Claims

1. A recombinant pre-fusion respiratory syncitial virus (RSV) Fusion (F) polypeptide, comprising at least one epitope that is specific to the pre-fusion conformation F protein, wherein the at least one epitope is recognized by a pre-fusion specific monoclonal antibody, comprising a heavy chain CDR1 region of SEQ ID NO: 54, a heavy chain CDR2 region of SEQ ID NO: 55, a heavy chain CDR3 region of SEQ ID NO: 56 and a light chain CDR1 region of SEQ ID NO: 62, a light chain CDR2 region of SEQ ID NO: 63, and a light chain CDR3 region of SEQ ID NO: 64 and/or a pre-fusion specific monoclonal antibody, comprising a heavy chain CDR1 region of SEQ ID NO: 58, a heavy chain CDR2 region of SEQ ID NO: 59, a heavy chain CDR3 region of SEQ ID NO: 60 and a light chain CDR1 region of SEQ ID NO: 66, a light chain CDR2 region of SEQ ID NO: 67, and a light chain CDR3 region of SEQ ID NO: 68.

2. Pre-fusion RSV F polypeptide according to claim 1, wherein the polypeptide is trimeric.

3. Pre-fusion RSV F polypeptide according to claim 1 or 2, wherein the polypeptide comprises a mutation of the amino acid residue on position 67 and/or a mutation of the amino acid residue on position 215.

4. Pre-fusion RSV F polypeptide according to claim 1, 2 or 3, wherein the polypeptide comprises a F1 domain and a F2 domain, and a linking sequence comprising from 1 to 10 amino acid residues, linking said F1 domain to said F2 domain.

5. Pre-fusion RSV F polypeptide according to any of the preceding claims 1-4, comprising a truncated F1 domain and a F2 domain, and a linking sequence comprising 1 to 10 amino acid residues, linking said F1 to said F2 domain, wherein polypeptide comprises at least one stabilizing mutation in the F1 and/or F2 domain as compared to the RSV F1 and/or F2 domain in the wild-type RSV F protein, wherein the at least one stabilizing mutation is selected from the group consisting of:
(a) a mutation of amino acid residue N/T on position 67.
(b) a mutation of amino acid residue S on position 215.

6. Pre-fusion RSV F polypeptide according to any of the preceding claims 1-5, wherein the at least one stabilizing mutation is selected from the group consisting of:
(a) a mutation of amino acid residue N/T on position 67 into I; and
(b) a mutation of amino acid residue S on position 215 into P.

7. Pre-fusion RSV F polypeptide according to claim 5 or 6, wherein the polypeptide comprises a heterologous trimerization domain linked to said truncated F1 domain.

8. Pre-fusion RSV F polypeptide according to any one of the claims 1-7, wherein the polypeptide comprises at least one further mutation, wherein said mutation is selected from the group consisting of:
(a) a mutation of the amino acid residue on position 46;
(b) a mutation of the amino acid residue on position 77;
(c) a mutation of the amino acid residue on position 80;
(d) a mutation of the amino acid residue on position 92;
(e) a mutation of the amino acid residue on position 175;

(f) a mutation of the amino acid residue on position 184;

(g) a mutation of the amino acid residue on position 185;

(h) a mutation of the amino acid residue on position 201;

(i) a mutation of the amino acid residue on position 209;

(j) a mutation of the amino acid residue on position 421;

(k) a mutation of the amino acid residue on position 426;

(l) a mutation of the amino acid residue on position 465;

(m)a mutation of the amino acid residue on position 486;

(n) a mutation of the amino acid residue on position 487; and

(o) a mutation of the amino acid residue on position 508.

