expressing a desired antigen to elicit an effective immune response so that it can be utilized broadly across a designated human subject population.

[0006] The envelope glycoproteins of Sindbis virus and other alphaviruses disclosed herein incorporate into the lipid bilayer of the viral particle membrane. Typically, the viral membrane (envelope) includes multiple copies of trimers of two glycoprotein heterodimers, E1 and E2, which are produced from cleavage of a single precursor protein. The precursor protein comprises, from its N- to C-terminus, the E3, E2, 6K and E1 proteins. The small E3 glycoprotein serves as a signal sequence for translocation of the E2 protein into the membrane, and is cleaved from E2 by furin or some other Ca^{2+}-dependent serine proteinase. The 6K protein serves as a signal sequence for translocation of the E1 protein into the membrane and is then cleaved from the precursor protein. WO 2008/011636 and US 2011/0064763 disclose lentiviral packaging systems.

SUMMARY OF THE INVENTION

[0007] The inventors have discovered that lentiviral vector particles that exhibit two characteristics (a) pseudotyped with a highly mannosylated alphavirus glycoprotein and (b) comprising a Vpx protein, have unexpectedly improved transduction efficiency for cells expressing DC-SIGN. These particles infect cells expressing DC-SIGN, particularly dendritic cells, significantly more efficiently than lentiviral vector particles having only one of these two characteristics. In particular instances, highly mannosylated pseudotyped lentiviral vector particles are provided that comprise a Vpx protein and a lentiviral genome comprising a sequence of interest (e.g., a polynucleotide encoding an antigen).

Methods of generating pseudotyped lentiviral vector particles

[0008] Disclosed herein is~~One aspect of the disclosure provides~~ a method of generating a pseudotyped lentiviral vector particle comprising: (a) culturing in a culture medium comprising a mannosidase inhibitor, preferably a mannosidase I inhibitor, and a virus packaging cell comprising: (1) a lentiviral vector genome comprising a polynucleotide encoding an exogenous antigen, (2) a polynucleotide encoding an alphavirus glycoprotein that preferentially binds cells expressing DC-SIGN, and (3) a polynucleotide encoding a SAMHD1 inhibitor; and (b) isolating a pseudotyped lentiviral vector particle that preferentially binds cells expressing DC-SIGN.

[0009] OneAnother aspect of the disclosure provides a method of generating a pseudotyped lentiviral vector particle comprising: (a) culturing in a culture medium comprising

[0017] In some or any of the embodiments described herein, the polynucleotide encoding the Vpx protein or Vpr protein is on the same or different plasmid as the polynucleotide encoding the rev protein, or the polynucleotide comprising the gag and pol genes.

[0018] In some or any of the embodiments described herein, the lentiviral vector genome is derived from HIV-1.

[0019] In some or any of the embodiments described herein, the lentiviral vector genome has an inactivated 3' long terminal repeat (LTR) or a self-inactivating 3' long terminal repeat (LTR). In some or any of the embodiments described herein, the lentiviral vector genome comprises a U3 element lacking at least one of an enhancer sequence, a TATA box, an Sp1 site, an NK-kappa B site, or a polypurine tract (PPT).

[0020] In some or any of the embodiments described herein, the lentiviral vector genome comprises the nucleotide sequence of any one of SEQ ID NOs: 21 [SIN vector], 22 [703 vector], or 23 [704 vector].

[0021] In some or any of the embodiments described herein, the lentiviral vector genome further comprises a nucleotide sequence encoding a dendritic cell maturation/stimulatory factor. In some or any of the embodiments described herein, the dendritic cell maturation/stimulatory factor is selected from the group consisting of GM-CSF, IL-2, IL-4, IL-6, IL-7, IL-15, IL-21, IL-23, TNFα, B7.1, B7.2, 4-1BB, CD40 ligand, and drug-inducible CD40.

[0022] In some or any of the embodiments described herein, the nucleotide sequence encoding an antigen is operably linked to a promoter selected from the group consisting of the human Ubiquitin-C promoter (UbiC), the cytomegalovirus immediate early promoter (CMV), the Rous sarcoma virus promoter (RSV), and the tetracycline-responsive promoter. In some or any of the embodiments described herein, the promoter is an intron-deficient promoter. In some or any of the embodiments described herein, the intron-deficient promoter is a UbiC

