CLAIMS

What is claimed is:

1. A SARS-CoV-2 vaccine comprising i) an inactivated SARS-CoV-2 particle; ii) a CpG-containing oligodeoxynucleotide (CpG-ODN); and iii) an alum adjuvant.

2. The SARS-CoV-2 vaccine according to claim 1, wherein said CpG-ODN is CpG 1018 as defined by SEQ ID NO: 4, and said alum adjuvant is aluminium hydroxide.

3. The SARS-Cov-2 vaccine according to any one of the preceding claims, wherein the alum (Al^{3+}):CpG (w/w) ratio in the vaccine composition is about 1:10, about 1:5, about 1:4, about 1:3, about 1:2, about 1:1, about 2:1, about 3:1, about 4:1, about 5:1, about 10:1, preferably between about 1:3 and 3:1, more preferably between about 1:2 and 1:1.

4. The SARS-CoV-2 vaccine according to any one of the preceding claims, wherein the alum:CpG (w/w) ratio in the vaccine composition is about 1:2.

5. The SARS-CoV-2 vaccine according to any one of the preceding claims, wherein the alum content of the vaccine is between about 0.1 and 2 mg/mL, between about 0.2 and 1.5 mg/mL, between about 0.5 and 1.3 mg/mL, especially between about 0.8 to 1.2 mg/mL, most preferably about 1 mg/mL, i.e., 0.5 mg/dose, wherein the vaccine is delivered in a volume of 0.5 mL.

6. The SARS-CoV-2 vaccine according to any one of the preceding claims, wherein the CpG content of the vaccine is between about 0.25 and 6 mg/mL, between about 0.5 and 3 mg/mL, between about 1 and 3 mg/mL, especially between about 1.5 to 2.5 mg/mL, most preferably about 2 mg/mL, i.e., 1 mg/dose, wherein the vaccine is delivered in a volume of 0.5 mL.

7. The SARS-CoV-2 vaccine according to any one of the preceding claims, wherein the amount of inactivated SARS-CoV-2 virus per dose in the vaccine is between about 0.01 and 25 mAU (milli-absorption units x minutes), preferably between about 0.05 and 10 mAU, more preferably between about 0.1 and 5 mAU, most preferably between about 0.25 and 2.5 mAU as assessed by SEC-HPLC, wherein the vaccine is delivered in a volume of 0.5 mL.

8. The SARS-CoV-2 vaccine according to any one of the preceding claims, wherein the amount of free (unbound) CpG in the vaccine composition is greater than 10%, greater than 20%, greater than 30%, greater than 40%, greater than 50%, greater than 60%, greater than 70%, greater than

80%, greater than 90%, greater than 95%, preferably about 70% to 95%, most preferably about 80% to 90% (w/w), based on the total amount of CpG in the vaccine composition.

9. The SARS-CoV-2 vaccine according to any one of the preceding claims, wherein the vaccine composition comprises at least one buffer, preferably a phosphate buffer.

10. The SARS-CoV-2 vaccine according to any one of claims 2 to 9, wherein said vaccine comprising aluminium hydroxide comprises less than 1.25 ppb Cu.

11. The SARS-CoV-2 vaccine according to any one of the preceding claims, wherein viral RNA in the inactivated SARS-CoV-2 particle is replication-deficient.

12. The SARS-CoV-2 vaccine according to any one of the preceding claims, wherein viral RNA in the inactivated SARS-CoV-2 particle (i) is alkylated and/or acylated (ii) comprises one or more modified purine (preferably guanine) residues or strand breaks and/or (iii) is cross-linked with one or more viral proteins.

13. The SARS-CoV-2 vaccine according to any one of the preceding claims, wherein the virus particle is a beta-propiolactone-inactivated SARS-CoV-2 particle, preferably at a concentration of 300 to 700ppm, more preferably 500ppm and inactivated for about 1 to 48h, preferably 20 to 28h, most preferred 24h.

14. The SARS-CoV-2 vaccine according to any one of the preceding claims, wherein the virus particle is an ultraviolet (UV)-inactivated SARS-CoV-2 particle.

15. The SARS-CoV-2 vaccine according to any one of the preceding claims, wherein surface proteins in the inactivated SARS-CoV-2 particle comprise reduced modifications compared to viral RNA in the inactivated SARS-CoV-2 particle, preferably wherein surface proteins comprise a reduced proportion of modified residues compared to viral RNA in the inactivated SARS-CoV-2 particle; said modifications being with respect to a native SARS-CoV-2 particles, preferably wherein said modifications comprise alkylated and/or acylated nucleotide or amino acid residues.

16. The SARS-CoV-2 vaccine according to any one of the preceding claims, wherein the inactivated SARS-CoV-2 particle comprises a native conformation of (i) spike (S) protein; (ii) nucleocapsid (N) protein; (iii) membrane (M) glycoprotein; and/or (iv) envelope (E) protein; preferably wherein the inactivated SARS-CoV-2 particle comprises a native conformation spike (S) protein.

