV viral vector for use in a method of treating a genetic disorder, the method comprising:

- (a) performing a method of immunoglobulin depletion on a patient;
- (b) monitoring the progress of depletion of immunoglobulin using the method of any one of claims 21 to 27; and
- 20 (c) administering the AAV viral vector 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.