

CLAIMS

WHAT IS CLAIMED:

1. A targeted lipid particle, comprising:
 - (a) a lipid bilayer enclosing a lumen,
 - (b) a henipavirus F protein molecule or biologically active portion thereof; and
 - (c) a targeted envelope protein comprising (i) a henipavirus envelope attachment glycoprotein G (G protein) or a biologically active portion thereof and (ii) single domain antibody (sdAb) variable domain, wherein the sdAb variable domain is attached to the C-terminus of the G protein or the biologically active portion thereof and/or wherein the sdAb is attached to the G protein or the biologically active portion thereof via a peptide linker, wherein the sdAb binds to a cell surface molecule of a target cell,

wherein the F protein molecule or the biologically active portion thereof and the targeted envelope protein are embedded in the lipid bilayer.
2. The targeted lipid particle of claim 1, wherein the cell surface molecule is a protein, glycan, lipid or low molecular weight molecule.
3. The targeted lipid particle of claim 1 or claim 2, wherein the target cell is selected from the group consisting of tumor-infiltrating lymphocytes, T cells, neoplastic or tumor cells, virus-infected cells, stem cells, central nervous system (CNS) cells, haematopoietic stem cells (HSCs), liver cells, and fully differentiated cells.
4. The targeted lipid particle of any of claims 1-3, wherein the target cell is selected from the group consisting of a CD3⁺ T cell, a CD4⁺ T cell, a CD8⁺ T cell, a hepatocyte, a haematopoietic stem cell, a CD34⁺ haematopoietic stem cell, a CD105⁺ haematopoietic stem cell, a CD117⁺ haematopoietic stem cell, a CD105⁺ endothelial cell, a B cell, a CD20⁺ B cell, a CD19⁺ B cell, a cancer cell, a CD133⁺ cancer cell, an EpCAM⁺ cancer cell, a CD19⁺ cancer cell, a Her2/Neu⁺ cancer cell, a GluA2⁺ neuron, a GluA4⁺ neuron, a NKG2D⁺ natural killer cell, a SLC1A3⁺ astrocyte, a SLC7A10⁺ adipocyte, or a CD30⁺ lung epithelial cell.
5. The targeted lipid particle of any of claims 1-4, wherein the single domain antibody binds to an antigen or portion thereof present on a hepatocyte; and/or the cell surface molecule or antigen is selected from the group consisting of ASGR1, ASGR2 and TM4SF.

6. The targeted lipid particle of any of claims 1-4, wherein the single domain antibody binds to an antigen or portion thereof present on a T cell; and/or the cell surface molecule or antigen is CD8 or CD4.

7. The targeted lipid particle of any of claims 1-4, wherein the cell surface molecule or antigen is LDL-R.

8. A targeted lipid particle, comprising:
(a) a lipid bilayer enclosing a lumen,
(b) a henipavirus F protein molecule or biologically active portion thereof; and
(c) a targeted envelope protein comprising (i) a henipavirus envelope attachment glycoprotein G (G protein) or a biologically active portion thereof and (ii) a binding domain, wherein the binding domain is attached to the C-terminus of the G protein or the biologically active portion thereof, and wherein the binding domain binds a cell surface molecule selected from the group consisting of ASGR1, ASGR2, TM4SF5, CD8, CD4, and low density lipoprotein receptor (LDL-R),

wherein the F protein molecule or the biologically active portion thereof and the targeted envelope protein are embedded in the lipid bilayer.

9. The targeted lipid particle of any of claims 1-8, wherein the lipid particle is a lentiviral vector.

10. A lentiviral vector, comprising:
(a) a henipavirus F protein molecule or biologically active portion thereof; and
(b) a targeted envelope protein comprising (i) a henipavirus envelope attachment glycoprotein G (G protein) or a biologically active portion thereof and (ii) a binding domain, wherein the binding domain is attached to the C-terminus of the G protein or the biologically active portion thereof, and wherein the binding domain binds CD4; and
(c) a cargo comprising nucleic acid encoding a chimeric antigen receptor (CAR), wherein the CAR comprises (i) an extracellular antigen binding domain that binds CD19, (ii) a transmembrane domain and (iii) an intracellular signaling region comprising a CD3zeta signaling domain.

11. A lentiviral vector, comprising:

(a) a henipavirus F protein molecule or biologically active portion thereof; and

(b) a targeted envelope protein comprising (i) a henipavirus envelope attachment glycoprotein G (G protein) or a biologically active portion thereof and (ii) a binding domain, wherein the binding domain is attached to the C-terminus of the G protein or the biologically active portion thereof, and wherein the binding domain binds a cell surface molecule selected from the group consisting of ASGR1, ASGR2 and TM4SF5.

12. The targeted lipid particle of claim 8 or claim 9 or the lentiviral vector of claim 10 or claim 11, wherein the binding domain is attached to the G protein via a linker.

