

CLAIMS

1. Use of an agent that induces a protective immune response against SARS-CoV-2 in the manufacture of a medicament for~~A method of inducing and/or activating a protective immune response against Severe Acute Respiratory Syndrome beta-coronavirus 2 (SARS-CoV-2) in a subject, wherein the subject is to be administered an effective amount of the agent comprising administering to the upper respiratory tract of the subject an effective amount of an agent that induces a protective immune response against SARS-CoV-2.~~

2. The ~~method~~use of claim 1, wherein the agent that induces a protective immune response against SARS-CoV-2 is a pseudotyped lentiviral vector particle encoding a Severe Acute Respiratory Syndrome beta-coronavirus 2 (SARS-CoV-2) spike (S) protein or a derivative or fragment thereof.

3. The ~~use method~~ of claim 1 or 2, wherein the agent is to be administered by aerosol inhalation.

4. The ~~use method~~ of claim 2, wherein the agent is to be administered by nasal instillation.

5. The ~~use method~~ of claim 2, wherein the agent is to be administered by nasal insufflation.

6. The ~~use method~~ of any one of claims 1 to 5, wherein the treatment course consists of a single administration to the upper respiratory tract or wherein the treatment course comprises more than one administration, in particular two administrations, to the upper respiratory tract.

7. The ~~use method~~ of any one of claims 1 to 5, wherein the treatment course comprises at least one priming administration outside of the respiratory tract, such as

intramuscular, intradermal or subcutaneous routes, followed by at least one boosting administration to the upper respiratory tract.

8. The use method of any one of claims 1 to 7, wherein the protective immune response comprises production of SARS-CoV-2 neutralizing antibodies in the subject.

9. The use method of claim 8, wherein the neutralizing antibodies comprise IgG antibodies.

10. The use method of any one of claims 1 to 9, wherein the protective immune response comprises production of SARS-CoV-2 S-specific T cells in the subject.

11. The use method of claim 10, wherein the SARS-CoV-2 S-specific T cells comprise CD4⁺ T cells, CD8⁺ T cells, or both CD4⁺ and CD8⁺ T cells.

12. The use method of claim 10 or 11, wherein the SARS-CoV-2 S-specific T cells comprise lung CD8⁺ T cells.

13. The use method of any one of claims 10 to 12, wherein the SARS-CoV-2 S-specific T cells comprise IFN- γ -producing T-cells.

14. The use method of any one of claims 10 to 13, wherein the CD8⁺ T cells comprise T cells with an effector memory (T_{em}) and/or resident memory (T_{rm}) phenotype.

15. The use method of any one of claims 10 to 14, wherein the SARS-CoV-2 S-specific T cells are recruited to the olfactory bulb.

16. The use method of any one of claims 1 to 15 wherein the protective immune response provides a reduced likelihood of developing SARS-CoV-2 infection-related inflammation in the subject.

17. The ~~use method~~ of any one of claims 2 to 16, wherein the SARS-CoV-2 S protein has an amino acid sequence identical to SEQ ID NO: 1 and the SARS-CoV-2 S protein derivative has an amino acid sequence at least 95% identical to SEQ ID NO: 1.

18. The ~~use method~~ of any one of claims 2 to 17, wherein the SARS-CoV-2 S protein is expressed from a coding sequence having a nucleotide sequence identical to SEQ ID NO: 2 and the SARS-CoV-2 S protein derivative is expressed from a coding sequence having a nucleotide sequence at least 80% identical to SEQ ID NO: 2.

19. The ~~use method~~ of any one of claims 2 to 18, wherein the SARS-CoV-2 S protein derivative or fragment thereof comprises a peptide selected from peptide 61-75 (NVTWFHAIHVSGTNG (SEQ ID NO: 15)), peptide 536-550 (NKCVNFNFNGLTGTG (SEQ ID NO: 16)) and peptide 576-590 (VRDPQTLEILDITPC (SEQ ID NO: 17)).

20. The ~~use method~~ of any one of claims 2 to 18, wherein the SARS-CoV-2 S derivative or fragment thereof comprises an amino acid modification relative to SEQ ID NO: 1, the modification selected from:

- (i) 986^{K→P} and 987^{V→P},
- (ii) 681^{PRRRAR}686 (SEQ ID NO: 22) → 681^{PGSAGS}686 (SEQ ID NO: 23), and
- (iii) 986^{K→P}, 987^{V→P}, and 675^{QTQTNSPRRAR}685 (SEQ ID NO: 24) deletion.

21. The ~~use method~~ of any one of claims 2 to 20, wherein the Severe Acute Respiratory Syndrome beta-coronavirus 2 (SARS-CoV-2) spike (S) protein or a derivative or fragment thereof comprises or consists of an amino acid sequence selected from SEQ ID NOS: 1, 5, 8, 11, 14, 108, 111, 114, 117, and 120.

