CLAIMS

We claim:

1. A lentiviral vector particle comprising:

(a) an envelope comprising a Sindbis virus E2 glycoprotein of SEQ ID NO: 1 in which 160X is absent or is an amino acid other than glutamic acid, or a variant of SEQ ID NO: 1 thereof having at least 80% identity to SEQ ID NO: 1 and in which 160X is absent or is an amino acid other than glutamic acid, capable of infecting dendritic cells; wherein E2 is not part of a fusion protein with Sindbis virus E3; and

(b) a lentiviral vector genome comprising a sequence of interest;

2. The lentiviral vector particle of claim 1, wherein the E2 glycoprotein or variant binds to DC-SIGN.

3. The lentiviral vector particle of claim 1 or claim 2, wherein the 160X is absent or is glycine, alanine, valine, leucine or isoleucine, such as selected from glycine, valine, leucine or isoleucine.

4. The lentiviral vector particle of claim 1 or claim 2, wherein 160X is glycine.

5. The lentiviral vector particle of claim 1, 2, 3 or 4, wherein the E2 glycoprotein variant has at least one amino acid altered to reduce its net positive charge.

6. The lentiviral vector particle of claim 5, wherein the altered amino acids are chosen from the group consisting of lysine 70, lysine 76, or lysine 159 of SEQ ID NO:1, and the substitutions are optionally independently chosen from glutamic acid or aspartic acid.

7. The lentiviral vector particle of claim 1, 2, 3, 4, 5 or 6, wherein the E2 variant sequence is SEQ ID No. 2, 3, or 4; (variants 1, 2, and 3), optionally comprising one or more further substitutions, insertions or deletions.

8. The lentiviral vector particle of claim 1, 2, 3, 4, 5, 6 or 7, which is a variant in which the sequence of residues 71-75 of SEQ ID NO:1 is unchanged or has one or two substitutions which do not affect the ability of the variant to infect DCs, but do not alter the number of amino acids in this region.

9. The lentiviral vector particle of any of the foregoing claims, wherein the sequence of interest encodes a tumor-specific antigen or a virus-derived antigen, such as an HIV or SIV antigen.

10. The lentiviral vector particle of any of the foregoing claims, wherein the particles have an IU of at least 10^5/mL.

11. A lentiviral vector genome pseudotyped in an envelope comprising a Sindbis virus E2 glycoprotein variant, the genome comprising a sequence of interest,

wherein the E2 glycoprotein variant facilitates infection of dendritic cells by the lentiviral vector particle; and wherein the E2 glycoprotein variant has an amino acid sequence as defined in any one of claims 1 to 10 above.

12. A lentiviral vector packaging system for producing a pseudotyped lentiviral vector particle, comprising:

(i) a first nucleic acid molecule encoding a Sindbis virus E2 glycoprotein of SEQ ID NO:1 in which 160X is absent or an amino acid other than glutamic acid, or a variant thereof capable of infecting dendritic cells having at least sequence 80% identity to SEQ ID NO:1 and in which 160X is absent or is an amino acid other than glutamic acid, and;

(ii) a second nucleic acid molecule encoding gag and pol proteins;

(iii) a third nucleic acid molecule encoding rev; and

(iv) a lentiviral vector genome comprising a sequence of interest;

13. The packaging system of claim 12, wherein the E2 glycoprotein has an amino acid sequence as defined in any one of claims 1 to 9 above.

14. The packaging system of claims 12 or 13, wherein the pol protein has a non-functional integrase.

15. The packaging system of claim 14, wherein the non-functional Integrase has a D64V mutation.

16. The packaging system of claims 12, 13, 14 or 15, wherein the fourth nucleic acid molecule is a non-integrating lentiviral genome.

17. The packaging system of claims 12, 13, 14, 15 or 16, wherein the lentiviral vector particles are produced to a titer of at least 10^5 IU/mL.

