CLAIMS

1. An influenza hemagglutinin stem domain polypeptide comprising (a) an influenza hemagglutinin HA1 domain that comprises an HA1 N- terminal stem segment, covalently linked by a linking sequence of 0-50 amino acid residues to an HA1 C- terminal stem segment, and (b) an influenza hemagglutinin HA2 domain, wherein the hemagglutinin stem domain polypeptide is resistant to protease cleavage at the junction between HA1 and HA2,and wherein one or more amino acids in the amino acid sequence connecting the A helix and the helix CD of HA2 have been mutated as compared to a wild-type influenza HA2 domain, and wherein the HA1 and HA2 domain are derived from an influenza A virus subtype selected from the group consisting of H1, H5 and H3.

2. A polypeptide according to claim 1, wherein the polypeptide does not comprise a full-length HA1 domain.

3. A polypeptide according to claim 1 or 2, wherein the polypeptide is glycosylated.

4. A polypeptide according to claim 1, 2 or 3, comprising (a) an HA1 N-terminal segment comprising the amino acids 1-x of HA1, covalently linked by a linking sequence of 0-50 amino acid residues to an HA1 C- terminal stem segment comprising the amino acids y-end of HA1, and (b) an influenza hemagglutinin HA2 domain.

5. A polypeptide according to any of the claims 1-4, wherein the polypeptide comprises an influenza hemagglutinin HA1 and/or HA2 domain based on influenza hemagglutinin from an influenza A virus comprising HA of the H1 subtype, and wherein x=any amino acid between 46 and 60 and y=any amino acid between 290 and 325.

6. A polypeptide according to claim 5, wherein the polypeptide comprises an influenza hemagglutinin HA1 and/or HA2 domain based on influenza hemagglutinin from an influenza A virus comprising HA of the H1 subtype selected from the group consisting of influenza A viruses of Table 7.

7. A polypeptide according to any of the claims 1-4, wherein the polypeptide comprises an influenza hemagglutinin HA1 and/or HA2 domain based on influenza hemagglutinin from an influenza A virus comprising HA of the H3 subtype, and wherein x=any amino acid between 56 and 69, and wherein and y=any amino acid between 292 and 325.

8. A polypeptide according to any one of the preceding claims, wherein the C- terminal amino acid residue of the HA1 C-terminal stem segment is any amino acid other than arginine (R) or lysine (K), preferably glutamine (Q).

9. A polypeptide according to any of the preceding claims, wherein the polypeptide comprises one or more further mutations in the HA1 domain and/or the HA2 domain.

10. A polypeptide according to any one of the preceding claims, wherein the polypeptide comprises one or more disulfide bridges.

11. A polypeptide according to any one of the preceding claims, wherein the polypeptide does not comprise the signal sequence.

12. A polypeptide according to claim 11, comprising an HA1 N-terminal segment comprising the amino acids p-x of HAl, covalently linked by a linking sequence of 0-50 amino acid residues to an HA1 C- terminal stem segment comprising the amino acids y-end of HA1, and (b) an influenza hemagglutinin HA2 domain, wherein p=the first amino acid of the mature HA.

13. A polypeptide according to any one of the preceding claims, wherein the polypeptide does not comprise the intracellular and transmembrane sequence of HA.

14. A polypeptide according to claim 13, wherein the polypeptide does not comprise the C-terminal part of the influenza H1 HA2 domain spanning from amino acid residue 520, 521, 522, 523, 524, 525, 526, 527, 528, 529 or 530 to the C-terminal amino acid of H1 HA2.

15. A polypeptide according to any one of the preceding claims, wherein the polypeptide selectively binds to the antibodies CR6261 and/or CR9114 and does not bind to the antibody CR8057.

16. A polypeptide according to any one of the preceding claims, wherein the polypeptide selectively binds to the antibodies CR8020, CR843 and/or CR9114 and does not bind to the antibody CR8057.