22. The ~~use method~~ of any one of claims 2 to 21, wherein the administered lentiviral vector particle is integrative.

23. The ~~use method~~ of any one of claims 2 to 21, wherein the administered lentiviral vector particle is nonintegrative.

24. The ~~use method~~ of claim 23, wherein the administered nonintegrative lentiviral particle comprises a D64V mutation in an integrase coding sequence.

25. The ~~use method~~ of any one of claims 2 to 24, wherein the administered lentiviral vector particle is pseudotyped with Vesicular Stomatitis Virus envelop Glycoprotein (VSV-G).

26. The ~~use method~~ of any one of claims 2 to 25, wherein lentiviral vector particle is to be administered as a vaccine formulation comprising the lentiviral vector particle and a pharmaceutically acceptable carrier.

27. A dosage form for administration to the upper respiratory tract of a subject of a pseudotyped lentiviral vector particle encoding a Severe Acute Respiratory Syndrome beta-coronavirus 2 (SARS-CoV-2) spike (S) protein or a derivative or fragment thereof.

28. The dosage form of claim 27, wherein the dosage form is ~~for administration~~ to be administered by aerosol inhalation.

29. The dosage form of claim 27, wherein the dosage form is to be administered ~~for administration~~ by nasal instillation.

30. The dosage form of claim 27, wherein the dosage form is to be administered ~~for administration~~ by nasal insufflation.

31. The dosage form of any one of claims 27 to 30, wherein the SARS-CoV-2 S protein has an amino acid sequence identical to SEQ ID NO: 1 and the SARS-CoV-2 S protein derivative has an amino acid sequence at least 95% identical to SEQ ID NO: 1.

32. The dosage form of any one of claims 27 to 30, wherein the SARS-CoV-2 S protein is expressed from a coding sequence having a nucleotide sequence identical to SEQ ID NO: 2 and the SARS-CoV-2 S protein derivative is expressed from a coding sequence having a nucleotide sequence at least 80% identical to SEQ ID NO: 2.

33. The dosage form of any one of claims 27 to 32, wherein the SARS-CoV-2 S protein derivative or fragment thereof comprises a peptide selected from peptide 61-75 (NVTWFHAIHVSGTNG (SEQ ID NO: 15)), peptide 536-550 (NKCVNFNFNGLTGTG (SEQ ID NO: 16)) and peptide 576-590 (VRDPQTLEILDITPC (SEQ ID NO: 17)).

34. The dosage form of any one of claims 27 to 33, wherein the SARS-CoV-2 S derivative or fragment thereof comprises an amino acid modification relative to SEQ ID NO: 1, the modification selected from:

- (i) 986^{K→P} and 987^{V→P},
- (ii) 681^{PRRARS}686 (SEQ ID NO: 22) → 681^{PGSAGS}686 (SEQ ID NO: 23), and
- (iii) 986^{K→P}, 987^{V→P}, and 675^{QTQTNSPRRAR}685 (SEQ ID NO: 24) deletion.

35. The dosage form of any one of claims 27 to 34, wherein the Severe Acute Respiratory Syndrome beta-coronavirus 2 (SARS-CoV-2) spike (S) protein or a derivative or fragment thereof comprises or consists of an amino acid sequence selected from SEQ ID NOS: 1, 5, 8, 11, 14, 108, 111, 114, 117, and 120

36. The dosage form of any one of claims 27 to 35, wherein the administered lentiviral vector particle is integrative.

37. The dosage form of any one of claims 27 to 35, wherein the administered lentiviral vector particle is nonintegrative.

38. The dosage form of claim 37, wherein the nonintegrative lentiviral particle comprises a D64V mutation in an integrase coding sequence.

39. The dosage form of any one of claims 27 to 38, wherein the lentiviral vector particle is pseudotyped with Vesicular Stomatitis Virus envelop Glycoprotein (VSV-G).

40. A kit comprising the dosage form of the pseudotyped lentiviral vector particle encoding a SARS-CoV-2 S protein or a derivative or fragment thereof according to any one of claims 27 to 39 and an applicator for administration to the upper respiratory tract.

41. The kit of claim 40, wherein the applicator for administration to the upper respiratory tract is an applicator for aerosol inhalation.

42. The kit of claim 40, wherein the applicator for administration to the upper respiratory tract is an applicator for nasal instillation.

43. The kit of claim 40, wherein the applicator for administration to the upper respiratory tract is an applicator for nasal insufflation.

