Claims

1. A mutant of a wild-type PIV1 F protein, which mutant comprises a F1 polypeptide and a F2 polypeptide, wherein the mutant comprises at least one amino acid mutation relative to the amino acid sequence of the wild-type PIV1 F protein, and wherein the amino acid mutation is selected from the group consisting of:

(1) at least one engineered disulfide bond mutation;

(2) at least one cavity filling mutation;

(3) at least one proline substitution mutation;

(4) at least one glycine replacement mutation; and,

(5) a cleavage site mutation.

2. The mutant according to claim 1 wherein the mutant comprises an engineered disulfide mutation wherein the engineered disulfide mutation is Q92C-G134C.

3. The mutant according to claim 1 or 2, wherein the mutant comprises a cavity filling mutation wherein the cavity filling mutation is selected from T198A, Q92A, Q92L, A466L, A466V, A466I, S473V, S473L, S473I, S473A, A480L and A480V.

4. The mutant according to claim 3, wherein the mutant comprises the cavity filling mutations A466L and S473L.

5. The mutant according to claim 4 further comprising the cavity filling mutation A480L or A480V.

6. The mutant according to any one of claims 1 to 5, wherein the mutant comprises a proline substitution mutation,, wherein the proline substitution mutation is A128P.

7. The mutant according to any one of claims 1 to 6, wherein the mutant comprises a glycine replacement mutation, wherein the glycine replacement mutation is G134A or G134L.

8. The mutant according to any one of claims 1 to 7 wherein the mutant comprises a cleavage site mutation wherein the cleavage site mutation is F113G and F114S.

9. The mutant according to claim 1, wherein the mutant comprises the mutations selected from the group consisting of:

(1) Q92C-G134C;

(2) A466L;

(3) A466V;

(4) S473V;

(5) S473L;

(6) A480L;

(7) A466L and S473A;

(8) A466L and S473L;

(9) T198A;

(10) G134A;

(11) A128P;

(12) F113G, F114S, Q92C-G134C, A466L, S473L and A480L;

(13) Q92C-G134C, A466L, S473L and A480L;

(14) Q92C-G134C, A466L and S473L;

(15) F113G, F114S, Q92C-G134C, A466V, S473V and A480V;

(16) Q92C-G134C, A466V, S473V and A480V;

(17) Q92C-G134C, A466V and S473V;

(18) F113G, F114S, A466L, S473L, A480L and G134A;

(19) A466L, S473L, A480L and G134A;

(20) A466L, S473L and G134A;

(21) F113G, F114S, A466L, S473L, A480L, Q92A and G134A;

(22) F113G, F114S, A466L, S473L and G134A;

(23) A466L, S473L, A480L, Q92A, G134A;

(24) A466L, S473L, Q92A, G134A;

(25) F113G, F114S, Q92L, G134A;

(26) A466L, S473L, A480L, Q92L and G134A;

(27) A466L, S473L, Q92L and G134A;

(28) F113G, F114S, A466L, S473L, A480L, Q92A and G134L;

(29) A466L, S473L, A480L, Q92A and G134L;

(30) F113G, F114S, Q92C-G134C, A466I, S473I and A480L;

(31) F113G, F114S, Q92C-G134C, A466I and, S473I; and,

(32) A466I, S473I, A480L, Q92L and G134A.

10. The mutant according to claim 1, wherein the mutant comprises the mutations

(1) A466L, S473L, A480L and G134A,

(2) F113G, F114S, A466L, S473L and G134A,

(3) F113G, F114S, A466L, S473L, A480L and G134A, or,

(4) F113G, F114S, Q92C, G134C, A466L, S473L and A480L.

11. The mutant according to claim 1 wherein the mutant comprises a leucine at position 466, 473 and 480 (466L, 473L and 480L) and an alanine at position 134 (134A) and wherein the mutant comprises a F1 polypeptide and a F2 polypeptide selected from the group consisting of:

(1) a F2 polypeptide comprising the amino acid sequence of SEQ ID NO:255 and a F1 polypeptide comprising the amino acid sequence of SEQ ID NO:254;

(2) a F2 polypeptide comprising an amino acid sequence that is at least 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO:255 and a F1 polypeptide comprising an amino acid sequence that is at least 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:254, or,

wherein the mutant comprises a glycine (G) at position 113 (113G), a serine at position 114 (114S), a leucine at position 466 and 473 (466L and 473L) and an alanine at position 134 (134A) and wherein the mutant comprises a F1 polypeptide and a F2 polypeptide selected from the group consisting of:

(1) a F2 polypeptide comprising the amino acid sequence of SEQ ID NO:291 and a F1 polypeptide comprising the amino acid sequence of SEQ ID NO:290;

(2) a F2 polypeptide comprising an amino acid sequence that is at least 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO:291 and a F1 polypeptide comprising an amino acid sequence that is at least 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:290, or,

wherein the mutant comprises a glycine (G) at position 113 (113G), a serine at position 114 (114S), a leucine at position 466, 473 and 480 (466L, 473L and 480L) and an alanine at position 134 (134A) and wherein the mutant comprises a F1 polypeptide and a F2 polypeptide selected from the group consisting of:

(1) a F2 polypeptide comprising the amino acid sequence of SEQ ID NO:277 and a F1 polypeptide comprising the amino acid sequence of SEQ ID NO:276;

(2) a F2 polypeptide comprising an amino acid sequence that is at least 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO:277 and a F1 polypeptide comprising an amino acid sequence that is at least 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:276, or,

wherein the mutant comprises a glycine (G) at position 113 (113G), a serine at position 114 (114S), a leucine at position 466, 473 and 480 (466L, 473L and 480L) and a cysteine at position 92 and 134 (92C and 134C) wherein the mutant comprises a F1 polypeptide and a F2 polypeptide selected from the group consisting of:

(1) a F2 polypeptide comprising the amino acid sequence of SEQ ID NO:273 and a F1 polypeptide comprising the amino acid sequence of SEQ ID NO:272;

(2) a F2 polypeptide comprising an amino acid sequence that is at least 97%, 98% or 99% identical to the amino acid sequence of SEQ ID NO:273 and a F1 polypeptide comprising an amino acid sequence that is at least 97%, 98%, or 99% identical to the amino acid sequence of SEQ ID NO:272.

12. The mutant according to any one of claims 1 to 11, wherein the F1 polypeptide lacks the cytoplasmic domain and the transmembrane domain.

13. The mutant according to any one of claims 1 to 11 wherein the F1 polypeptide comprises or consists of amino acid residues 113 to 477 or comprises or consists of amino acid residues 113 to 480.

14. The mutant according to any one of claims 1 to 11, wherein the F1 polypeptide comprises the ectodomain, the transmembrane domain and the cytoplasmic domain.

15. The mutant according to any one of claims 1 to 14, wherein the mutant is linked to a trimerization domain, wherein the trimerization domain is a phage T4 fibritin foldon, wherein the trimerization domain is linked to the C-terminus of the F1 polypeptide via a linker, and the linker is GGGS.

16. The mutant according to any one of claims 1 to 14, wherein the mutant is in the form of a trimer and/or wherein the mutant is in the prefusion conformation.

17. The mutant of any one of claims 1 to 16 wherein the wild-type PIV1 is SEQ ID NO:206.

18. The mutant of any one of claim 1 to 17 wherein the amino acid positions correspond to the amino acid sequence of a reference of SEQ ID NO:206.

19. A nucleic acid comprising at least one coding sequence encoding at least one mutant of a wild-type PIV 1 F protein according to any one of claims 1 to 18, or an immunogenic fragment or immunogenic variant thereof, wherein the nucleic acid comprises at least one heterologous untranslated region (UTR).

20. A nucleic acid according to claim 19, wherein the at least one heterologous untranslated region is selected from at least one heterologous 5'-UTR and/or at least one heterologous 3'-UTR.

21. A nucleic acid according to claim 19 or 20, wherein the at least one heterologous 3'-UTR comprises or consists of a nucleic acid sequence having at least, at most, exactly, or between any two of 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to CΨCGAGCΨGGΨ ACΨGCAΨGCA CGCAAΨGCΨA GCΨGCCCCΨΨ ΨCCCGΨCCΨG GGΨACCCCGA GΨCΨCCCCCG ACCΨCGGGΨC CCAGGΨAΨGC ΨCCCACCΨCC ACCΨGCCCCA CΨCACCACCΨ CΨGCΨAGΨΨC CAGACACCΨC CCAAGCACGC AGCAAΨGCAG CΨCAAAACGC ΨΨAGCCΨAGC CACACCCCCA CGGGAAACAG CAGΨGAΨΨAA CCΨΨΨAGCAA ΨAAACGAAAG ΨΨΨAACΨAAG CΨAΨACΨAAC CCCAGGGΨΨG GΨCAAΨΨΨCG ΨGCCAGCCAC ACCCΨGGAGC ΨAGC.

