

CLAIMS

What is claimed is:

1. A recombinant human immunodeficiency virus (HIV) envelope (Env) protein, comprising two or more of the following amino acid residues:

- (i) Phe, Leu, Met, or Trp, preferably Phe, at position 651;
- (ii) Phe, Ile, Met, or Trp, preferably Ile, at position 655;
- (iii) Asn or Gln, preferably Asn, at position 535;
- (iv) Val, Ile or Ala, preferably Val, at position 589;
- (v) Phe or Trp, preferably Phe, at position 573;
- (vi) Ile at position 204; and/or
- (vii) Phe, Met, or Ile, preferably Phe, at position 647,

wherein the numbering of the positions is according to the numbering in gp160 of HIV-1 isolate HXB2.

2. A recombinant HIV Env protein, comprising one or more of the following amino acid residues:

- (i) Phe, Leu, Met, or Trp, preferably Phe, at position 651;
- (ii) Phe, Ile, Met, or Trp, preferably Ile, at position 655;
- (iii) Asn or Gln, preferably Asn, at position 535;
- (iv) Val, Ile or Ala, preferably Val, at position 589;
- (v) Phe or Trp, preferably Phe, at position 573;
- (vi) Ile at position 204; and/or
- (vii) Phe, Met, or Ile, preferably Phe, at position 647,

wherein the HIV Env protein is selected from the group consisting of:

- (1) an HIV Env consensus sequence, for instance from clade C for instance comprising the amino acid sequence of SEQ ID NO: 2 or 3 or for instance from clade B for instance comprising the amino acid sequence of SEQ ID NO: 4 or 5;
- (2) a synthetic HIV Env protein, for instance comprising the amino acid sequence of (a): SEQ ID NO: 6; or (b): SEQ ID NO: 6 with a mutation of Glu to Arg at position 166; or (c): (a) or (b) with a mutation of the amino acids at positions 501 and 605 into Cys residues and a mutation of the amino acid at position 559 into a Pro residue; or (d): (a), (b) or (c) having a further furin cleavage site mutation,

e.g. replacement of the amino acids at positions 508-511 by RRRRRR (SEQ ID NO: 10); or (e) SEQ ID NO: 7; or (f) SEQ ID NO: 8 or SEQ ID NO: 9; and

- (3) a parent HIV Env protein which is preferably a wild-type HIV Env protein, preferably of clade C, comprising at least one repair mutation at an amino acid residue that is present at the corresponding position at a frequency of less than 7.5%, preferably less than 2%, of HIV Env sequences in a collection of at least 1000, preferably at least 10000, wild-type HIV Env sequences, wherein the repair mutation is a substitution by an amino acid residue that is present at the corresponding position at a frequency of at least 10% of HIV Env sequences in said collection and preferably the repair mutation is a substitution by the amino acid residue that is present at the corresponding position most frequently in said collection;
- and

wherein the numbering of the positions is according to the numbering in gp160 of HIV-1 isolate HXB2.

3. A recombinant HIV Env protein, comprising one or more of the following amino acid residues:

- (i) Phe, Leu, Met, or Trp, preferably Phe, at position 651;
- (ii) Phe, Ile, Met, or Trp, preferably Ile, at position 655;
- (iii) Asn or Gln, preferably Asn, at position 535;
- (iv) Val, Ile or Ala, preferably Val, at position 589;
- (v) Phe or Trp, preferably Phe, at position 573;
- (vi) Ile at position 204; and/or
- (vii) Phe, Met, or Ile, preferably Phe, at position 647,

wherein the HIV Env protein is a HIV Env protein comprising at least one of the following:

- (a) Cys at positions 501 and 605;
- (b) Pro at position 559;
- (c) Cys at positions 501 and 605 and Pro at position 559; and

the numbering of the positions is according to the numbering in gp160 of HIV-1 isolate HXB2.