13. The targeted lipid particle or the lentiviral vector of claim 12, wherein the linker is a peptide linker.

14. The targeted lipid particle of any of claims 8, 9, 12, and 13 or the lentiviral vector of any of claims 10-13, wherein the binding domain is a single domain antibody or is a single chain variable fragment (scFv).

15. The targeted lipid particle of any of claims 1-7, 13, and 14 or the lentiviral vector of claim 13 or claim 14, wherein the peptide linker comprises up to 65 amino acids in length.

16. The targeted lipid particle of any of claims 1-7 and 13-15 or the lentiviral vector of any of claims 13-15, wherein the peptide linker is a flexible linker that comprises GS, GGS, GGGGS (SEQ ID NO:43), GGGGGS (SEQ ID NO:41) or combinations thereof, or wherein the peptide linker comprises (GGS)_n, wherein n is 1 to 10; (GGGGS)_n (SEQ ID NO:42), wherein n is 1 to 10; or (GGGGGS)_n (SEQ ID NO:27), wherein n is 1 to 6.

17. The targeted lipid particle of any of claims 1-9, 15, and 16 or the lentiviral vector of any of claims 10-16, wherein the G protein or the biologically active portion thereof is a wild-type Nipah virus G (NiV-G) protein or a Hendra virus G protein, or is a functionally active variant or biologically active portion thereof.

18. The targeted lipid particle of any of claims 1-9 and 15-17 or the lentiviral vector of any of claims 10-17, wherein the G-protein or the biologically active portion thereof is a mutant NiV-G protein that exhibits reduced binding to Ephrin B2 or Ephrin B3.

19. The targeted lipid particle or lentiviral vector of claim 18, wherein the mutant NiV-G protein comprises:

one or more amino acid substitutions corresponding to amino acid substitutions selected from the group consisting of E501A, W504A, Q530A and E533A with reference to numbering set forth in SEQ ID NO:28.

20. The targeted lipid particle or lentiviral vector of claim 18 or claim 19, wherein the mutant NiV-G protein or the biologically active portion has the amino acid sequence set forth in SEQ ID NO: 16 or an amino acid sequence having at or about 80%, at least at or about 81%, at least at or about 82%, at least at or about 83%, at or about 84%, at least at or about 85%, at least at or about 86%, or at least at or about 87%, at least at or about 88%, or at least at or about 89%, at least at or about 90%, at least at or about 91%, at least at or about 92%, at least at or about 93%, at least at or about 94%, at least at or about 95%, at or about 96%, at least at or about 97%, at least at or about 98%, or at least at or about 99% sequence identity to SEQ ID NO:16.

21. The targeted lipid particle of any of claims 1-9 and 15-20 or the lentiviral vector of any of claims 10-20, wherein the F protein or the biologically active portion thereof is a wild-type Nipah virus F (NiV-F) protein or a Hendra virus F protein or is a functionally active variant or biologically active portion thereof.

22. The targeted lipid particle of any of claims 1-9 and 15-21 or the lentiviral vector of any of claims 10-21, wherein the NiV-F protein is a biologically active portion thereof that has a 22 amino acid truncation at or near the C-terminus of the wild-type NiV-F protein (SEQ ID NO:2).

23. The targeted lipid particle or lentiviral vector of claim 22, wherein the NiV-F protein or the biologically active portion has the sequence set forth in SEQ ID NO:8 or SEQ ID NO:23 or an amino acid sequence that is encoded by a sequence of nucleotides encoding a sequence having at or about 80%, at least at or about 81%, at least at or about 82%, at least at or about 83%, at or about 84%, at least at or about 85%, at least at or about 86%, or at least at or about 87%, at least at or about 88%, or at least at or about 89%, at least at or about 90%, at least at or about 91%, at least at or about 92%, at least at or about 93%, at least at or about 94%, at least at or about 95%, at or about 96%, at least at or about 97%, at least at or about 98%, or at least at or about 99% sequence identity to SEQ ID NO: 8 or SEQ ID NO:23.

24. The targeted lipid particle of any of claims 1-9 and 15-23 or the lentiviral vector of any of claims 10-23, wherein the F protein comprises the sequence set forth in SEQ ID NO:23 and the G protein comprises the sequence set forth in SEQ ID NO:16.

25. The targeted lipid particle of any of claims 1-9 and 15-24 or the lentiviral vector of any of claims 10-24, wherein the lipid particle or lentiviral vector further comprises an exogenous agent.

26. The targeted lipid particle or lentiviral vector of claim 25, wherein the exogenous agent:

is a protein or a nucleic acid;

is a nucleic acid encoding a cargo for delivery to the target cell;

encodes a therapeutic agent or a diagnostic agent;

encodes a membrane protein; or

is a nucleic acid comprising a payload gene for correcting a genetic deficiency.