22. A nucleic acid according to any one of claims 19 to 21, wherein the at least one heterologous 5'-UTR comprises or consists of a nucleic acid sequence having at least, at most, exactly, or between any two of 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to GAAΨAAAC ΨAGΨAΨΨCΨΨ CΨGGΨCCCCA CAGACΨCAGA GAGAACCCGC CACC.

23. A nucleic acid according to any one of claims 19 to 22, wherein the nucleic acid comprises at least one poly(A) sequence, preferably comprising 30 to 200 adenosine

nucleotides and/or at least one poly(C) sequence, preferably comprising 10 to 40 cytosine nucleotides.

24. A nucleic acid according to any one of claims 19 to 23, wherein the nucleic acid is a DNA or an RNA.

25. A nucleic acid according to claim 24, wherein the nucleic acid is an mRNA, a self-replicating RNA, a circular RNA, or a replicon RNA.

26. A nucleic acid according to claim 25, wherein the nucleic acid is an mRNA.

27. A nucleic acid according to any one of claims 24 to 26, wherein the RNA comprises a 5'-cap structure, preferably m7G, capO, cap1 , cap2, a modified capO or a modified cap1 structure, preferably a 5'- cap1 structure.

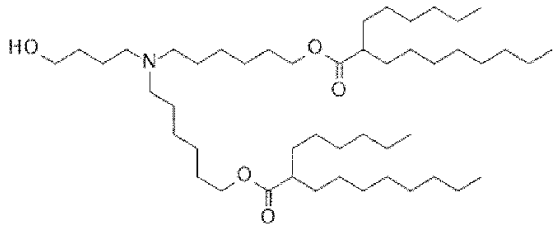
28. A nucleic acid according to any one of claims 25 to 27, wherein the RNA comprises 1-methylpseudouridine substitution and wherein all the uridines of the RNA are replaced by 1-methylpseudouridine.

29. A nucleic according to any one of claims 24 to 28 wherein the RNA comprises the nucleic acid sequence of any of SEQ ID NO:403, SEQ ID NO:405, SEQ ID NO:407 and SEQ ID NO:409.

30. A composition comprising at least one nucleic acid according to any one of claims 19 to 29, wherein the composition comprises at least one pharmaceutically acceptable carrier.

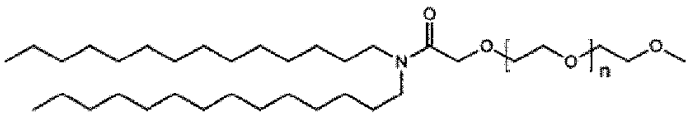
31. A composition comprising at least one nucleic acid according to any one of claims 19 to 29, wherein the at least one nucleic acid is complexed with one or more lipids thereby forming lipid nanoparticles (LNP).

32. A composition according to claim 31 , wherein the LNP comprises a cationic lipid according to formula III-3:



(III-3).

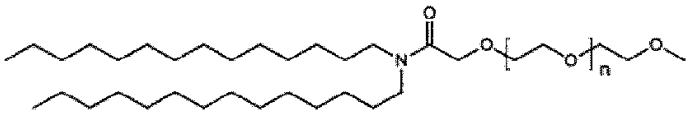
33. A composition according to claim 31 or 32, wherein the LNP comprises a PEG lipid of formula (IVa):



(IVa),

wherein *n* has a mean value ranging from 30 to 60.

34. A composition according to any one of claims 31 to 33, wherein the LNP comprises a PEG lipid of formula (IVa):



(IVa),

wherein n is an integer selected such that the average molecular weight of the PEG lipid is about 2500g/mol.

35. A composition according to any one of claims 31 to 34, wherein the LNP comprises one or more neutral lipids and/or one or more steroid or steroid analogueswherein the neutral lipid is 1 ,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and wherein the steroid is cholesterol.

36. A composition according to any one of claims 31 to 35, wherein the LNP comprises (i) at least one cationic lipid, preferably a lipid of formula (III), more preferably lipid Ill-3; (ii) at least one neutral lipid, preferably 1 ,2-distearoyl-sn-glycero-3-phosphocholine (DSPC); (iii) at least one steroid or steroid analogue, preferably cholesterol; and (iv) at least one polymer conjugated lipid, preferably a PEG-lipid derived from formula (IVa, with n = 49), wherein (i) to (iv) are in a molar ratio of about 20-60% cationic lipid, 5-25% neutral lipid, 25-55% sterol, and 0.5-15% PEG-lipid.