17. A method for providing an influenza hemagglutinin stem domain polypeptide comprising the steps of:
(a) Providing an influenza HA0 amino acid sequence;
(b) Removing the cleavage site between HA1 and HA2;
(c) Removing the amino acid sequence of the globular head domain from the HA0 sequence;
(d) Introducing one or more mutations in the amino acid sequence connecting the C-terminal residue of helix A to the N-terminal residue of helix CD; and
(e) Introducing one or more disulfide bridges in the HA stem domain polypeptide.

18. An influenza hemagglutinin stem domain polypeptide obtainable by the method according to claim 17.

19. A nucleic acid encoding the polypeptide of any one of claims 1 to 16.

20. An immunogenic composition comprising the polypeptide of any one of claims 1 to 16, and/or a nucleic acid molecule according to claim 19.

21. A polypeptide according to any of the claims 1-16, a nucleic acid according to claim 19, and/or an immunogenic composition according to claim 20 for use as a medicament, in particular for use as a vaccine.

NEW CLAIMS

1. An influenza hemagglutinin stem domain polypeptide comprising (a) an H1 HA1 N-terminal segment comprising the amino acids 1-x, wherein x is any amino acid between the amino acid on position 47 and the amino acid on position 60, covalently linked by a linking sequence of 0-50 amino acid residues to an H1 HA1 C- terminal stem segment comprising the amino acids y-end of HA1, wherein y is any amino acid between the amino acids on position 290 and the amino acid on position 325 of H1 HA1, and (b) an influenza hemagglutinin HA2 domain, wherein the C- terminal amino acid residue of the HA1 C-terminal stem segment is any amino acid other than arginine (R) or lysine (K) and wherein one or more amino acids in the amino acid sequence connecting the C-terminal residue of the A helix to the N-terminal residue of the helix CD of HA2 have been mutated as compared to a wild-type influenza HA2 and wherein the amino acid sequence connecting the C-terminal residue of the A helix to the N-terminal residue of the helix CD comprises the amino acid sequence between residues 402-418 of influenza HA2, comprising the amino acid sequence MNTQFTAVGKEFN(H/K)LE(K/R) (SEQ ID NO: 17, and wherein one or more of the amino acids on position 406, 409, 413 and 416 have been changed into an amino acid selected from the group consisting of S,T,N,Q,R,H,K,D,E and G, and wherein the polypeptides comprise a disulphide bridge between the amino acids of position 324 and 436; wherein the numbering is based on the numbering of amino acids of the H1N1 influenza strain A/Brisbane/59/2007 (SEQ ID NO: 1).

2. An influenza hemagglutinin stem domain polypeptide according to claim 1, wherein the polypeptide comprises hemagglutinin stem domains from or based on HA of the influenza virus A/Brisbane/59/2007 (SEQ ID NO: 1) or A/California/04/2009 (SEQ ID NO: 159).

3. An influenza hemagglutinin stem domain polypeptide comprising (a) an H3 HA1 N-terminal segment comprising the amino acids 1-x, wherein x is any amino acid between the amino acid on position 56 and the amino acid on position 69, covalently linked by a linking sequence of 0-50 amino acid residues to an H3 HA1 C- terminal stem segment comprising the amino acids y-end of HA1, wherein y is any amino acid between the amino acids on position 292 and the amino acid on position 325 of H3 HA1, and (b) an influenza hemagglutinin HA2 domain, wherein the

AMENDED SHEET
Duration: 13.03.2014 15:47:58 - 13.03.2014 15:50:11. This page 11 of 14.2014 15:49:44
Received at the EPO on Mar 13, 2014 15:50:11. Page 11 of 14