9. Pre-fusion RSV F polypeptide according to claim 8, wherein the at least one further mutation is selected from the group consisting of:

(a) a mutation of the amino acid residue S on position 46 into G;

(b) a mutation of the amino acid residue K on position 77 into E;

(c) a mutation of the amino acid residue K on position 80 into E;

(d) a mutation of the amino acid residue E on position 92 into D;

(e) a mutation of the amino acid residue N on position 175 into P;

(f) a mutation of the amino acid residue G on position 184 into N;

(g) a mutation of the amino acid residue V on position 185 into N;

(h) a mutation of the amino acid residue K on position 201 into Q;

(i) a mutation of the amino acid residue K on position 209 into Q;

(j) a mutation of the amino acid residue K on position 421 into N;

(k) a mutation of the amino acid residue N on position 426 into S;

(l) a mutation of the amino acid residue K on position 465 into E or Q;

(m) a mutation of the amino acid residue D on position 486 into N;

(n) a mutation of the amino acid residue E on position 487 into Q, N or I; and

(o) a mutation of the amino acid residue K on position 508 into E.

10. Pre-fusion RSV F polypeptide according to any of the preceding claims 1-9, wherein the polypeptide comprises at least two mutations.

11. Pre-fusion RSV F polypeptide according to claim 10, wherein the at least two mutations comprise a mutation of amino acid residue N/T on position 67 into I; and a mutation of amino acid residue S on position 215 into P.

12. Pre-fusion RSV F polypeptide according to claim 11, wherein the polypeptide comprises at least one further mutation selected from the group consisting of:

(a) a mutation of the amino acid residue S on position 46 into G;

(b) a mutation of the amino acid residue K on position 77 into E;

(c) a mutation of the amino acid residue K on position 80 into E;

(d) a mutation of the amino acid residue E on position 92 into D;

(e) a mutation of the amino acid residue N on position 175 into P;

(f) a mutation of the amino acid residue G on position 184 into N;

(g) a mutation of the amino acid residue V on position 185 into N;

(h) a mutation of the amino acid residue K on position 201 into Q;

(i) a mutation of the amino acid residue K on position 209 into Q;

(j) a mutation of the amino acid residue K on position 421 into N;

(k) a mutation of the amino acid residue N on position 426 into S;

(l) a mutation of the amino acid residue K on position 465 into E or Q;

(m) a mutation of the amino acid residue D on position 486 into N;

(n) a mutation of the amino acid residue E on position 487 into Q, N or I; and

(o) a mutation of the amino acid residue K on position 508 into E.

13. Pre-fusion RSV F polypeptide according to any one of the preceding claims 1-12, wherein the heterologous trimerization domain comprises the amino acid sequence EKKIEAIEKKIEAIEKKIEA (SEQ ID NO: 3)

14. Pre-fusion RSV F polypeptide according to claim 13, wherein the trimerization domain is linked to amino acid residue 495 of the RSV F protein.

15. Pre-fusion RSV F polypeptide according to any one of the claims 1-14, wherein the heterologous trimerization domain comprises the amino acid sequence GYIPEAPRDGQAYVRKDGEWVLLSTFL (SEQ ID NO: 4).

16. Pre-fusion RSV F polypeptide according to claim 15, wherein the trimerization domain is linked to amino acid residue 513 of the RSV F protein.

17. Pre-fusion RSV F polypeptide according to any one of the preceding claims, wherein the linker between the F1 and the F2 domain comprises 5 amino acid residues.

18. Pre-fusion RSV F polypeptide according to claim 17, wherein the linker comprises the amino acid sequence GSGSG (SEQ ID NO: 5).

19. Pre-fusion RSV F polypeptide according to any of the preceding claims, wherein the F1 domain and/or the F2 domain are from an RSV A strain.

20. Pre-fusion RSV F polypeptide according to any of the preceding claims, wherein the F1 domain and/or the F2 domain are from an RSV B strain.

21. Pre-fusion RSV F polypeptide according to any of the preceding claims, wherein the polypeptide is stable for at least 30 minutes at 55° C, preferably at 58° C, more preferably at 60° C.

22. Pre-fusion RSV F polypeptide according to any of the preceding claims, wherein the polypeptide is stable after storage at 4°C for at least 30 days, preferably at least 60 days, preferably at least 6 months, even more preferably at least 1 year.