44. A vector selected from:
pFlap-ieCMV-S2PdeltaF-WPREm (CNCM I-5537),
pFlap-ieCMV-S2P3F-WPREm (CNCM I-5538),
pFlap-ieCMV-S2P-WPREm (CNCM I-5539),
pFlap-ieCMV-SFL-WPREm (CNCM I-5540),
pFlap-ieCMV-S-B1.1.7-WPREm (CNCM I-5708),
pFlap-ieCMV-S-B351-WPREm (CNCM I-5709),
pFlap-ieCMV-S-B351-2P-WPREm (CNCM I-5710),
pFlap-ieCMV-SFL-D614G-WPREm (CNCM I-5711), and
pFlap-ieCMV-S-P1-WPREm (CNCM I-5712).

45. A host cell comprising a vector of claim 38.

46. A pseudotyped lentiviral vector particle encoding a Severe Acute Respiratory Syndrome beta-coronavirus 2 (SARS-CoV-2) spike (S) protein or a derivative or fragment thereof.

47. A pseudotyped lentiviral vector particle according to claim 46 wherein the encoded SARS-CoV-2 spike protein derivative or fragment thereof is as defined in any one of claims 31, 32, 33, 34 or 35.

48. A pseudotyped lentiviral vector particle according to claim 46 or 47 wherein the SARS-CoV-2 spike protein is selected from the SARS-CoV-2 spike protein that has the amino acid sequence of SEQ ID No. 1; the SARS-CoV-2 S protein derivative that has an amino acid sequence at least 95% identical or at least 99% identical to SEQ ID NO:1; the SARS-CoV-2 spike protein derivative that has the amino acid sequence of SEQ ID NO: 8, SEQ ID No. 11, SEQ ID No. 108, SEQ ID No. 111, SEQ ID No. 114, SEQ ID No. 117, or SEQ ID No. 120; the SARS-CoV-2 S protein derivative that has an amino acid sequence at least 95% identical or at least 99% identical to SEQ ID NO: 8, SEQ ID No. 11, SEQ ID No. 108, SEQ ID No. 111, SEQ ID No. 114, SEQ ID No. 117, or SEQ ID No. 120; and the SARS-CoV-2 spike protein fragment that has the amino acid sequence of SEQ ID No. 14 or the SARS-CoV-2 S protein derivative that has an amino acid sequence at least 95% identical or at least 99% identical to SEQ ID NO: 14 .

49. A pseudotyped lentiviral vector particle according to any one of claims 46 to 48 wherein the pseudotyped lentiviral vector particle is as defined in any one of claims 36, 37, 38, or 39.

50. A pseudotyped lentiviral vector particle encoding a Severe Acute Respiratory Syndrome beta-coronavirus 2 (SARS-CoV-2) spike (S) protein or a derivative or fragment thereof, wherein the pseudotyped lentiviral vector particle is made by a method comprising co-transfection of a host cell with a vector selected from:

pFlap-ieCMV-S2PdeltaF-WPREm (CNCM I-5537),

pFlap-ieCMV-S2P3F-WPREm (CNCM I-5538),

pFlap-ieCMV-S2P-WPREm (CNCM I-5539),
pFlap-ieCMV-SFL-WPREm (CNCM I-5540),
pFlap-ieCMV-S-B1.1.7-WPREm (CNCM I-5708),
pFlap-ieCMV-S-B351-WPREm (CNCM I-5709),
pFlap-ieCMV-S-B351-2P-WPREm (CNCM I-5710),
pFlap-ieCMV-SFL-D614G-WPREm (CNCM I-5711), and
pFlap-ieCMV-S-P1-WPREm (CNCM I-5712).

51. A pseudotyped lentiviral vector particle according to any one of claims 46 to 49, wherein the genome of the vector particle comprises a polynucleotide selected from:

a polynucleotide encoding S2P Δ F (S2PdeltaF) of SEQ ID No. 13 or a coding sequence having a nucleotide sequence at least 80% identical to SEQ ID No.13, in particular a coding sequence having a mutation, in particular a deletion, in the RBD,

a polynucleotide encoding S2P3F of SEQ ID No. 10 or a coding sequence having a nucleotide sequence at least 80% identical to SEQ ID No.10 having a mutation in the RBD, in particular wherein the coding sequence having a nucleotide sequence at least 80% identical to SEQ ID No.10 comprises mutations 986^{K→P} and 987^{V→P}.