C- terminal amino acid residue of the HA1 C-terminal stem segment is any amino acid other than arginine (R) or lysine (K) and wherein one or more amino acids in the amino acid sequence connecting the C-terminal residue of the A helix to the N-terminal residue of the helix CD of HA2 have been mutated as compared to a wild-type influenza HA2 domain and wherein the amino acid sequence connecting the C-terminal residue of the A helix to the N-terminal residue of the helix CD comprises the amino acid sequence between residues 400-420 of influenza HA2, comprising the amino acid sequence I(E/G)KTNEKFHQIEKEFSEVEGR (SEQ ID NO: 104) and wherein one or more of the amino acids on position 401, 408, 411, 415 and 418 have been changed into an amino acid selected from the group consisting of S,T,N,Q,R,H,K,D,E and G, and wherein the polypeptides comprise a disulphide bridge between the amino acids of position 326 and 438; wherein the numbering is based on the numbering of amino acids of the H3N2 influenza strain A/Wisconsin/67/2005 (SEQ ID NO: 89).

4. An influenza hemagglutinin stem domain polypeptide according to claim 3, wherein the polypeptide comprises HA domains of A/Wisconsin/67/2005 (SEQ ID NO: 89) or A/Hong Kong/1/1968 (SEQ ID NO: 121)

5. An influenza hemagglutinin stem domain polypeptide according to any one of the claims 1-4, wherein the C- terminal amino acid residue of the HA1 C-terminal stem segment is glutamine (Q), serine (S), threonine (T), asparagine (N), aspartic acid (D) or glutamic acid (E)

6. A polypeptide according to any one of the preceding claims, wherein the polypeptide does not comprise a full-length HA1 domain.

7. A polypeptide according to any one of the preceding claims, wherein the polypeptide is glycosylated.

8. A polypeptide according to any one of the preceding claims, wherein the polypeptide comprises an influenza hemagglutinin HA1 and/or HA2 domain based on influenza hemagglutinin from an influenza A virus comprising HA of the H1 subtype selected from the group consisting of influenza A viruses of Table 7.

AMENDED SHEET 2014 15:49:55
Duration: 13.03.2014 15:47:58 - 13.03.2014 15:50:11. This page 12 of
Received at the EPO on Mar 13, 2014 15:50:11. Page 12 of 14

9. A polypeptide according to any of the preceding claims, wherein the polypeptide comprises one or more further mutations in the HA1 domain and/or the HA2 domain.

10. A polypeptide according to any one of the preceding claims, wherein the polypeptide does not comprise the signal sequence.

11. A polypeptide according to claim 10, comprising an HA1 N-terminal segment comprising the amino acids p-x of HA1, covalently linked by a linking sequence of 0-50 amino acid residues to an HA1 C- terminal stem segment comprising the amino acids y-end of HA1, and (b) an influenza hemagglutinin HA2 domain, wherein p=the first amino acid of the mature HA.

12. A polypeptide according to any one of the preceding claims, wherein the polypeptide does not comprise the intracellular and transmembrane sequence of HA.

13. A polypeptide according to claim [[9]] 12, wherein the amino acid sequence from position 523, 524, 525, 526, 527, 528, 529 or 530 of the HA2 domain to the C-terminus has been removed.

14. A polypeptide according to any one of claim 1, 2 or claim 5-13, wherein the polypeptide selectively binds to the antibodies CR6261 and/or CR9114 and does not bind to the antibody CR8057.

15. A polypeptide according to any one of claim 4-13, wherein the polypeptide selectively binds to the antibodies CR8020, CR843 and/or CR9114.

16. A method for providing an influenza hemagglutinin stem domain polypeptide comprising the steps of:
(a) Providing an influenza H1 or H3 HA0 amino acid sequence;
(b) Removing the cleavage site between HA1 and HA2;
(c) Removing the amino acid sequence of the globular head domain from the HA0 sequence;

Duration: 13.03.2014 15:47:58 - 13.03.2014 15:50:11. This page 13 of
Received at the EPO on Mar 13, 2014 15:50:11. Page 13 of 14

(d) Introducing one or more mutations in the amino acid sequence connecting the C-terminal residue of helix A to the N-terminal residue of helix CD; and
(e) Introducing one or more a disulfide bridges in the HA stem domain.