[0022A] Another aspect of the disclosure provided a method of generating a pseudotyped lentiviral vector particle comprising: (a) culturing in a culture medium comprising kifunensine a virus packaging cell comprising: (1) a lentiviral vector genome comprising a polynucleotide encoding MAGE-A3 and NY-ESO-1, wherein a polynucleotide encoding a self-cleaving TA2 peptide is positioned between the polynucleotide encoding MAGE-A3 and NY-ESO-1, wherein the lentiviral genome lacks a polypurine tract (PPT), and

wherein expression of MAGE-A3 and NY-ESO-1 is controlled by a UbiC promoter lacking an intron, (2) a polynucleotide encoding a Sindbis E2 glycoprotein that preferentially binds dendritic cells expressing DC-SIGN, (3) a polynucleotide comprising human codon optimized gag and pol genes, wherein the polynucleotide lacks a functional rev responsive element (RRE) and wherein the pol gene encodes an inactive integrase enzyme, (4) a polynucleotide encoding a Vpx protein or a Vpr protein that retains SAMHD1-inhibiting activity; and (b) isolating a pseudotyped lentiviral vector particle that preferentially binds dendritic cells expressing DC-SIGN.

[0023] Related aspects of the disclosure provide a lentiviral vector particle produced by any of the methods recited above.

Compositions comprising pseudotyped lentiviral vector particles

[0023A] Another aspect of the disclosure provides a composition comprising pseudotyped lentiviral vector particles comprising (a) a Vpx protein or a Vpr protein that retains SAMHD1-inhibiting activity, (b) an exogenous polynucleotide encoding an antigen, and (c) a plurality of envelope glycoproteins that preferentially bind cells expressing DC-SIGN, wherein said composition is more highly mannosylated compared to a control composition of the same pseudotyped lentiviral vector particles prepared in the absence of a mannosidase inhibitor.

[0024] Also disclosed is~~Another aspect of the disclosure provides~~ a composition comprising pseudotyped lentiviral vector particles comprising (a) a SAMHD1 inhibitor, (b) an exogenous

polynucleotide encoding an antigen, and (c) an envelope glycoprotein that preferentially binds dendritic cells expressing DC-SIGN, wherein at least 60%, or at least 70%, or at least 80%, preferably at least 90% of N-linked glycans in said composition comprise a Man_5 through Man_9 structure, preferably Man_9.

[0025] Also disclosed is~~Another aspect of the disclosure provides~~ a composition comprising pseudotyped lentiviral vector particles comprising (a) a Vpx protein, (b) an exogenous polynucleotide encoding an antigen, and (c) an envelope glycoprotein that preferentially binds dendritic cells expressing DC-SIGN, wherein at least 80% of N-linked glycans in said composition comprise a Man_9 structure.

[0026] In some or any of the embodiments described herein, the Vpx protein comprises an amino acid sequence that is at least 80% identical to SIVmac Vpx protein (SEQ ID NO: 44).

[0027] In some or any of the embodiments described herein, the Vpx protein comprises an amino acid sequence at least 90% identical to SIVmac Vpx (SEQ ID NO: 44), SIVsm Vpx (SEQ ID NO: 45), SIVrcm Vpx (SEQ ID NO: 46), or HIV-2 Vpx (SEQ ID NO: 47).

[0028] In some or any of the embodiments described herein, the Vpr protein comprises an amino acid sequence at least 90% identical to SIVdeb Vpr (SEQ ID NO: 48) or SIVmus Vpr (SEQ ID NO: 49).

[0029] In some or any of the embodiments described herein, the pseudotyped lentiviral vector particle infects dendritic cells expressing DC-SIGN with an *in vitro* transduction efficiency of at least 1%, or at least 5%, or at least 10%, or at least 20%. See, e.g., the procedure of Example 8.

[0030] In some or any of the embodiments described herein, the glycoprotein is a Sindbis virus E2 glycoprotein. In some or any of the embodiments described herein, the E2 glycoprotein has at least 90% identity to SEQ ID NO: 30 [SIN-Var1]. In some or any of the embodiments described herein, (i) residue 160 of the E2 glycoprotein is absent or is an amino acid other than glutamic acid, (ii) one or more of residues 70, 76, or 159 of the E2 glycoprotein variant is a non-basic residue, and (iii) the E2 glycoprotein variant is not part of a fusion protein with Sindbis virus E3 glycoprotein.

[0031] In some or any of the embodiments described herein, the antigen is a tumor-specific antigen or a virus-specific antigen. In some or any of the embodiments described herein, the tumor-specific antigen is selected from the group consisting of NY-ESO-1, MAGE, e.g., MAGE-A3 and MAGE-A1, MART-1/Melan-A, BAGE, RAGE, gp100, gp75,

WHAT IS CLAIMED:

1. A composition comprising pseudotyped lentiviral vector particles comprising (a) a Vpx protein or a Vpr protein that retains SAMHD1-inhibiting activity, (b) an exogenous polynucleotide encoding an antigen, and (c) a plurality of envelope glycoproteins that preferentially bind cells expressing DC-SIGN, wherein said composition is more highly mannosylated compared to a control composition of the same pseudotyped lentiviral vector particles prepared in the absence of a mannosidase inhibitor.

