

CLAIMS

1. A ~~method of delivering a nucleic acid to a cell, the method~~retrovirus comprising:
(i) ~~providing a retrovirus comprising (a) the a nucleic acid, and (b) a viral envelope~~
that comprises (i) a viral envelope protein comprising at least one mutation that diminishes its native function viral tropism, wherein the viral envelope protein is a vesicular stomatitis virus (VSV)-G envelope protein or a cocal virus G envelope protein -; and (ii) a non-viral membrane-bound protein comprising a transmembrane-bound domain and an extracellular targeting domain that is capable of binding to a cognate ligand expressed on the surface of the a cell; and
(ii) ~~contacting the retrovirus with the cell, thereby delivering the nucleic acid to the~~
cell.
2. The ~~method-retrovirus~~ of claim 1, wherein the nucleic acid encodes a gene of interest.
3. The ~~method-retrovirus~~ of claim 2, wherein the gene of interest encodes a protein.
4. The ~~method-retrovirus~~ of any one of claims 1-3, wherein the cognate ligand is a protein.
5. The ~~method-retrovirus~~ of claim 4, wherein the cognate ligand is a protein receptor.
6. The ~~method-retrovirus~~ of any one of claims 1-5, wherein the cell is an antigen-specific cell T cell or an antigen-specific B cell.
7. The ~~method-retrovirus~~ of any one of claims 1-6, wherein the extracellular targeting domain is capable of binding to a cognate protein that is present on the cell surface of a T cell or B cell.
8. The ~~method-retrovirus~~ of any one of claims 1 to 7, wherein the retrovirus is a lentivirus.

9. ~~The method-retrovirus~~ of any one of claims 1-8, wherein the VSV-G envelope protein an amino acid substitution at position K47 and/or R354 of SEQ ID NO: 13 or a cocal virus G protein comprising an amino acid substitution at position K64 mutation and/or position R371 of SEQ ID NO: 51; and

(a) the VSV-G envelope protein comprises a K47A or K47Q amino acid substitution and an R354A or R354Q amino acid substitution;

(b) the VSV-G envelope protein comprises a K47A amino acid substitution and an R354A amino acid substitution;

(c) the VSV-G envelope protein comprises a K47A amino acid substitution and an R354Q amino acid substitution;

(d) the VSV-G envelope protein comprises a K47Q amino acid substitution and an R354A amino acid substitution; or

(e) the VSV-G envelope protein comprises a K47Q amino acid substitution and an R354Q amino acid substitution~~the viral envelope protein is a VSV-G envelope protein, a measles virus-envelope protein, a nipah virus envelope protein, or a cocal virus G protein.~~

10. ~~The retrovirus method of claim 9~~any one of claims 1-8, wherein:

(a) the cocal virus G envelope protein comprises a K64A or K64Q amino acid substitution and an R371A or R371Q amino acid substitution~~the at least one mutation of a VSV-G envelope protein is a mutation selected from any one or more of H8, K47, Y209, and R354;~~

(b) the cocal virus G envelope protein comprises a K64A amino acid substitution and an R371A amino acid substitution~~the at least one mutation of the measles virus envelope protein is a mutation selected from any one or more of Y481, R533, S548, and F549;~~

(c) the cocal virus G envelope protein comprises a K64A amino acid substitution and an R371Q amino acid substitution~~the at least one mutation of the nipah virus envelope protein is a mutation selected from any one or more of E501, W504, Q530, and E533; or~~

(d) the cocal virus G envelope protein comprises a K64Q amino acid substitution and an R371A amino acid substitution~~the at least one mutation of the cocal virus G protein is a K64 mutation and/or an R371 mutation~~

(e) the cocal virus G envelope protein comprises a K64Q amino acid substitution and an R371Q amino acid substitution.