37. A composition according to any one of claims 31 to 36, wherein the LNP comprises (i) at least one cationic lipid, preferably a lipid of formula (III), more preferably lipid Ill-3; (ii) at least one neutral lipid, preferably 1 ,2-distearoyl-sn-glycero-3-phosphocholine (DSPC); (iii) at least one steroid or steroid analogue, preferably cholesterol; and (iv) at least one polymer conjugated lipid, preferably a PEG-lipid derived from formula (IVa, with n = 45), wherein (i) to (iv) are in a molar ratio of about 20-60% cationic lipid, 5-25% neutral lipid, 25-55% sterol, and 0.5-15% PEG-lipid.

38. A composition according to any one of claims 31 to 37, wherein the LNPs have a Z-average size in a range of about 60nm to about 120nm.

39. A composition according to any one of claims 31 to 38, wherein the LNP comprises (i) at least one cationic lipid according to formula III-3; (ii) DSPC; (iii) cholesterol; and (iv) a PEG-lipid, according to formula IVa, wherein (i) to (iv) are in a molar ratio of about 20-60% cationic lipid, 5-25% neutral lipid, 25-55% sterol, and 0.5-15% PEG-lipid.

40. An immunogenic composition comprising a mutant according to any one of claims 1 to 18 or a nucleic acid according to any one of claims 19 to 29 or a composition according to any one of claims 30 to 39.

41. An immunogenic composition according to claim 40, further comprising

a hMPV A antigen selected from the group consisting of a mutant of a wild-type hMPV A F protein and a nucleic acid encoding a mutant of a wild-type hMPV A F protein and/or,
a hMPV B antigen selected from the group consisting of a mutant of a wild-type hMPV B F protein and a nucleic acid encoding a mutant of a wild-type hMPV B F protein, and/or,
a PIV1 antigen selected from the group consisting of a mutant of a wild-type PIV1 F protein and a nucleic acid encoding a mutant of a wild-type PIV1 F protein and/or,
a RSV antigen selected from the group consisting of a mutant of a wild-type RSV F protein of subtype A and a nucleic acid encoding a mutant of a wild-type RSV F protein of subtype A, and/or,
a RSV antigen selected from the group consisting of a mutant of a wild-type RSV F protein of subtype B and a nucleic acid encoding a mutant of a wild-type RSV F protein of subtype B.

42. A mutant of a wild-type hMPV F protein as defined in any of embodiment E1 to E73 disclosed in above section B.

43. A nucleic acid comprising at least one coding sequence encoding at least one mutant of a wild-type hMPV F protein, said nucleic acid being as defined in any of embodiments E74 to E89 disclosed in above section B.

44. A mutant of a wild-type PIV3 F protein as defined in any of embodiment E1 to E52 disclosed in above section D.

45. The mutant according to claim 44 wherein the mutant comprises the mutations E209C and L234C.

46. The mutant according to claim 45 wherein the mutant comprises the mutations E209C, L234C, S160C and V170C.

47. The mutant according to claim 44 wherein the mutant comprises the mutations selected from the group consisting of

(1) G230A, S470A and S477A;
(2) S160C, V170C, G230A and A463L;
(3) S160C, V170C, S470A and S477A;
(4) S160C, V170C, G230A, S470A and S477A;
(5) S160C, V170C, G230A, A463L, S470A and S477A
(6) S160C, V170C, E209C, L234C, A463L and S470L;
(7) S160C, V170C, E209C, L234C, A463L and I474F;
(8) S160C, V170C, E209C, L234C, A463L, S470L, F110G, F111S;
(9) S160C, V170C, A463L and S470L, and,
(10) E209C, L234C.

48. A nucleic acid comprising at least one coding sequence encoding at least one mutant of a wild-type PIV3 F protein, said nucleic acid being as defined in any of embodiments E53 to E69 disclosed in above section D.

49. A nucleic acid according to claim 48 wherein the nucleic acid is an mRNA and comprises the nucleic acid sequence of any of SEQ ID NO:411, SEQ ID NO:413, SEQ ID NO:415, SEQ ID NO:417 and SEQ ID NO:419, SEQ ID NO:504, SEQ ID NO:506, SEQ ID NO:508 and SEQ ID NO:510, SEQ ID NO: 295, SEQ ID NO: 297 and SEQ ID NO: 299.