2. The composition of claim 1, wherein the envelope glycoproteins are alphavirus glycoproteins.

3. The composition of claim 2, wherein the envelope glycoproteins are Sindbis glycoproteins.

4. The composition of any of claims 1-3, wherein high mannosylation is characterized by being more EndoH-sensitive than a control composition prepared in the absence of a mannosidase inhibitor.

5. The composition of any of claims 1-3, wherein EndoH sensitivity is determined by assessing the molecular weight of the envelope glycoproteins by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) after EndoH treatment.

6. The composition of any one of claims 1-3, wherein the molecular weight of the envelope glycoproteins after treatment with EndoH has shifted about 45% or more of the distance between (a) envelope glycoproteins not treated with an endoglycosidase, and (b) envelope glycoproteins treated with PNGase F.

7. The composition of claim 6, wherein the molecular weight of the envelope glycoproteins after treatment with EndoH has shifted approximately 70% or more of the distance between (a) envelope glycoproteins not treated with endoglycosidase, and (b) envelope glycoproteins treated with PNGase F.

8. The composition of claim 6, wherein the molecular weight of the envelope glycoproteins after treatment with EndoH has shifted approximately 90% or more of the distance between (a) envelope glycoproteins not treated with endoglycosidase, and (b) envelope glycoproteins treated with PNGase F.

9. The composition of any one of claims 1-8, wherein at least 30% of the envelope glycoproteins in said composition have an increased amount of EndoH-sensitive

glycan as compared to control glycoproteins having the same amino acid sequence(s) in a control composition of pseudotyped lentiviral vector particles prepared in the absence of a mannosidase inhibitor.

10. The composition of any one of claims 1-9, wherein a majority of the envelope glycoproteins are highly mannosylated.

11. The composition of any one of claims 1-10, wherein the pseudotyped lentiviral vector particles are integration deficient.

12. The composition of any one of claims 1-11, wherein the composition comprises a Sindbis E2 glycoprotein.

13. The composition of claim 12, wherein the E2 glycoprotein is 90% identical to SEQ ID NO: 30 [SIN-Var1].

14. The composition of claim 12 or 13, wherein (i) residue 160 of the E2 glycoprotein is absent or is an amino acid other than glutamic acid, (ii) one or more of residues 70, 76, or 159 of the E2 glycoprotein variant is a non-basic residue, and (iii) the E2 glycoprotein variant is not part of a fusion protein with Sindbis virus E3 glycoprotein.

15. The composition of claim 14, wherein the E2 glycoprotein is SEQ ID NO: 30 [SIN-Var1].

16. The composition of any one of claims 1-15, wherein the Vpx protein comprises an amino acid sequence that is at least 80% identical to SIVmac Vpx [SEQ ID NO: 44].

17. The composition of any one of claims 1-15, wherein the Vpx protein comprises an amino acid sequence at least 90% identical to SIVmac Vpx (SEQ ID NO: 44), SIVsm Vpx (SEQ ID NO: 45), SIVrcm Vpx (SEQ ID NO: 46), or HIV-2 Vpx (SEQ ID NO: 47).

18. The composition of any one of claims 1-14, wherein the Vpx protein comprises an amino acid sequence at least 90% identical to SIVdeb Vpr (SEQ ID NO: 48) or SIVmus Vpr (SEQ ID NO: 49).

19. The composition of any one of claims 1-18, wherein the antigen is a tumor-specific antigen or a virus-specific antigen.

20. The composition of claim 19, wherein the tumor-specific antigen is selected from the group consisting of NY-ESO-1, MAGE, MART-1/Melan-A, BAGE, RAGE, gp100, gp75, mda-7, tyrosinase, tyrosinase-related protein, renal cell carcinoma antigen, 5T4, SM22-alpha, carbonic anhydrase I, carbonic anhydrase IX (also known as G250), HIF-1alpha, HIF-2alpha, VEGF, prostate specific membrane antigen (PSMA), prostate-specific antigen (PSA), prostatic acid phosphates, six-transmembrane epoithelial antigen of the prostate (STEAP), NKX3.1, telomerase enzyme, survivin, mesothelin, mutated ras, bcr/abl rearrangement, Her2/neu, mutated p53, wild-type p53, cytochrome P450 1B1, N-acetylglucosaminyltransferase-V, human papilloma virus protein E6, human papilloma virus protein E7, carcinoembryonic antigen, and alpha-fetoprotein.