27. The targeted lipid particle or lentiviral vector of claim 26, wherein the membrane protein is a chimeric antigen receptor (CAR).

28. A polynucleotide comprising a nucleic acid sequence encoding:

(i) a henipavirus envelope attachment glycoprotein G (G protein) or a biologically active portion thereof and (ii) a single domain antibody (sdAb) variable domain, wherein the sdAb variable domain is attached to the C-terminus of the G protein or the biologically active portion thereof; or

(ii) a henipavirus envelope attachment glycoprotein G (G protein) or a biologically active portion thereof and (ii) a binding domain that binds a cell surface molecule selected from the group consisting of ASGR1, ASGR2, TM4SF5, CD4, CD8, and low density lipoprotein receptor (LDL-R).

29. The polynucleotide of claim 28, wherein the polynucleotide is of (ii) and wherein the binding domain is a single domain antibody (sdAb) or is a single chain variable fragment (scFv).

30. The polynucleotide of claim 28 or claim 29, wherein the nucleic acid sequence is a first nucleic acid sequence and the polynucleotide further comprises a second nucleic acid sequence encoding a henipavirus F protein molecule or a biologically active portion thereof.

31. A vector, comprising the polynucleotide of any of claims 28-30.

32. A plasmid, comprising the polynucleotide of any of claims 28-30.

33. A cell comprising the polynucleotide of any of claims 28-30, or the vector of claim 31, or the plasmid of claim 32.

34. A method of making a targeted lipid particle comprising a henipavirus F protein molecule or biologically active portion thereof and a targeted envelope protein comprising a henipavirus envelope attachment glycoprotein G (G protein) or a biologically active portion thereof and a single domain antibody (sdAb) variable domain, the method comprising:

- a) providing a cell that comprises a nucleic acid encoding a henipavirus F protein molecule or biologically active portion thereof and a nucleic acid encoding a targeted envelope protein, the targeted envelope protein comprising a henipavirus envelope attachment glycoprotein G (G protein) or a biologically active portion thereof and a single domain antibody (sdAb) variable domain;
- b) culturing the cell under conditions that allow for production of a targeted lipid particle, and
- c) separating, enriching, or purifying the targeted lipid particle from the cell, thereby making the targeted lipid particle.

35. A method of making a pseudotyped lentiviral vector, the method comprising:

- a) providing a producer cell that comprises a lentiviral viral nucleic acid(s), a nucleic acid encoding a henipavirus F protein molecule or biologically active portion thereof, and a nucleic acid encoding a targeted envelope protein, said targeted envelope protein comprising a henipavirus envelope attachment glycoprotein G (G protein) or a biologically active portion thereof and a single domain antibody;
- b) culturing the cell under conditions that allow for production of the lentiviral vector, and
- c) separating, enriching, or purifying the lentiviral vector from the cell, thereby making the pseudotyped lentiviral vector.

36. A method of making a targeted lipid particle comprising a henipavirus F protein molecule or biologically active portion thereof and a targeted envelope protein comprising a henipavirus envelope attachment glycoprotein G (G protein) or a biologically active portion thereof and a binding domain, the method comprising:

- a) providing a cell that comprises a nucleic acid encoding a henipavirus F protein molecule or biologically active portion thereof and a nucleic acid encoding a targeted envelope protein, the targeted envelope protein comprising a henipavirus envelope attachment glycoprotein G (G protein) or a biologically active portion thereof and binding domain, wherein the binding domain:
 - (i) binds a cell surface molecule selected from the group consisting of ASGR1, ASGR2, and TM4SF5;
 - (ii) binds a cell surface molecule selected from the group consisting of CD4 or CD8; or

(iii) binds a cell surface molecule that is low density lipoprotein receptor (LDL-R);
b) culturing the cell under conditions that allow for production of a targeted lipid particle, and
c) separating, enriching, or purifying the targeted lipid particle from the cell, thereby making the targeted lipid particle,

wherein the targeted lipid particle is a pseudotyped lentiviral vector.

37. A method of making a targeted lipid particle comprising a henipavirus F protein molecule or biologically active portion thereof and a targeted envelope protein, comprising:

a) providing a cell that comprises the polynucleotide of any of claims 28-30, or the vector of claim 31, or the plasmid of claim 32;
b) culturing the cell under conditions that allow for production of a targeted lipid particle, and
c) separating, enriching, or purifying the targeted lipid particle from the cell, thereby making the targeted lipid particle.

38. A method of making a pseudotyped lentiviral vector, comprising:

a) providing a producer cell that comprises a lentiviral viral nucleic acid(s), and the polynucleotide of any of claims 28-30, or the vector of claim 31, or the plasmid of claim 32;
b) culturing the cell under conditions that allow for production of the lentiviral vector, and
c) separating, enriching, or purifying the lentiviral vector from the cell, thereby making the pseudotyped lentiviral vector.