4. The recombinant HIV Env protein of claim 2 or 3, comprising two or more of the amino acid residues indicated in (i) to (vii).
5. The recombinant HIV Env protein of any of claims 1, 2, and 4, comprising Cys at positions 501 and 605 or Pro at position 559, preferably Cys at positions 501 and 605 and Pro at position 559.
6. The recombinant HIV Env protein of any of claims 1 to 5, comprising three or more of the amino acid residues indicated in (i) to (vii).
7. The recombinant HIV Env protein of any of claims 1 to 5, comprising four or more of the amino acid residues indicated in (i) to (vii).
8. The recombinant HIV Env protein according to any one of claims 1 to 7, comprising Phe at position 651, Ile at position 655, Asn at position 535, and Val at position 589.
9. The recombinant HIV Env protein of any of claims 1 to 8, comprising five or more of the amino acid residues indicated in (i) to (vii).
10. The recombinant HIV Env protein of any of claims 1 to 9, further comprising one or more of the following:
 - (viii) Gln, Glu, Ile, Met, Val, Trp, or Phe, preferably Gln or Glu, at position 588;
 - (ix) Lys at position 64 or Arg at position 66 or Lys at position 64 and Arg at position 66;
 - (x) Trp at position 316;
 - (xi) Cys at both positions 201 and 433;
 - (xii) Pro at position 556 or 558 or at both positions 556 and 558;
 - (xiii) replacement of the loop at amino acid positions 548-568 (HR1-loop) by a loop having 7-10 amino acids, preferably a loop of 8 amino acids, for example having a sequence chosen from any one of (SEQ ID NOs: 12-17);
 - (xiv) Gly at position 568, or Gly at position 569, or Gly at position 636, or Gly at both positions 568 and 636, or Gly at both positions 569 and 636; and/or
 - (xv) Tyr at position 302, or Arg at position 519, or Arg at position 520, or Tyr at position 302 and Arg at position 519, or Tyr at position 302 and Arg at position 520, or Tyr at position 302 and Arg at both positions 519 and 520.

11. The recombinant HIV Env protein of any of claims 1 to 10, further comprising a mutation in a furin cleavage sequence of the HIV Env protein, preferably a replacement at positions 508-511 by RRRRRR (SEQ ID NO: 10).
12. The recombinant HIV Env protein of any of claims 1 to 11, comprising an amino acid sequence that is at least 95% identical to any one of SEQ ID NOs: 3, 5, 20, 22, 24, 26, 27, 28, 29, 30, 31, or 32.
13. The recombinant HIV Env protein of any of claims 1 to 12, further comprising: (xvi) an amino acid residue chosen from Val, Ile, Phe, Met, Ala, or Leu, preferably Val or Ile, most preferably Val, at position 658.
14. The recombinant HIV Env protein of any of claims 1 to 13, being a gp140 or gp160 protein.
15. The recombinant HIV Env protein of any of claims 1 to 14, which is from clade C or clade A, preferably from clade C.
16. A trimeric complex comprising a noncovalent oligomer of three of the recombinant HIV Env proteins of any of claims 1 to 15.
17. A particle, preferably a liposome or nanoparticle, displaying on its surface a recombinant HIV Env protein of any of claims 1 to 15 or a trimeric complex of claim 16.
18. An isolated nucleic acid molecule encoding a recombinant HIV Env protein of any of claims 1 to 15.
19. A vector comprising the isolated nucleic acid molecule of claim 18 operably linked to a promoter.
20. The vector of claim 19, which is an adenovirus vector.
21. A host cell comprising the isolated nucleic acid molecule of claim 18 or the vector of claim 19 or 20.
22. A method of producing a recombinant HIV Env protein, comprising growing the host cell of claim 21 under conditions suitable for production of the recombinant HIV Env protein.
23. A composition comprising the recombinant HIV Env protein of any of claims 1 to 15, the trimeric complex of claim 16, the particle of claim 17, the isolated nucleic acid molecule of claim 18, or the vector of claim 19 or 20, and a pharmaceutically acceptable carrier.
24. A method of improving the trimer formation of an HIV Env protein, the method comprising substituting one or more amino acid residues in a parent HIV Env protein, wherein the one or more substitutions result in one or more of the following amino acids:

- (i) Phe, Leu, Met, or Trp, preferably Phe, at position 651;
- (ii) Phe, Ile, Met, or Trp, preferably Ile, at position 655;
- (iii) Asn or Gln, preferably Asn, at position 535;
- (iv) Val, Ile or Ala, preferably Val, at position 589;
- (v) Phe or Trp, preferably Phe, at position 573;
- (vi) Ile at position 204; and/or
- (vii) Phe, Met, or Ile, preferably Phe, at position 647,

wherein the numbering of the positions is according to the numbering in gp160 of HIV-1 isolate HXB2.