21. The composition of claim 19, wherein the virus-specific antigen is an HIV antigen, an SIV antigen, an adenovirus antigen, an enterovirus antigen, a coronavirus antigen, a calicivirus antigen, a distemper virus antigen, an Ebola virus antigen, a flavivirus antigen, a hepatitis virus antigen, a herpesvirus antigen, an infectious peritonitis virus antigen, an influenza virus antigen, a leukemia virus antigen, a Marburg virus antigen, an orthomyxovirus antigen, a papilloma virus antigen, a parainfluenza virus antigen, a paramyxovirus antigen, a parvovirus antigen, a pestivirus antigen, a picorna virus antigen, a poliovirus antigen, a pox virus antigen, a rabies virus antigen, a reovirus antigen, a retrovirus antigen, or a rotavirus antigen.

22. The composition of any one of claims 1-21, wherein the lentiviral vector genome further comprises a nucleotide sequence encoding a second antigen.

23. The composition of claim 22 wherein the first and second antigen are expressed as a fusion protein that comprises a self-cleaving A2 peptide between the two antigens.

24. The composition of claim 23, wherein self-cleaving A2 peptide comprises the amino acid sequence of SEQ ID NO: 56 or SEQ ID NO: 57.

25. The composition of any one of claims 22-24, wherein the first antigen is NY-ESO-1 and the second antigen is MAGE-A3.

26. The composition of claim 22, wherein the first and second antigen are expressed from a bi-directional promoter.

27. The composition of claim 1 wherein the envelope glycoproteins bind cells expressing mouse SIGNR1.

28. The composition of claim 1, wherein the pseudotyped lentiviral vector particles more efficiently transduce cells expressing mouse SIGNR1 compared to cells not expressing mouse SIGNR1.

29. A method of generating a pseudotyped lentiviral vector particle comprising:

(a) culturing in a culture medium comprising kifunensine a virus packaging cell comprising:

(1) a lentiviral vector genome comprising a polynucleotide encoding an exogenous antigen,

(2) a polynucleotide encoding a Sindbis E2 glycoprotein that preferentially binds dendritic cells expressing DC-SIGN, and

(3) a polynucleotide encoding a Vpx protein or a Vpr protein that retains SAMHD1-inhibiting activity; and

(b) isolating a pseudotyped lentiviral vector particle that preferentially binds dendritic cells expressing DC-SIGN.

30. The method of claim 29, wherein the E2 glycoprotein is 90% identical to SEQ ID NO: 30 [SIN-Var1].

31. The method of claim 29 or 30, wherein (i) residue 160 of the E2 glycoprotein is absent or is an amino acid other than glutamic acid, (ii) one or more of residues 70, 76, or 159 of the E2 glycoprotein variant is a non-basic residue, and (iii) the E2 glycoprotein variant is not part of a fusion protein with Sindbis virus E3 glycoprotein.

32. The method of claim 30, wherein the E2 glycoprotein is SEQ ID NO: 30 [SIN-Var1].

33. The method of any one claims 29-32, wherein the Vpx protein comprises an amino acid sequence that is at least 80% identical to SIVmac Vpx [SEQ ID NO: 44].

34. The method of any one of claims 29-32, wherein the Vpx protein comprises an amino acid sequence at least 90% identical to SIVmac Vpx (SEQ ID NO: 44), SIVsm Vpx (SEQ ID NO: 45), SIVrcm Vpx (SEQ ID NO: 46), or HIV-2 Vpx (SEQ ID NO: 47).

35. The method of any one of claims 29-32, wherein the Vpx protein comprises an amino acid sequence at least 90% identical to SIVdeb Vpr (SEQ ID NO: 48) or SIVmus Vpr (SEQ ID NO: 49).

36. The method of any one of claims 29-35, wherein the antigen is a tumor-specific antigen or a virus-specific antigen.

37. The method of claim 36, wherein the tumor-specific antigen is selected from the group consisting of NY-ESO-1, MAGE, MART-1/Melan-A, BAGE, RAGE, gp100, gp75, mda-7, tyrosinase, tyrosinase-related protein, renal cell carcinoma antigen, 5T4, SM22-alpha, carbonic anhydrase I, carbonic anhydrase IX (also known as G250), HIF-1alpha, HIF-2alpha, VEGF, prostate specific membrane antigen (PSMA), prostate-specific antigen (PSA), prostatic acid phosphates, six-transmembrane epoithelial antigen of the prostate (STEAP), NKX3.1, telomerase enzyme, survivin, mesothelin, mutated ras, bcr/abl rearrangement, Her2/neu, mutated p53, wild-type p53, cytochrome P450 1B1, N-acetylglucosaminyltransferase-V, human papilloma virus protein E6, human papilloma virus protein E7, carcinoembryonic antigen, and alpha-fetoprotein.