17. A SARS-CoV-2 vaccine according to any preceding claim, wherein the inactivated SARS-CoV-2 particle comprises one or more beta-propiolactone-modified cysteine, methionine and/or histidine residues.

18. A SARS-CoV-2 vaccine according to any preceding claim, wherein an inactivated SARS-CoV-2 particle comprises fewer than 200, 100, 50, 30, 20, 15, 10, 9, 8, 7 or 6 beta-propiolactone-modified amino acid residues; preferably wherein a spike (S) protein of the inactivated SARS-CoV-2 particle comprises fewer than 100, 50, 30, 20, 15, 10, 9, 8, 7 or 6 beta-propiolactone-modified amino acid residues; more preferably wherein the inactivated SARS-CoV-2 particle or spike protein thereof comprises 20 or fewer beta-propiolactone-modified amino acid residues; most preferably wherein the inactivated SARS-CoV-2 particle or spike protein thereof comprises 1 to 100, 2 to 50, 3 to 30, 10 to 20 or about 15 beta-propiolactone-modified amino acid residues.

19. A SARS-CoV-2 vaccine according to any preceding claim, wherein fewer than 20%, 15%, 10%, 5% or 4% of SARS-CoV-2 polypeptides in the particle are beta-propiolactone-modified; preferably wherein 0.1 to 10%, more preferably 1 to 5%, more preferably 2 to 8% or about 3-6% of SARS-CoV-2 polypeptides in the particle, comprise at least one beta-propiolactone modification; preferably as detected in the vaccine by mass spectroscopy, optionally following enzymatic digestion with trypsin, chymotrypsin and/or PNGase F or acid hydrolysis.

20. A SARS-CoV-2 vaccine according to any preceding claim, wherein:

(i) a spike (S) protein of the inactivated SARS-CoV-2 particle comprises a beta-propiolactone modification at one or more of the following residues: 49, 146, 166, 177, 207, 245, 379, 432, 519, 625, 1029, 1032, 1058, 1083, 1088, 1101, 1159 and/or 1271; preferably H49, H146, C166, M177, H207, H245, C432, H519, H625, M1029, H1058, H1083, H1088, H1101, H1159 and/or H1271; or H207, H245, C379, M1029 and/or C1032, e.g. in SEQ ID NO:3, or a corresponding position in SEQ ID NO: 19, 21, 23, 25 or 27; and/or

(ii) a membrane (M) glycoprotein of the inactivated SARS-CoV-2 particle comprises a beta-propiolactone modification at one or more of the following residues: 125, 154, 155, 159 and/or 210; preferably H154, H155, C159 and/or H210, e.g. in SEQ ID NO: 29;

(iii) a nucleocapsid (N) protein of the inactivated SARS-CoV-2 particle comprises a beta-propiolactone modification at M234, e.g. in SEQ ID NO: 28.

21. A SARS-CoV-2 vaccine according to any preceding claim, wherein fewer than 30%, 20%, 10%, 5%, 3% or 1% of one or more of the following residues, preferably of at least 2, 3, 4, 5, 6, 7, 8,

9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or all of the following residues, in the inactivated SARS-CoV-2 particles are beta-propiolactone modified:

(i) in the spike (S) protein, residues 49, 146, 166, 177, 207, 245, 379, 432, 519, 625, 1029, 1032, 1058, 1083, 1088, 1101, 1159 and/or 1271; preferably H49, H146, C166, M177, H207, H245, C432, H519, H625, M1029, H1058, H1083, H1088, H1101, H1159 and/or H1271; or H207, H245, C379, M1029 and/or C1032; e.g. in SEQ ID NO: 3, or a corresponding position in SEQ ID NO: 19, 21, 23, 25 or 27; and/or (ii) in the membrane (M) glycoprotein, residues 125, 154, 155, 159 and/or 210; preferably H154, H155, C159 and/or H210; e.g. in SEQ ID NO: 29; and/or (iii) M234 of the nucleocapsid (N) protein, e.g. in SEQ ID NO: 28.

22. A SARS-CoV-2 vaccine according to any preceding claim, wherein the proportion of beta-propiolactone-modified residues at each of the following positions in the inactivated SARS-CoV-2 particles is:

(i) in the spike (S) protein (e.g. of SEQ ID NO: 3, or a corresponding position in SEQ ID NO: 19, 21, 23, 25 or 27):

(a) residues H49, H146, C166, H207, H519, M1029, H1083, H1088, H1101, H1159 and/or H1271: less than 20%, preferably 0.01 to 10%, more preferably 0.1 to 5%; and/or

(b) residues M177, C432, H625: less than 30%, preferably 0.1 to 20%, more preferably 1 to 10%; and/or

(c) residues H245, H1058: less than 30%, preferably 0.1 to 20%, more preferably 5 to 15%;

(ii) in the membrane (M) glycoprotein (e.g. of SEQ ID NO: 29):

(f) H154: less than 5%, less than 1% or less than 0.1%; and/or

(g) H155: less than 10%, preferably 0.1 to 5%; and/or

(h) C159: less than 5%, less than 1% or less than 0.1%; and/or

(i) H210: less than 20%, preferably 0.1 to 10%; and/or

(iii) in the nucleocapsid (N) protein (e.g. of SEQ ID NO: 28):

(j) M234: less than 90%, less than 10% or less than 0.1%.