23. Pre-fusion RSV F polypeptide according to any one of the preceding claims, wherein the polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 21 – SEQ ID NO: 52 and 71-89.

24. Pre-fusion RSV F polypeptide according to any of the preceding claims, wherein the polypeptide does not comprise a HIS-Tag.

25. Nucleic acid molecule encoding a pre-fusion RSV F polypeptide according to any one of the preceding claims 1-24.

26. Nucleic acid molecule according to claim 25, wherein the nucleic acid molecule has been codon-optimized for expression in mammalian cells.

27. Vector comprising a nucleic acid molecule according to claim 25 or claim 26.

28. Composition comprising a pre-fusion RSV F polypeptide according to any of the claims 1-24, a nucleic acid molecule according to claim 25 or claim 26 and/or a vector according to claim 27.

29. Pre-fusion RSV F polypeptide according to any of the claims 1-24, a nucleic acid molecule according to claim 25 or claim 26 and/or a vector according to claim 27 for use in inducing an immune response against RSV F protein.

30. Pre-fusion RSV F polypeptide according to any of the claims 1-24, a nucleic acid molecule according to claim 25 or claim 26 and/or a vector according to claim 27for use as a vaccine.

31. Pre-fusion RSV F polypeptide according to any of the claims 1-24, a nucleic acid molecule according to claim 25 or claim 26 and/or a vector according to claim 27 for use in the prophylaxis and/or treatment of RSV infection.

32. A method for stabilizing the pre-fusion conformation of an RSV F polypeptide, comprising introducing one or more mutations in a RSV F1 and/or F2 domain, as compared to the wild-type RSV F1 and/or F2 domain, wherein the one or more mutations are selected from the group consisting of:

(e) a stabilizing mutation in a region that locks the HRA domain from hinging adjacent to the conserved 69-212 disulfide bridge, said region comprising the amino acid residues 66-68 and 214-216,

(f) a mutation in the helix comprising the amino acid residues 76-98 at the C-terminus of the F2 domain;

(g) a mutation that reduces the negative charge repulsion between the top of the HRB stem region comprising amino acids 486, 487; and

(h) a stabilizing mutation in the HRA region.

33. Method according to claim 32, wherein the mutation in the HRA hinge region is at position 67.

34. Method according to claim 32 or 33, wherein the mutation in the HRA hinge region is at position 215.

35. Method according to claim 32, 33 or 34, wherein the mutation in the HRA hinge region is at position 66 or 68, and/or at position 214 or 216.

36. Method according to claim 32, wherein the mutation in the helix is at position 77.

37. Method according to claim 32, wherein the mutation in the helix is at position 80.

38. Method according to claim 36 or 37, wherein the amino acid residue at position 77 and/or 80 is changed into a negatively charged amino acid.

39. Method according to any one of the claims 32-38, wherein the mutation is at position 92.

40. Method according to claim 32, wherein the mutation that reduces the negative charge repulsion between the top of the HRB stem region comprising amino acids 486, 487, 489.

41. Method according to claim 40, wherein the mutation is at position 489.

42. Method according to claim 40, wherein the mutation is at position 486.

43. Method according to claim 32, wherein the mutation stabilizes the beta-turns between the amino acid residues 175-193.