38. The method of claim 36, wherein the virus-specific antigen is an HIV antigen, an SIV antigen, an adenovirus antigen, an enterovirus antigen, a coronavirus antigen, a calicivirus antigen, a distemper virus antigen, an Ebola virus antigen, a flavivirus antigen, a hepatitis virus antigen, a herpesvirus antigen, an infectious peritonitis virus antigen, an influenza virus antigen, a leukemia virus antigen, a Marburg virus antigen, an orthomyxovirus antigen, a papilloma virus antigen, a parainfluenza virus antigen, a paramyxovirus antigen, a parvovirus antigen, a pestivirus antigen, a picorna virus antigen, a poliovirus antigen, a pox virus antigen, a rabies virus antigen, a reovirus antigen, a retrovirus antigen, or a rotavirus antigen.

39. The method of any one of claims 29-38, wherein the lentiviral vector genome further comprises a nucleotide sequence encoding a second antigen.

40. The method of claim 39 wherein the first and second antigen are expressed as a fusion protein that comprises a self-cleaving A2 peptide between the two antigens.

41. The method of claim 40, wherein self-cleaving A2 peptide comprises the amino acid sequence of SEQ ID NO: 56 or SEQ ID NO: 57.

42. The method of any one of claims 39 to 41, wherein the first antigen is NY-ESO-1 and the second antigen is MAGE-A3.

43. The method of claim 39, wherein the first and second antigen are expressed from a bi-directional promoter.

44. The method of any one of claims 29-43, wherein the kifunensine is present in the culture medium at a concentration of about 0.1 μg/ml to about 10 μg/ml.

45. The method of claim 44, wherein the kifunensine is present in the culture medium at a concentration of about 0.25 μg/ml to about 2 μg/ml.

46. The method of any one of claims 29-45, wherein the virus packaging cell further comprises:

(i) a polynucleotide comprising gag and pol genes; and

(ii) a polynucleotide encoding a rev protein.

47. The method of claim 46, wherein the gag and pol genes are human codon optimized and comprise a non-optimized window around position 1228 to 1509 of SEQ ID NO: 54.

48. The method of claim 46 or 47, wherein the polynucleotide comprising gag and pol genes lacks a functional rev responsive element (RRE).

49. The method of any one of claims 46 to 48, wherein the pol gene encodes an inactive integrase enzyme.

50. The method of claim 49, wherein the integrase enzyme has a D64V mutation.

51. The method of any one of claims 46 to 50, wherein the polynucleotide encoding the Vpx protein is on the same or different plasmid as the polynucleotide encoding the rev protein, or the polynucleotide comprising the gag and pol genes.

52. The method of any one of claims 29 to 51, wherein the lentiviral vector genome is derived from HIV-1.

53. The method of any one of claims 29 to 52, wherein the lentiviral vector genome has an inactivated 3' long terminal repeat (LTR) or a self-inactivating 3' long terminal repeat (LTR).

54. The method of claim 53, wherein the lentiviral vector genome comprises a U3 element lacking at least one of an enhancer sequence, a TATA box, an Sp1 site, an NK-kappa B site, or a polypurine tract (PPT).

55. The method of any one of claims 29 to 54, wherein the lentiviral vector genome comprises the nucleotide sequence of any one of SEQ ID NOs: 21, 22, or 23.

56. The method of any one of claims 29 to 55, wherein the lentiviral vector genome further comprises a nucleotide sequence encoding a dendritic cell maturation/stimulatory factor.

57. The method of claim 56, wherein the dendritic cell maturation/stimulatory factor is selected from the group consisting of GM-CSF, IL-2, IL-4, IL-6, IL-7, IL-15, IL-21, IL-23, TNFα, B7.1, B7.2, 4-1BB, CD40 ligand, and drug-inducible CD40.

58. The method of any one of claims 29 to 57, wherein the nucleotide sequence encoding an antigen is operably linked to a promoter selected from the group consisting of the human Ubiquitin-C promoter (UbiC), the cytomegalovirus immediate early promoter (CMV), the Rous sarcoma virus promoter (RSV), and the tetracycline-responsive promoter.

59. The method of claim 58, wherein the promoter is an intron-deficient promoter.

60. The method of claim 59, wherein the intron-deficient promoter is a UbiC promoter.

61. The lentiviral vector particle produced by the method of claim 29-45.

62. The lentiviral vector particle produced by the method of claim 46-60.

63. A method of generating a pseudotyped lentiviral vector particle comprising:

(a) culturing in a culture medium comprising kifunensine a virus packaging cell comprising:

(1) a lentiviral vector genome comprising a polynucleotide encoding MAGE-A3 and NY-ESO-1, wherein a polynucleotide encoding a self-cleaving TA2 peptide is positioned between the polynucleotide encoding MAGE-A3 and NY-ESO-1, wherein the lentiviral genome lacks a polypurine tract (PPT), and wherein expression of MAGE-A3 and NY-ESO-1 is controlled by a UbiC promoter lacking an intron,

(2) a polynucleotide encoding a Sindbis E2 glycoprotein that preferentially binds dendritic cells expressing DC-SIGN,

(3) a polynucleotide comprising human codon optimized gag and pol genes, wherein the polynucleotide lacks a functional rev responsive element (RRE) and wherein the pol gene encodes an inactive integrase enzyme,

(4) a polynucleotide encoding a Vpx protein or a Vpr protein that retains SAMHD1-inhibiting activity; and

(b) isolating a pseudotyped lentiviral vector particle that preferentially binds dendritic cells expressing DC-SIGN.