39. A producer cell comprising the polynucleotide of any of claims 28-30, or the vector of claim 31, or the plasmid of claim 32.

40. The producer cell of claim 39, further comprising nucleic acid encoding a henipavirus F protein or a biologically active portion thereof.

41. A producer cell comprising (i) a viral nucleic acid(s) and (ii) nucleic acid encoding a henipavirus F protein molecule or biologically active portion thereof and (iii) a nucleic acid encoding a targeted envelope protein comprising a henipavirus envelope attachment glycoprotein G (G protein) or a biologically active portion thereof and a single domain antibody (sdAb) variable domain.

42. A producer cell comprising (i) a viral nucleic acid(s) and (ii) nucleic acid encoding a henipavirus F protein molecule or biologically active portion thereof and (iii) a nucleic acid encoding a targeted envelope protein comprising a henipavirus envelope attachment glycoprotein G (G protein) or a biologically active portion thereof and a binding domain, wherein the binding domain:

(i) binds a cell surface molecule selected from the group consisting of ASGR1, ASGR2, and TM4SF5;

(ii) binds a cell surface molecule selected from the group consisting of CD4 or CD8;

or

(iii) binds a cell surface molecule that is low density lipoprotein receptor (LDL-R).

43. A targeted lipid particle or pseudotyped lentiviral vector produced by the method of any of claims 34-38 or from the producer cell of any of claims 39-42.

44. A composition comprising a plurality of targeted lipid particles of any one of claims 1-9, 12-27, and 43 or a plurality of lentiviral vectors of any of claims 10-27 and 43.

45. A method of transducing a cell comprising transducing a cell with a lentiviral vector of any of claims 9-27 and 43 or a composition comprising a lentiviral vector or plurality of lentiviral vectors of any of claims 9-27 and 43.

46. Use of the targeted lipid particle or lentiviral vector of any of claims 25-27, in the manufacture of a medicament for delivering an exogenous agent to a subject, wherein the targeted lipid particle or lentiviral vector comprises the exogenous agent.

47. Use of the composition of claim 44, in the manufacture of a medicament for delivering an exogenous agent to a subject, wherein the targeted lipid particles or lentiviral vectors of the plurality comprise the exogenous agent.

48. A method of delivering a chimeric antigen receptor (CAR) to a cell, comprising contacting a cell with the lentiviral vector of any of claims 10 and 12-24 or a targeted lipid particle of claim 27, wherein the lentiviral vector or targeted lipid particle comprises a nucleic acid encoding the CAR.

49. A method of delivering a chimeric antigen receptor (CAR) to a cell, comprising contacting a cell with the composition of claim 44, wherein the lentiviral vectors or the targeted lipid particles of the plurality comprise a nucleic acid encoding the CAR.

50. A method of delivering an exogenous agent to a hepatocyte, comprising contacting a cell with the lentiviral vector of any of claims 11-24 or a targeted lipid particle or

lentiviral vector of claim 26, wherein the exogenous agent is a nucleic acid comprising a payload gene for correcting a genetic deficiency.

51. A method of delivering an exogenous agent to a hepatocyte, comprising contacting a cell with the composition of claim 44, wherein the lentiviral vectors or the targeted lipid particles of the plurality comprise an exogenous agent for delivery to the hepatocyte.

52. Use of a targeted lipid particle of any of claims 1-9, 14-27, and 43, or the lentiviral vector of any of claims 9-27 and 43, or the composition of claim 44, in the manufacture of a medicament for treating a disease or disorder in a subject.

53. Use of a targeted lipid particle of any of claims 1-9, 14-27, and 43, the lentiviral vector of any of claims 9-27 and 43, or the composition of claim 44, in the manufacture of a medicament for fusing a mammalian cell to a targeted lipid particle in a subject.

54. The use of claim 53, wherein the fusing of the mammalian cell to the targeted lipid particle delivers an exogenous agent to the subject.

55. A targeted lipid particle of any one of claims 1-9, 14-27, and 43, or a lentiviral vector of any one of claims 9-27 and 43, for use in treating a disease or disorder in a subject.

56. A targeted lipid particle of any one of claims 1-9, 14-27, and 43, or a lentiviral vector of any one of claims 9-27 and 43, for use in fusing a mammalian cell to a targeted lipid particle in a subject.

57. A targeted lipid particle of any one of claims 1-9, 14-27, and 43, or a lentiviral vector of any one of claims 9-27 and 43, for use in delivering an exogenous agent to a subject, wherein the targeted lipid particle or lentiviral vector comprises the exogenous agent.

58. A composition of claim 44, for use in delivering an exogenous agent to a subject, wherein the targeted lipid particles or lentiviral vectors of the plurality comprise the exogenous agent.