44. Method according to claim 43, wherein the mutation is stabilizing the turn at position 175.

45. Method according to claim 43, wherein the mutation is stabilizing the turn at position 184 -185.

46. Stabilized pre-fusion RSV F polypeptide, obtainable by a method according to any one of the claims 32-45.

Claims

1. A recombinant pre-fusion respiratory syncitial virus (RSV) Fusion (F) polypeptide, comprising at least one epitope that is specific to the pre-fusion conformation F protein, wherein the at least one epitope is recognized by a pre-fusion specific monoclonal antibody, comprising a heavy chain CDR1 region of SEQ ID NO: 54, a heavy chain CDR2 region of SEQ ID NO: 55, a heavy chain CDR3 region of SEQ ID NO: 56 and a light chain CDR1 region of SEQ ID NO: 62, a light chain CDR2 region of SEQ ID NO: 63, and a light chain CDR3 region of SEQ ID NO: 64 and/or a pre-fusion specific monoclonal antibody, comprising a heavy chain CDR1 region of SEQ ID NO: 58, a heavy chain CDR2 region of SEQ ID NO: 59, a heavy chain CDR3 region of SEQ ID NO: 60 and a light chain CDR1 region of SEQ ID NO: 66, a light chain CDR2 region of SEQ ID NO: 67, and a light chain CDR3 region of SEQ ID NO: 68, wherein the polypeptide comprises a mutation of the amino acid residue on position 67 and/or a mutation of the amino acid residue on position 215 and wherein the amino acid positions are given in reference to the sequence of RSV F protein from the A2 strain (SEQ ID NO: 1).

2. Pre-fusion RSV F polypeptide according to claim 1, wherein the polypeptide is trimeric.

3. Pre-fusion RSV F polypeptide according to claim 1 or 2, wherein the polypeptide comprises a F1 domain and a F2 domain, and a linking sequence comprising from 1 to 10 amino acid residues, linking said F1 domain to said F2 domain.

4. Pre-fusion RSV F polypeptide according to claim 1, 2 or 3, comprising a truncated F1 domain and a F2 domain, and a linking sequence comprising 1 to 10 amino acid residues, linking said F1 to said F2 domain, wherein polypeptide comprises at least one stabilizing mutation in the F1 and/or F2 domain as compared to the RSV F1 and/or F2 domain in the wild-type RSV F protein, wherein the at least one stabilizing mutation is selected from the group consisting of:
   (a) a mutation of amino acid residue N/T on position 67.
   (b) a mutation of amino acid residue S on position 215.

5. Pre-fusion RSV F polypeptide according to claim 4, wherein the at least one stabilizing mutation is selected from the group consisting of:
   (a) a mutation of amino acid residue N/T on position 67 into I; and
   (b) a mutation of amino acid residue S on position 215 into P.

6. Pre-fusion RSV F polypeptide according to claim 4 or 5, wherein the polypeptide comprises a heterologous trimerization domain linked to said truncated F1 domain.

7. Pre-fusion RSV F polypeptide according to any one of the claims 1-6, wherein the polypeptide comprises at least one further mutation, wherein said mutation is selected from the group consisting of:
   (a) a mutation of the amino acid residue S on position 46 into G;
   (b) a mutation of the amino acid residue K on position 77 into E;
   (c) a mutation of the amino acid residue K on position 80 into E;

(d) a mutation of the amino acid residue E on position 92 into D;

(e) a mutation of the amino acid residue N on position 175 into P;

(f) a mutation of the amino acid residue G on position 184 into N;

(g) a mutation of the amino acid residue V on position 185 into N;

(h) a mutation of the amino acid residue K on position 201 into Q;

(i) a mutation of the amino acid residue K on position 209 into Q;

(j) a mutation of the amino acid residue K on position 421 into N;

(k) a mutation of the amino acid residue N on position 426 into S;

(l) a mutation of the amino acid residue K on position 465 into E or Q;

(m) a mutation of the amino acid residue D on position 486 into N;

(n) a mutation of the amino acid residue E on position 487 into Q, N or I; and

(o) a mutation of the amino acid residue K on position 508 into E.

8. Pre-fusion RSV F polypeptide according to any of the preceding claims 1-7, wherein the polypeptide comprises at least two mutations, wherein the at least two mutations comprise a mutation of amino acid residue N/T on position 67 into I; and a mutation of amino acid residue S on position 215 into P.