WHAT IS CLAIMED:

1. A composition comprising pseudotyped lentiviral vector particles comprising (a) a Vpx protein or a Vpr protein that retains SAMHD1-inhibiting activity, (b) an exogenous polynucleotide encoding an antigen, and (c) a plurality of envelope glycoproteins that preferentially bind cells expressing DC-SIGN, wherein said composition is more highly mannosylated compared to a control composition of the same pseudotyped lentiviral vector particles prepared in the absence of a mannosidase inhibitor.

2. The composition of claim 1, wherein the envelope glycoproteins are selected from the group consisting of alphavirus glycoproteins or Sindbis glycoproteins.

3. The composition of any of claim 1, wherein high mannosylation is characterized by being more EndoH-sensitive than a control composition prepared in the absence of a mannosidase inhibitor.

4. The composition of any of claim 1, wherein EndoH sensitivity is determined by assessing the molecular weight of the envelope glycoproteins by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) after EndoH treatment.

5. The composition of any one of claim 1, wherein the molecular weight of the envelope glycoproteins after treatment with EndoH has shifted about 45% or more, 70% or more, or 90% or more of the distance between (a) envelope glycoproteins not treated with endoglycosidase, and (b) envelope glycoproteins treated with PNGase F.

6. The composition of any one of claim 1, wherein at least 30% of the envelope glycoproteins in said composition have an increased amount of EndoH-sensitive glycan as compared to control glycoproteins having the same amino acid sequence(s) in a control composition of pseudotyped lentiviral vector particles prepared in the absence of a mannosidase inhibitor.

7. The composition of any one of claim 1, wherein the pseudotyped lentiviral vector particles are integration deficient.

8. Thcomposition of any one of claim 1, wherein the composition comprises glycoprotein selected from the group consisting of a Sindbis E2 glycoprotein, a Sindbis E2 glycoprotein is that 90% identical to SEQ ID NO: 30 [SIN-Var1] a Sindbis E2 glycoprotein is

that 90% identical to SEQ ID NO: 30 [SIN-Var1] wherein (i) residue 160 of the E2 glycoprotein is absent or is an amino acid other than glutamic acid, (ii) one or more of residues 70, 76, or 159 of the E2 glycoprotein variant is a non-basic residue, and (iii) the E2 glycoprotein variant is not part of a fusion protein with Sindbis virus E3 glycoprotein or a Sindbis E2 glycoprotein as set out in SEQ ID NO: 30 [SIN-Var1].

9. The composition of any one of claim 1, wherein the Vpx protein comprises an amino acid sequence that is at least 80% identical to SIVmac Vpx [SEQ ID NO: 44], or wherein the Vpx protein comprises an amino acid sequence at least 90% identical to SIVmac Vpx (SEQ ID NO: 44), SIVsm Vpx (SEQ ID NO: 45), SIVrcm Vpx (SEQ ID NO: 46), or HIV-2 Vpx (SEQ ID NO: 47), or

wherein the Vpx protein comprises an amino acid sequence at least 90% identical to SIVdeb Vpr (SEQ ID NO: 48) or SIVmus Vpr (SEQ ID NO: 49).

10. The composition of any one of claim 1, wherein the antigen (a) is a tumor-specific antigen or a virus-specific antigen; (b) is selected from the group consisting of NY-ESO-1, MAGE, MART-1/Melan-A, BAGE, RAGE, gp100, gp75, mda-7, tyrosinase, tyrosinase-related protein, renal cell carcinoma antigen, 5T4, SM22-alpha, carbonic anhydrase I, carbonic anhydrase IX (also known as G250), HIF-1alpha, HIF-2alpha, VEGF, prostate specific membrane antigen (PSMA), prostate-specific antigen (PSA), prostatic acid phosphates, six-transmembrane epoithelial antigen of the prostate (STEAP), NKX3.1, telomerase enzyme, survivin, mesothelin, mutated ras, bcr/abl rearrangement, Her2/neu, mutated p53, wild-type p53, cytochrome P450 1B1, N-acetylglucosaminyltransferase-V, human papilloma virus protein E6, human papilloma virus protein E7, carcinoembryonic antigen, and alpha-fetoprotein; (c) is an HIV antigen, an SIV antigen, an adenovirus antigen, an enterovirus antigen, a coronavirus antigen, a calicivirus antigen, a distemper virus antigen, an Ebola virus antigen, a flavivirus antigen, a hepatitis virus antigen, a herpesvirus antigen, an infectious peritonitis virus antigen, an influenza virus antigen, a leukemia virus antigen, a Marburg virus antigen, an orthomyxovirus antigen, a papilloma virus antigen, a parainfluenza virus antigen, a paramyxovirus antigen, a parvovirus antigen, a pestivirus antigen, a picorna virus antigen, a poliovirus antigen, a pox virus antigen, a rabies virus antigen, a reovirus antigen, a retrovirus antigen, or a rotavirus antigen.