18. A cell transfected with the first and fourth nucleic acid molecules of any one of claims 12, 13, 14, 15, 16, or 17.

19. An isolated nucleic acid molecule encoding the protein of any one of claims 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10, or an E3/E2 glycoprotein optionally in the form of a Sindbis E3/E2/6K/E1 polyprotein, or an E3/E2 glycoprotein wherein the E3 sequence corresponds to residues 1-65 of SEQ ID NO:20, or a variant thereof having at least 80% sequence identity to residues 1-65 of SEQ ID NO:20, wherein residues 62-65 are RSKR (SEQ ID NO: 27) and the variant is capable of being incorporated into a pseudotyped viral envelope, optionally further wherein residue 1 of the E2 polyprotein is Ser.

20. An expression vector comprising the nucleic acid molecule of claim 19.

21. A host cell comprising the expression vector of claim 20.

22. A method of making a lentiviral vector particle of any one of claims 1 to 11, comprising expressing in a cell

(i) a first nucleic acid molecule encoding a Sindbis virus E2 glycoprotein of SEQ ID NO:1 in which 160X is other than glutamic acid, or a variant thereof capable of infecting dendritic cells having at least sequence 80% identity to SEQ ID NO:1 and in which 160X is absent or is an amino acid other than glutamic acid, and;

(ii) a second nucleic acid molecule wherein the second nucleic acid molecule can be transcribed and the transcript assembled into a pseudotyped lentiviral vector particle.

23. A lentiviral vector particle according to any one of claims 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or 11 for use in a method of treatment of a human or animal subject.

24. A method of delivering lentiviral vector particles to cells in vitro, comprising mixing the cells with the lentiviral vector particles of any one of claims 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11.

25. A therapeutic or prophylactic vaccine comprising the lentiviral vector particles of any one of claims 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or 11 and a pharmaceutically acceptable excipient.

Claims

1. A lentiviral vector particle comprising:

(a) an envelope comprising

(i) a Sindbis virus E2 glycoprotein of SEQ ID NO:1 in which 160X is absent or is an amino acid other than glutamic acid, or a variant of SEQ ID NO:1 thereof having at least 80% identity to SEQ ID NO:1 and in which 160X is absent or is an amino acid other than glutamic acid, capable of infecting dendritic cells;

wherein E2 is not part of a fusion protein with Sindbis virus E3; and

(b) a lentiviral vector genome comprising one or more sequences of interest.

2. The lentiviral vector particle of claim 1, wherein the 160X is absent or is glycine, alanine, valine, leucine or isoleucine, such as selected from glycine, valine, leucine or isoleucine.

3. The lentiviral vector particle of claim 1 or 2, wherein the E2 glycoprotein variant has at least one amino acid altered to reduce its net positive charge.

4. The lentiviral vector particle of claim 3, wherein the at least one amino acid to be altered to reduce the net positive charge is chosen from lysine 70, lysine 76 or lysine 159 of SEQ ID NO:1, and preferably wherein the substitutions are independently chosen from glutamic acid or aspartic acid.

5. The lentiviral vector particle of claim 1, wherein the E2 variant sequence is amino acid residues 66 to 488 of SEQ ID NO:3, amino acid residues 66 to 488 of SEQ ID NO: 4, or amino acid residues 66 to 486 of SEQ ID NO:5; (variants 1, 2, and 3).

6. The lentiviral vector particle of any of claims 1- 5, which is a variant in which the sequence of residues 71-75 of SEQ ID NO:1 is unchanged or has one or two substitutions which do not affect the ability of the variant to infect DCs, but do not alter the number of amino acids in this region.

7. The lentiviral vector particle of any of claims 1-6, wherein the sequence of interest expresses a product that is an antigen of a disease-causing agent or a diseased cell.