17. An influenza hemagglutinin stem domain polypeptide obtainable by the method according to claim 18.

18. A nucleic acid encoding the polypeptide of any one of claims 1 to 15 or claim 17.

19. An immunogenic composition comprising the polypeptide of any one of claims 1 to 15 or claim 17, and/or a nucleic acid molecule according to claim 18.

20. A polypeptide according to any of the claims 1-15 or claim 17, a nucleic acid according to claim 18, and/or an immunogenic composition according to claim 19 for use as a medicament, in particular for use as a vaccine.

Duration: 13.03.2014 15:47:58 - 13.03.2014 15:50:11. This page 14 of
Received at the EPO on Mar 13, 2014 15:50:11. Page 14 of 14

CLAIMS

1. An influenza hemagglutinin stem domain polypeptide comprising (a) an H1 HA1 N-terminal segment comprising the amino acids 1-52, covalently linked by a linking sequence of 0-10 amino acid residues to an H1 HA1 C- terminal stem segment comprising the amino acids 321-end of HA1, and (b) an influenza hemagglutinin HA2 domain, wherein the C- terminal amino acid residue of the HA1 C-terminal stem segment is any amino acid other than arginine (R) or lysine (K), and wherein one or more of the amino acids on position 406, 409, 413 and 416 have been changed into an amino acid selected from the group consisting of S,T,N,Q,R,H,K,D,E and G, and wherein the polypeptides comprise a disulphide bridge between the amino acids on position 324 and 436;
wherein the numbering is based on the numbering of amino acids of the H1N1 influenza strain A/Brisbane/59/2007 (SEQ ID NO: 1).

2. An influenza hemagglutinin stem domain polypeptide according to claim 1, wherein the polypeptide comprises hemagglutinin stem domains from or based on HA of the influenza virus A/Brisbane/59/2007 (SEQ ID NO: 1) or A/California/04/2009 (SEQ ID NO: 159).

3. An influenza hemagglutinin stem domain polypeptide comprising (a) an H3 HA1 N-terminal segment comprising the amino acids 1-61, covalently linked by a linking sequence of 0-10 amino acid residues to an H3 HA1 C- terminal stem segment comprising the amino acids 323-end of HA1, and (b) an influenza hemagglutinin HA2 domain, wherein the C- terminal amino acid residue of the HA1 C-terminal stem segment is any amino acid other than arginine (R) or lysine (K) and wherein one or more of the amino acids on position 401, 408, 411, 415 and 418 have been changed into an amino acid selected from the group consisting of S,T,N,Q,R,H,K,D,E and G, and wherein the polypeptides comprise a disulphide bridge between the amino acids of position 326 and 438; wherein the numbering is based on the numbering of amino acids of the H3N2 influenza strain A/Wisconsin/67/2005 (SEQ ID NO: 89).

4. An influenza hemagglutinin stem domain polypeptide according to claim 3, wherein the polypeptide comprises HA domains of A/Wisconsin/67/2005 (SEQ ID NO: 89) or A/Hong Kong/1/1968 (SEQ ID NO: 121).

5. An influenza hemagglutinin stem domain polypeptide according to any one of the claims 1-4, wherein the C- terminal amino acid residue of the HA1 C-terminal stem segment is glutamine (Q), serine (S), threonine (T), asparagine (N), aspartic acid (D) or glutamic acid (E)

6. A polypeptide according to any one of the preceding claims, wherein the polypeptide does not comprise a full-length HA1 domain.

7. A polypeptide according to any one of the preceding claims, wherein the polypeptide is glycosylated.

8. A polypeptide according to any one of the preceding claims, wherein the polypeptide comprises an influenza hemagglutinin HA1 and/or HA2 domain based on influenza hemagglutinin from an influenza A virus comprising HA of the H1 subtype selected from the group consisting of influenza A viruses of Table 7.

9. A polypeptide according to any of the preceding claims, wherein the polypeptide comprises one or more further mutations in the HA1 domain and/or the HA2 domain.