11. The composition of any one of claim 1, wherein the lentiviral vector further comprises a nucleotide sequence encoding a second antigen.

12. The composition of claim 11 wherein the first and second antigen are expressed as a fusion protein that comprises a self-cleaving A2 peptide between the two antigens.

13. The composition of claim 12, wherein self-cleaving A2 peptide comprises the amino acid sequence of SEQ ID NO: 56 or SEQ ID NO: 57.

14. The composition of 22, wherein the first antigen is NY-ESO-1 and the second antigen is MAGE-A3.

15. The composition of claim 11, wherein the first and second antigen are expressed from a bi-directional promoter.

16. The composition of claim 1 wherein the envelope glycoproteins bind cells expressing mouse SIGNR1.

17. The composition of claim 1, wherein the pseudotyped lentiviral vector particles more efficiently transduce cells expressing mouse SIGNR1 compared to cells not expressing mouse SIGNR1.

18. A method of generating a pseudotyped lentiviral vector particle comprising:

(a) culturing in a culture medium comprising kifunensine a virus packaging cell comprising:

(1) a lentiviral vector genome comprising a polynucleotide encoding an exogenous antigen,

(2) a polynucleotide encoding a Sindbis E2 glycoprotein that preferentially binds dendritic cells expressing DC-SIGN, and

(3) a polynucleotide encoding a Vpx protein or a Vpr protein that retains SAMHD1-inhibiting activity; and

(b) isolating a pseudotyped lentiviral vector particle that preferentially binds dendritic cells expressing DC-SIGN.

19. The method of claim 18, wherein (a) the E2 glycoprotein is 90% identical to SEQ ID NO: 30 [SIN-Var1] (b) (i) residue 160 of the E2 glycoprotein is absent or is an amino acid other than glutamic acid, (ii) one or more of residues 70, 76, or 159 of the E2 glycoprotein variant is a non-basic residue, and (iii) the E2 glycoprotein variant is not part of a fusion protein with Sindbis virus E3 glycoprotein; or (c) the E2 glycoprotein is SEQ ID NO: 30 [SIN-Var1].

20. The method of claim 18, wherein (a) the Vpx protein comprises an amino acid sequence that is at least 80% identical to SIVmac Vpx [SEQ ID NO: 44]; (b) the Vpx protein comprises an amino acid sequence at least 90% identical to SIVmac Vpx (SEQ ID NO: 44), SIVsm Vpx (SEQ ID NO: 45), SIVrcm Vpx (SEQ ID NO: 46), or HIV-2 Vpx (SEQ ID NO: 47);

or (c) the Vpx protein comprises an amino acid sequence at least 90% identical to SIVdeb Vpr (SEQ ID NO: 48) or SIVmus Vpr (SEQ ID NO: 49).

21. The method of claim 18, wherein the antigen is (a) a tumor-specific antigen or a virus-specific antigen; (b) selected from the group consisting of NY-ESO-1, MAGE, MART-1/Melan-A, BAGE, RAGE, gp100, gp75, mda-7, tyrosinase, tyrosinase-related protein, renal cell carcinoma antigen, 5T4, SM22-alpha, carbonic anhydrase I, carbonic anhydrase IX (also known as G250), HIF-1alpha, HIF-2alpha, VEGF, prostate specific membrane antigen (PSMA), prostate-specific antigen (PSA), prostatic acid phosphates, six-transmembrane epoithelial antigen of the prostate (STEAP), NKX3.1, telomerase enzyme, survivin, mesothelin, mutated ras, bcr/abl rearrangement, Her2/neu, mutated p53, wild-type p53, cytochrome P450 1B1, N-acetylglucosaminyltransferase-V, human papilloma virus protein E6, human papilloma virus protein E7, carcinoembryonic antigen, and alpha-fetoprotein; or (c) an HIV antigen, an SIV antigen, an adenovirus antigen, an enterovirus antigen, a coronavirus antigen, a calicivirus antigen, a distemper virus antigen, an Ebola virus antigen, a flavivirus antigen, a hepatitis virus antigen, a herpesvirus antigen, an infectious peritonitis virus antigen, an influenza virus antigen, a leukemia virus antigen, a Marburg virus antigen, an orthomyxovirus antigen, a papilloma virus antigen, a parainfluenza virus antigen, a paramyxovirus antigen, a parvovirus antigen, a pestivirus antigen, a picorna virus antigen, a poliovirus antigen, a pox virus antigen, a rabies virus antigen, a reovirus antigen, a retrovirus antigen, or a rotavirus antigen.