8. The lentiviral vector particle of any of claims 1-6 wherein the sequence of interest encodes a tumor-specific antigen, optionally NY-ESO-1, MAGE, MART-1/Melan-A, BAGE, RAGE, a melanocyte-melanoma lineage antigen, such as gp100, gp75, mda-7, tyrosinase or tyrosinase-related protein; renal cell carcinoma antigen, 5T4, SM22-alpha, carbonic anhydrase I, carbonic anhydrase IX (also known as G250), hypoxia-inducible factors (optionally, HIF-1 alpha or HIF-2alpha), VEGF or prostate specific membrane antigen (PSMA), prostate-specific antigen (PSA), prostatic acid phosphates, six-

transmembrane epoithelial antigen of the prostate (STEAP), NKX3.1, epitope proteins/peptides derived from genes mutated in tumor cells or genes transcribed at different levels in tumor compared to normal cells, such as telomerase enzyme, survivin, mesothelin, mutated ras, bcr/abl rearrangement, Her2/neu, mutated or wild-type p53, cytochrome P450 1B1, abnormally expressed intron sequences such as N-acetylglucosaminyltransferase-V; clonal rearrangements of immunoglobulin genes generating unique idiotypes in myeloma or B-cell lymphomas; epitope proteins/peptides derived from oncoviral processes, such as human papilloma virus proteins E6 or E7; or nonmutated oncofetal proteins with a tumor-selective expression, such as carcinoembryonic antigen or alpha-fetoprotein.

9. The lentiviral vector particle of any of claims 1- 7, wherein the sequence of interest encodes a virus-derived antigen, such as an HIV or SIV antigen, adenovirus polypeptide, alphavirus polypeptides, calicivirus polypeptides, e.g., a calicivirus capsid antigen, coronavirus polypeptides, distemper virus polypeptide, Ebola virus polypeptide, enterovirus polypeptide, flavivirus polypeptide, hepatitis virus (AE) polypeptide, e.g., a hepatitis B core or surface antigen, or a hepatitis C virus E1 or E2 glycoproteins, core, or non-structural proteins, herpesvirus polypeptide, e.g., a herpes simplex virus or varicella zoster virus glycoprotein, immunodeficiency virus polypeptide, e.g., the human immunodeficiency virus envelope or protease, infectious peritonitis virus polypeptide, influenza virus polypeptide, e.g., an influenza A hemagglutinin, neuraminidase; or nucleoprotein, leukemia virus polypeptide, Marburg virus polypeptide, orthomyxovirus polypeptide, papilloma virus polypeptide, parainfluenza virus polypeptide, e.g., the hemagglutinin/ neuraminidase, paramyxovirus polypeptide, parvovirus polypeptide, pestivirus polypeptide, picorna virus polypeptide, e.g., a poliovirus capsid polypeptide, pox virus polypeptide, e.g., a vaccinia virus polypeptide, rabies virus polypeptide, e.g., a rabies virus glycoprotein G, reovirus polypeptide, retrovirus polypeptide, or rotavirus polypeptide.

10. A composition comprising a lentiviral vector particle of any of claims 1-9, wherein the composition has an IU of at least 10^5/mL.

11. A lentiviral vector packaging system for producing a pseudotyped lentiviral vector particle of any of claims 1-9, or the composition of claim 10, comprising:
(i) a first nucleic acid molecule encoding the Sindbis virus E2 glycoprotein of claim 1 (a)(i), and;
(ii) a second nucleic acid molecule encoding gag and pol proteins,
(iii) a third nucleic acid molecule encoding rev;
(iv) a lentiviral vector genome comprising a sequence of interest; and
(v) a packaging cell.

12. The lentiviral vector packaging system of claim 11, wherein:
(a) the pol protein has a non-functional integrase; and/or
(b) the pol protein has a non-functional integrase with a D64V mutation; and/or
(c) the lentiviral vector genome is a non-integrating lentiviral genome; and/or
(d) the packaging cell is stably transformed with (ii) and (iii); and/or
(e) the packaging cell is transfected with (i) and (iv).

13. An isolated nucleic acid molecule comprising a nucleotide sequence encoding the E2 glycoprotein or variant of claim 1 (a)(i), or any one of claims 2-9.