10. A polypeptide according to any one of the preceding claims, wherein the polypeptide does not comprise the signal sequence.

11. A polypeptide according to any one of the preceding claims, wherein the polypeptide does not comprise the intracellular and transmembrane sequence of HA.

12. A polypeptide according to claim 11, wherein the amino acid sequence from position 523, 524, 525, 526, 527, 528, 529 or 530 of the HA2 domain to the C-terminus has been removed.

13. A polypeptide according to any one of claim 1, 2 or claim 5-12, wherein the polypeptide selectively binds to the antibodies CR6261 and/or CR9114 comprising a heavy chain CDR1 region of SEQ ID NO: 25, a heavy chain CDR2 region of SEQ ID NO: 26, a heavy chain CDR3 region of SEQ ID NO: 27, and a light chain CDR1 region of SEQ ID NO: 28, a light chain CDR2 region of SEQ ID NO: 29 and a light chain CDR3 region of SEQ ID NO: 30, and does not bind to the antibody CR8057.

14. A polypeptide according to any one of claim 4-12, wherein the polypeptide selectively binds to the antibodies CR8020, CR843 and/or CR9114 comprising a heavy chain CDR1 region of SEQ ID NO: 25, a heavy chain CDR2 region of SEQ ID NO: 26, a heavy chain CDR3 region of SEQ ID NO: 27, and a light chain CDR1 region of SEQ ID NO: 28, a light chain CDR2 region of SEQ ID NO: 29 and a light chain CDR3 region of SEQ ID NO: 30 and does not bind to the antibody CR8057.

15. A method for providing an influenza H1 hemagglutinin stem domain polypeptide comprising the steps of:
(a) Providing an influenza H1 HA0 amino acid sequence;
(b) Removing the cleavage site between HA1 and HA2 by mutating the C-terminal amino acid of HA1 into an amino acid other that arginine (R) or lysine (K);
(c) Removing the amino acid sequence comprising the amino acid residues from position 53 to and including the amino acid residue on position 320 of the globular head domain from the HA0 sequence and joining the remaining parts of the sequence directly, or by introducing a linker sequences of 1 to 10 amino acids in length;
(d) Introducing one or more mutations in the amino acid sequence connecting the C-terminal residue of helix A to the N-terminal residue of helix CD; and
(e) Introducing at least a disulfide bridges in the HA stem domain between amino acids of (or the equivalent of) position 324 and 436 (numbering according to SEQ ID NO: 1).

16. A method for providing an influenza H3 hemagglutinin stem domain polypeptide comprising the steps of:
(a) Providing an influenza H3 HA0 amino acid sequence;

(b) Removing the cleavage site between HA1 and HA2 by mutating the C-terminal amino acid of HA1 into an amino acid other that arginine (R) or lysine (K);
(c) Removing the amino acid sequence comprising the amino acid residues from position 62 to and including the amino acid residue on position 322 of the globular head domain from the HA0 sequence and joining the remaining parts of the sequence directly, or by introducing a linker sequences of 1 to 10 amino acids in length;
(d) Introducing one or more mutations in the amino acid sequence connecting the C-terminal residue of helix A to the N-terminal residue of helix CD; and
(e) Introducing at least a disulfide bridges in the HA stem domain between amino acids of (or the equivalent of) position 326 and 438 (numbering according to SEQ ID NO: 89).

17. An influenza hemagglutinin stem domain polypeptide obtainable by the method according to claim 15 or 16.

18. A nucleic acid encoding the polypeptide of any one of claims 1 to 14 or claim 17.

19. An immunogenic composition comprising the polypeptide of any one of claims 1 to 14 or claim 17, and/or a nucleic acid molecule according to claim 18.

20. A polypeptide according to any of the claims 1-14 or claim 17, a nucleic acid according to claim 18, and/or an immunogenic composition according to claim 19 for use as a medicament, in particular for use as a vaccine.