11. The retrovirus ~~method~~ of any one of claims 1-10, wherein the cognate ligand is:
- (a) a T cell receptor (TCR);
 - (b) a cytokine receptor;
 - (c) a cytokine; or
 - (d) a cell surface marker.
12. The retrovirus ~~method~~ of claim 10, wherein the cognate ligand is a TCR specific for a cancer antigen, an autoimmune disease antigen, or an infectious agent antigen.
13. The retrovirus ~~method~~ of claim 10, wherein the cognate ligand is a cytokine receptor and the cytokine receptor is IL-13R.
14. The retrovirus ~~method~~ of claim 11, wherein the cognate ligand is a cell surface marker selected from CD3, CD19, and CD20.
15. The retrovirus ~~method~~ of any one of claims 1 to 14, wherein the non-viral membrane-bound protein comprises a Major Histocompatibility Complex (MHC) protein.
16. The retrovirus ~~method~~ of claim 15, wherein the MHC protein is a class I MHC protein or class II MHC protein.
17. The retrovirus ~~method~~ of claim 15, wherein the MHC protein is an HLA-A2 protein.
18. The retrovirus ~~method~~ of any one of claims 15-17, wherein the MHC protein is a disulfide stabilized MHC protein.

19. The retrovirus method of claim 18, wherein the disulfide stabilized MHC protein comprises a Y84C mutation.

20. The retrovirus method of any one of claims 1 to 19, wherein the extracellular targeting domain is a protein, a peptide, or an antibody.

21. The retrovirus method of claim 20, wherein the extracellular targeting domain is a peptide that is displayed by the MHC protein.

22. The retrovirus method of claim 21, wherein the peptide that is displayed by the MHC protein binds to a TCR on a T cell.

23. The retrovirus method of claim 22, wherein increasing the binding affinity of the peptide displayed by the MHC protein for the TCR increases the transduction efficiency of the T cell.

24. The retrovirus method of claim 22 or 23, wherein transduction of the T cell leads to activation of the T cell.

25. The retrovirus method of any one of claims 20-24, wherein the peptide is a NLV peptide, SL9 peptide, GL9 peptide, NY-ESO1 peptide, or EBV peptide.

26. The retrovirus method of claim 20, wherein the protein is interleukin-13 or a CD80 protein domain.

27. The retrovirus method of claim 20, wherein the antibody is an anti-CD19 antibody, an anti-TCR antibody, or an anti-CD3 antibody.

28. The retrovirus method of any one of claims 1 to 27, wherein a linker is

positioned between the membrane-bound domain and the extracellular targeting domain, optionally wherein the linker is:

- (a) a rigid linker, optionally comprising a PDGFR stalk or a CD8 α stalk;
- (b) a flexible linker, optionally comprising an amino acid sequence comprising GAPGAS (SEQ ID NO: 5) or GGGGS (SEQ ID NO: 7); or
- (c) an oligomerized linker, optionally comprising an IgG4 hinge or an amino acid sequence that can form a tetrameric coiled coil.

29. A retrovirus, comprising

- i) a nucleic acid; and
- ii) a viral envelope comprising
a CD80 protein comprising a transmembrane domain; and
a VSV-G viral envelope protein comprising an amino acid substitution at position K47 and/or R354 of SEQ ID NO: 13 or a cocal virus G protein comprising an amino acid substitution at position K64 mutation and/or position R371 of SEQ ID NO: 51~~a mutated viral envelope protein comprising at least one mutation that diminishes its native function.~~

30. The retrovirus of claim 29, wherein the retrovirus is a lentivirus.

31. The retrovirus of either one of claims 29 or 30, wherein the VSV-G envelope protein comprises an amino acid substitution at position K47 and/or R354 of SEQ ID NO: 13 and:

- (a) the VSV-G envelope protein comprises a K47A or K47Q amino acid substitution and an R354A or R354Q amino acid substitution;
- (b) the VSV-G envelope protein comprises a K47A amino acid substitution and an R354A amino acid substitution;
- (c) the VSV-G envelope protein comprises a K47A amino acid substitution and an R354Q amino acid substitution;
- (d) the VSV-G envelope protein comprises a K47Q amino acid substitution and an R354A

amino acid substitution; or

(e) the VSV-G envelope protein comprises a K47Q amino acid substitution and an R354Q amino acid substitution~~the viral envelope protein is a VSV-G envelope protein, a measles virus envelope protein, a nipah virus envelope protein, or a coccal virus G protein.~~

32. The retrovirus of claim 31, wherein the coccal virus G protein comprises an amino acid substitution at position K64 mutation and/or position R371 of SEQ ID NO: 51 and:

(a) the coccal virus G envelope protein comprises a K64A or K64Q amino acid substitution and an R371A or R371Q amino acid substitution~~the at least one mutation of a VSV-G envelope protein is a mutation selected from any one or more of H8, K47, Y209, and R354;~~

(b) the coccal virus G envelope protein comprises a K64A amino acid substitution and an R371A amino acid substitution~~the at least one mutation of the measles virus envelope protein is a mutation selected from any one or more of Y481, R533, S548, and F549;~~

(c) the coccal virus G envelope protein comprises a K64A amino acid substitution and an R371Q amino acid substitution~~the at least one mutation of the nipah virus envelope protein is a mutation selected from any one or more of E501, W504, Q530, and E533; or~~

(d) the coccal virus G envelope protein comprises a K64Q amino acid substitution and an R371A amino acid substitution;~~the at least one mutation of the coccal virus G protein is a mutation selected from K64 mutation and/or R371 mutation~~

(e) the coccal virus G envelope protein comprises a K64Q amino acid substitution and an R371Q amino acid substitution.

33. The retrovirus of any one of claims 29 to 32, wherein the CD80 protein domain ~~is an extracellular domain, optionally wherein the extracellular domain binds to a receptor on a target cell.~~

34. The retrovirus of any one of claims 29 to 33, further comprising a second non-viral membrane-bound protein comprising a membrane-bound domain and an extracellular targeting domain that is capable of binding to a cognate ligand of a cell.

35. The retrovirus of claim 34, wherein the second non-viral membrane-bound protein comprises a Major Histocompatibility Complex (MHC) protein.

36. The retrovirus of claim 34 or 35, wherein the extracellular targeting domain is a protein, a peptide, or an antibody.

37. The retrovirus of claim 36, wherein the extracellular targeting domain is a peptide that is displayed by the MHC protein.

38. The retrovirus of claim 37, wherein the peptide that is displayed by the MHC protein binds to a TCR on a T cell.

39. The retrovirus of claim 37 or claim 38, wherein the peptide is a NLV peptide, SL9 peptide, GL9 peptide, NY-ESO1 peptide, or EBV peptide.

40. The retrovirus of claim 36, wherein the protein is interleukin-13.

41. The retrovirus of claim 36, wherein the antibody is an anti-CD19 antibody, an anti-TCR antibody, or an anti-CD3 antibody.

37.42. An *in vitro* method of delivering a nucleic acid to a cell, the method comprising:
(i) ~~providing a retrovirus comprising the nucleic acid, a viral envelope protein comprising at least one mutation that diminishes its native function, and a CD80 protein domain; and~~
(ii) contacting the retrovirus of any one of claim 1 -36, with the cell, thereby delivering the nucleic acid to the cell.

43. A retrovirus of any one of claims 1-36, for use in delivering a nucleic acid to a cell
~~38. The method of claim 37, wherein the nucleic acid encodes a gene of interest, optionally wherein the gene of interest encodes a protein.~~

~~39. The method of claim 30 or 31, wherein the cell is an antigen-specific T cell or an~~

~~antigen-specific B-cell.~~

~~40. The method of any one of claims 37 to 39, wherein the retrovirus is a lentivirus.~~

~~41. The method of any one of claims 37 to 40, wherein the viral envelope protein is a VSV-G envelope protein, a measles virus envelope protein, a nipah virus envelope protein, or a coccal virus G protein.~~

~~42. The method of claim 41, wherein:~~

~~(a) the at least one mutation of a VSV-G envelope protein is a mutation selected from any one or more of H8, K47, Y209, and R354;~~

~~(b) the at least one mutation of the measles virus envelope protein is a mutation selected from any one or more of Y481, R533, S548, and F549;~~

~~(c) the at least one mutation of the nipah virus envelope protein is a mutation selected from any one or more of E501, W504, Q530, and E533; or~~

~~(d) the at least one mutation of the coccal virus G protein is a K64 mutation and/or R371 mutation.~~

~~43. The method of any one of claims 37 to 42, wherein the CD80 protein domain is an extracellular domain, optionally wherein the extracellular domain binds to a receptor on the cell.~~