14. The nucleic acid molecule of claim 13 wherein the protein is a Sindbis E3/E2/6K/E1 polyprotein that is processed such that the E2 protein is not part of a fusion protein with E3 when incorporated into a viral envelope.

15. The nucleic acid molecule of claim 14, wherein the E3 sequence corresponds to (a) residues 1-65 of SEQ ID NO:20, or (b) a variant thereof having at least 80% sequence identity to residues 1-65 of SEQ ID NO:20, wherein residues 62-65 are RSKR (SEQ ID NO: 27), and the variant is capable of being incorporated into a pseudotyped viral envelope.

16. A method of making a lentiviral vector particle of any one of claims 1-9, or the composition of claim 10, comprising culturing the packaging cell of claim 11.

17. The lentiviral particle of any one of claims 1-9, or the composition of claim 10, wherein the lentiviral vector genome
(a) has an inactivated or self-inactivating 3' long terminal repeat (LTR); and/or
(b) comprises a U3 element lacking at least one of:
(i) an enhancer sequence;
(ii) a TATA box (iii) an Sp1 site (iv) an NK-kappa B site and (v) a polypurine tract (PPT); and/or
(c) comprises the nucleotide sequence of SEQ ID NO: 21, 22 or 23; and/or,
(d) comprises a nucleotide sequence encoding a dendritic cell maturation/stimulatory factor.

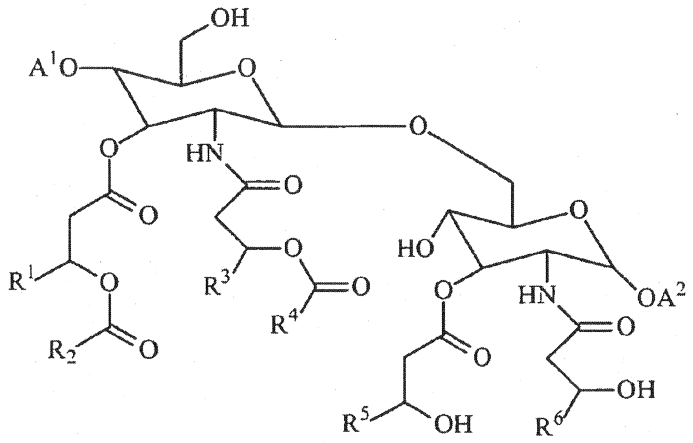
18. A lentiviral vector particle according to any one of claims 1-9, or the composition of claim 10, for use in a method of treatment of a human or animal subject, optionally comprising delivering lentiviral vector particles to dendritic cells ex vivo.

19. A lentiviral vector particle according to any one of claims 1-9, or the composition of claim 10, for use in inducing an antigen-specific immune response, optionally a humoral response and/or cellular response.

20. A therapeutic or prophylactic vaccine comprising the lentiviral vector particles of any one of claims 1-9, or the composition of claim 10, and a pharmaceutically acceptable excipient.

21. The therapeutic or prophylactic vaccine according to claim 20 further comprising an adjuvant.

22. The therapeutic or prophylactic vaccine according to claim 21, wherein the adjuvant is an adjuvant of formula (I):



(I)

wherein A1 and A2 are independently selected from the group of hydrogen, phosphate, and phosphate salts and the moieties R^1, R^2, R^3, R^4, R^5 and R^6 are independently selected from the group of hydrocarbyl having 3 to 23 carbons, represented by C_3-C_{23}.

23. The therapeutic or prophylactic vaccine according to claim 22, wherein
(a) A1 is phosphate or phosphate salt and A2 is hydrogen, and
(b)
(i) R^1, R^3, R^5 and R^6 are C_{11}-C_{20} alkyl, and R^2 and R^4 are C_{12}-C_{20} hydrocarbyl; or
(ii) R^1, R^3, R^5 and R^6 are C_{11} alkyl, and R^2 and R^4 are C_{13} hydrocarbyl; or
(iii) R^1, R^3, R^5 and R^6 are undecyl, and R^2 and R^4 are tridecyl.