22. The method of claim 18, wherein the lentiviral vector genome further comprises a nucleotide sequence encoding a second antigen.

23. The method of claim 22 wherein the first and second antigen are expressed as a fusion protein that comprises a self-cleaving A2 peptide between the two antigens.

24. The method of claim 23, wherein self-cleaving A2 peptide comprises the amino acid sequence of SEQ ID NO: 56 or SEQ ID NO: 57.

25. The method of claim 22, wherein the first antigen is NY-ESO-1 and the second antigen is MAGE-A3.

26. The method of claim 22, wherein the first and second antigen are expressed from a bi-directional promoter.

27. The method of claim 18, wherein the kifunensine is present in the culture medium at a concentration of about 0.1 μg/ml to about 10 μg/ml, or

wherein the kifunensine is present in the culture medium at a concentration of about 0.25 μg/ml to about 2 μg/ml.

28. The method of claim 18, wherein the virus packaging cell further comprises:

(i) a polynucleotide comprising gag and pol genes; and

(ii) a polynucleotide encoding a rev protein.

29. The method of claim 28, wherein the gag and pol genes are human codon optimized and comprise a non-optimized window around position 1228 to 1509 of SEQ ID NO: 54.

30. The method of claim 28, wherein the polynucleotide comprising gag and pol genes lacks a functional rev responsive element (RRE).

31. The method of claim 28, wherein the pol gene encodes an inactive integrase enzyme.

32. The method of claim 31, wherein the integrase enzyme has a D64V mutation.

33. The method of claim 18, wherein the lentiviral vector genome is derived from HIV-1.

34. The method of claim 18, wherein the lentiviral vector genome has an inactivated 3' long terminal repeat (LTR) or a self-inactivating 3' long terminal repeat (LTR).

35. The method of claim 34, wherein the lentiviral vector genome comprises a U3 element lacking at least one of an enhancer sequence, a TATA box, an Sp1 site, an NK-kappa B site, or a polypurine tract (PPT).

36. The method of claim 18, wherein the lentiviral vector genome comprises the nucleotide sequence of any one of SEQ ID NOs: 21, 22, or 23.

37. The method of claim 18, wherein the lentiviral vector genome further comprises a nucleotide sequence encoding a dendritic cell maturation/stimulatory factor.

38. The method of claim 37, wherein the dendritic cell maturation/stimulatory factor is selected from the group consisting of GM-CSF, IL-2, IL-4, IL-6, IL-7, IL-15, IL-21, IL-23, TNFα, B7.1, B7.2, 4-1BB, CD40 ligand, and drug-inducible CD40.

39. The method of claim 18, wherein the nucleotide sequence encoding an antigen is operably linked to a promoter selected from the group consisting of the human Ubiquitin-C promoter (UbiC), the cytomegalovirus immediate early promoter (CMV), the Rous sarcoma virus promoter (RSV), and the tetracycline-responsive promoter.

40. The method of claim 39, wherein the promoter is an intron-deficient promoter.

41. The method of claim 40, wherein the intron-deficient promoter is a UbiC promoter.

42. The lentiviral vector particle produced by the method of claim 18.

43. The lentiviral vector particle produced by the method of claim 28.

44. A method of generating a pseudotyped lentiviral vector particle comprising:

(a) culturing in a culture medium comprising kifunensine a virus packaging cell comprising:

(1) a lentiviral vector genome comprising a polynucleotide encoding MAGE-A3 and NY-ESO-1, wherein a polynucleotide encoding a self-cleaving TA2 peptide is positioned between the polynucleotide encoding MAGE-A3 and NY-ESO-1, wherein the

lentiviral genome lacks a polypurine tract (PPT), and wherein expression of MAGE-A3 and NY-ESO-1 is controlled by a UbiC promoter lacking an intron,

(2) a polynucleotide encoding a Sindbis E2 glycoprotein that preferentially binds dendritic cells expressing DC-SIGN,

(3) a polynucleotide comprising human codon optimized gag and pol genes, wherein the polynucleotide lacks a functional rev responsive element (RRE) and wherein the pol gene encodes an inactive integrase enzyme,

(4) a polynucleotide encoding a Vpx protein or a Vpr protein that retains SAMHD1-inhibiting activity; and

(b) isolating a pseudotyped lentiviral vector particle that preferentially binds dendritic cells expressing DC-SIGN.