a polynucleotide encoding S2P of SEQ ID No. 7 or a coding sequence having a nucleotide sequence at least 80% identical to SEQ ID No.7 having a mutation in the RBD,

a polynucleotide encoding SFL of SEQ ID No. 2 or a coding sequence having a nucleotide sequence at least 80% identical to SEQ ID No. 2 having a mutation in the RBD,

a polynucleotide encoding S-B1.1.7 of SEQ ID No. 107 or a coding sequence having a nucleotide sequence at least 80% identical to SEQ ID No. 107 having a mutation in the RBD,

a polynucleotide encoding S-B351 of SEQ ID No. 110 or a coding sequence having a nucleotide sequence at least 80% identical to SEQ ID No. 110 having a mutation in the RBD,

a polynucleotide encoding S-B1.1.7 S-B351-2P of SEQ ID No. 113 or a coding sequence having a nucleotide sequence at least 80% identical to SEQ ID No. 113 having a mutation in the RBD,

a polynucleotide encoding SFL-D614G of SEQ ID No. 116 or a coding sequence having a nucleotide sequence at least 80% identical to SEQ ID No. 116 having a mutation in the RBD, and

a polynucleotide encoding S-P1 of SEQ ID No. 119 or a coding sequence having a nucleotide sequence at least 80% identical to SEQ ID No. 119 having a mutation in the RBD.

52. An immunogenic composition that comprises a dosage form according to any one of claims 27 to 39 or a pseudotyped lentiviral particle according to any one of claims 46 to 51.

53. Use of a An immunogenic composition according to claim 52 ~~for use in the manufacture of a medicament for~~ inducing and/or activating a protective immune response against Severe Acute Respiratory Syndrome beta-coronavirus 2 (SARS-CoV-2) in a subject, wherein said use comprises a prime administration outside of the upper respiratory tract, in particular systemic, especially intramuscular administration and a boost or target administration to the upper respiratory tract.

54. The ~~immunogenic composition according to claim 52 for use~~ according to claim 53, wherein the administered doses or LV particles are identical in the prime and boost/target administration steps, or wherein the administered doses or LV particles are different in the prime and boost/target administration steps, in particular may be higher for the administration to the upper respiratory tract.

55. The ~~immunogenic composition according to claim 52 for use~~ according to claim 53 or 54, wherein the lentiviral vector particles are LV::SFL, in particular NILV::SFL and the administration regimen consists in a systemic, especially i.m. prime and a boost to the upper respiratory tract, in particular by i.n. boost.

56. The ~~immunogenic composition according to claim 52 for use~~ according to claim 53 or 54, wherein the lentiviral vector particles are LV::S_{prefusion}, in particular

NILV::S_{prefusion}, such as LV::S2PΔF (LV::S2deltaF) or NILV::S2PΔF (NILV::S2deltaF), or LV::S2P3F or NILV::S2P3F and the administration regimen consists in a systemic, especially i.m. prime and a boost to the upper respiratory tract, in particular by i.n. boost.

57. Use of Tthe immunogenic composition according to claim 52 ~~for use in the manufacture of a medicament to induce for inducing~~ a protective immune response against SARS-CoV-2 in the upper respiratory tract of a subject and/or in the brain against SARS-CoV-2.

58. Use of tThe immunogenic composition according to claim 52 ~~for use in the manufacture of a medicament to induce for inducing~~ a cross protective immune response of lungs and brain against ancestral including SARS-CoV-2 selected from the group of SARS-CoV-2 Wuhan strain, SARS-CoV-2 D614G strain and SARS-CoV-2 B.1.117 strain and against emerging SARS-CoV-2 variants such as SARS-CoV-2 P.1 variant, by eliciting B and T cell-responses.

59. ~~The immunogenic composition according to claim 52 for use~~ according to claim 53 to 58 wherein the dosage form or the pseudotyped lentiviral particle comprises pseudotyped lentiviral particles according to any one of claims 46 to 51 wherein the pseudotyped lentiviral particles are non-integrative.

60. ~~The immunogenic composition according to claim 52 for use~~ according to claim 53 or 58 to elicit a protective immune response against SARS-CoV-2 wherein the response elicits SARS-CoV-2 S-specific T cells, in particular SARS-CoV-2 S-specific T cells that comprise lung CD8⁺ T cells and/or IFN-γ-producing T-cells.

61. ~~The immunogenic composition according to claim 52 for use~~ according to any one of claims 53 to 60 to elicit a protective immune response against SARS-CoV-2 wherein the response elicits CD8⁺ T cells that comprise T cells with an effector memory (T_{em}) and/or resident memory (T_{rm}) phenotype.

62. The ~~immunogenic composition according to claim 52~~ for use according to any one of claims 53 to 61, the SARS-CoV-2 S-specific T cells are recruited to the olfactory bulb.

63. The ~~immunogenic composition according to claim 52~~ for use according to claim 53 to 62 wherein the Severe Acute Respiratory Syndrome beta-coronavirus 2 (SARS-CoV-2) spike (S) protein or a derivative or fragment thereof comprises or consists of an amino acid sequence selected from SEQ ID NOS: 1, 5, 8, 11, 14, 108, 111, 114, 117, and 120.

64. The ~~immunogenic composition according to claim 52~~ for use according to any one of claims 53 to 63 to prevent or to alleviate SARS-CoV-2 infection-related inflammation in the subject.