9. Pre-fusion RSV F polypeptide according to claim 8, wherein the polypeptide comprises at least one further mutation selected from the group consisting of:

(a) a mutation of the amino acid residue S on position 46 into G;

(b) a mutation of the amino acid residue K on position 77 into E;

(c) a mutation of the amino acid residue K on position 80 into E;

(d) a mutation of the amino acid residue E on position 92 into D;

(e) a mutation of the amino acid residue N on position 175 into P;

(f) a mutation of the amino acid residue G on position 184 into N;

(g) a mutation of the amino acid residue V on position 185 into N;

(h) a mutation of the amino acid residue K on position 201 into Q;

(i) a mutation of the amino acid residue K on position 209 into Q;

(j) a mutation of the amino acid residue K on position 421 into N;

(k) a mutation of the amino acid residue N on position 426 into S;

(l) a mutation of the amino acid residue K on position 465 into E or Q;

(m) a mutation of the amino acid residue D on position 486 into N;

(n) a mutation of the amino acid residue E on position 487 into Q, N or I; and

(o) a mutation of the amino acid residue K on position 508 into E.

10. Pre-fusion RSV F polypeptide according to any one of the preceding claims 6-9, wherein the heterologous trimerization domain comprises the amino acid sequence EKKIEAIEKKIEAIEKKIEA (SEQ ID NO: 3)

11. Pre-fusion RSV F polypeptide according to claim 10, wherein the trimerization domain is linked to amino acid residue 495 of the RSV F protein.

12. Pre-fusion RSV F polypeptide according to any one of the claims 6-9, wherein the heterologous trimerization domain comprises the amino acid sequence GYIPEAPRDGQAYVRKDGEWVLLSTFL (SEQ ID NO: 4).

13. Pre-fusion RSV F polypeptide according to claim 12, wherein the trimerization domain is linked to amino acid residue 513 of the RSV F protein.

14. Pre-fusion RSV F polypeptide according to any one of the preceding claims, wherein the linker between the F1 and the F2 domain comprises 5 amino acid residues.

15. Pre-fusion RSV F polypeptide according to claim 14, wherein the linker comprises the amino acid sequence GSGSG (SEQ ID NO: 5).

16. Pre-fusion RSV F polypeptide according to any of the preceding claims, wherein the F1 domain and/or the F2 domain are from an RSV A strain.

17. Pre-fusion RSV F polypeptide according to any of the preceding claims, wherein the F1 domain and/or the F2 domain are from an RSV B strain.

18. Pre-fusion RSV F polypeptide according to any of the preceding claims, wherein the polypeptide is stable for at least 30 minutes at 55° C, preferably at 58° C, more preferably at 60° C.

19. Pre-fusion RSV F polypeptide according to any of the preceding claims, wherein the polypeptide is stable after storage at 4°C for at least 30 days, preferably at least 60 days, preferably at least 6 months, even more preferably at least 1 year.

20. Pre-fusion RSV F polypeptide according to any one of the preceding claims, wherein the polypeptide comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 21 – SEQ ID NO: 52 and 71-89.

21. Pre-fusion RSV F polypeptide according to any of the preceding claims, wherein the polypeptide does not comprise a HIS-Tag.

22. Nucleic acid molecule encoding a pre-fusion RSV F polypeptide according to any one of the preceding claims 1-21.

23. Nucleic acid molecule according to claim 22, wherein the nucleic acid molecule has been codon-optimized for expression in mammalian cells.

24. Vector comprising a nucleic acid molecule according to claim 22 or claim 23.

25. Composition comprising a pre-fusion RSV F polypeptide according to any of the claims 1-21, a nucleic acid molecule according to claim 22 or claim 23 and/or a vector according to claim 24.

26. Pre-fusion RSV F polypeptide according to any of the claims 1-21, a nucleic acid molecule according to claim 22 or claim 23 and/or a vector according to claim 24 for use in inducing an immune response against RSV F protein.

27. Pre-fusion RSV F polypeptide according to any of the claims 1-21, a nucleic acid molecule according to claim 22 or claim 23 and/or a vector according to claim 24 for use as a vaccine.

28. Pre-fusion RSV F polypeptide according to any of the claims 1-21, a nucleic acid molecule according to claim 22 or claim 23 and/or a vector according to claim 24 for use in the prophylaxis and/or treatment of RSV infection.