23. The SARS-CoV-2 vaccine according to any one of the preceding claims, wherein infectivity of mammalian cells by the inactivated SARS-CoV-2 particle is reduced by at least 99%, 99.99% or 99.9999% compared with a native SARS-CoV-2 particle, or wherein infectivity of mammalian cells by the inactivated SARS-CoV-2 particle is undetectable.

24. A SARS-CoV-2 vaccine according to any one of the preceding claims, further comprising one or more pharmaceutically acceptable excipients.

25. The SARS-CoV-2 vaccine according to any one of the preceding claims, wherein the SARS-CoV-2 particle comprises an RNA sequence (and/or fragments thereof, optionally comprising modified (preferably alkylated or acylated) nucleotide residues) corresponding to a DNA sequence (i) as defined by SEQ ID NO: 9; or (ii) having at least 80%, at least 85%, at least 90%, at least 95% or at least 99% sequence identity to SEQ ID NO: 9; preferably wherein a native (non-inactivated) SARS-CoV-2 particle comprising the RNA sequence is able to pack a virulent SARS-CoV-2.

26. The SARS-CoV-2 vaccine according to any one of the preceding claims, wherein the said vaccine comprises an additional SARS-CoV-2 particle that comprises an RNA sequence (and/or fragments thereof, optionally comprising modified (preferably alkylated or acylated) nucleotide residues) corresponding to a DNA sequence (i) as defined by SEQ ID NO: 18; or (ii) having at least 80%, at least 85%, at least 90%, at least 95% or at least 99% sequence identity to SEQ ID NO: 18; preferably wherein a native (non-inactivated) SARS-CoV-2 particle comprising the RNA sequence is able to pack a virulent SARS-CoV-2.

27. The SARS-CoV-2 vaccine according to any one of the preceding claims, wherein the said vaccine comprises an additional SARS-CoV-2 particle that comprises an RNA sequence (and/or fragments thereof, optionally comprising modified (preferably alkylated or acylated) nucleotide residues) corresponding to a DNA sequence (i) as defined by SEQ ID NO: 22; or (ii) having at least 80%, at least 85%, at least 90%, at least 95% or at least 99% sequence identity to SEQ ID NO: 22; preferably wherein a native (non-inactivated) SARS-CoV-2 particle comprising the RNA sequence is able to pack a virulent SARS-CoV-2.

28. The SARS-CoV-2 vaccine according to any one of the preceding claims, wherein the CpG ODN is CpG 1018, comprising the sequence 5' TGACTGTGAACGTTCGAGATGA 3' as defined by SEQ ID NO: 4.

29. The SARS-CoV-2 vaccine according to any one of the preceding claims, wherein the alum adjuvant comprises aluminium hydroxide.

30. The SARS-CoV-2 vaccine according to any one of the preceding claims, wherein the vaccine is able to seroconvert a subject that is administered the SARS-CoV vaccine with at least a 70% probability.

31. The SARS-CoV-2 vaccine according to any one of the preceding claims, wherein the vaccine is able to seroconvert a subject that is administered the SARS-CoV vaccine with at least 90% probability.

32. The SARS-CoV-2 vaccine according to claim 30, wherein the SARS-CoV-2 vaccine is able to serocovert the subject that is administered the SARS-CoV-2 vaccine with at least 80%, 85%, 90%, or 95% probability.

33. The SARS-CoV-2 vaccine according to any one of the preceding claims, wherein the vaccine is obtained or obtainable from Vero cells.

34. The SARS-CoV-2 vaccine according to any one of the preceding claims, wherein on administration to a human subject the vaccine (i) does not induce antibody-dependent enhancement (ADE) of SARS-CoV-2-associated disease (COVID-19), and/or (ii) does not induce immunopathology in the subject.

35. A method of preventing or treating SARS-CoV-2 infection and/or SARS-CoV-2-associated disease (COVID-19) in a human subject in need thereof, comprising administering a prophylactically or therapeutically effective amount of the SARS-CoV-2 vaccine of any preceding claim to the subject.

36. The method according to claim 35, further comprising administering a second dose of a prophylactically or therapeutically effective amount of the SARS-CoV-2 vaccine, preferably wherein the second dose of the vaccine is the same formulation as the first.

37. The method according to claim 35 or 36, wherein said prophylactically or therapeutically effective amount of the SARS-CoV-2 vaccine per dose is defined as about 1 to 100 AU/dose, preferably between about 2 to 75 AU/dose, preferably between about 3 and 60 AU/dose, more preferably between about 3 and 55 AU/dose, more preferably between about 3 and 53 AU/dose, as assessed by ELISA, even more preferably between about 3 and 40 AU/dose, more preferably about 10 to 60 AU/dose, 20 to 50 AU/dose, 25 to 45 AU/dose or 30 to 40 AU/dose such as e.g. 35 AU/dose or 40 AU/dose.

38. The method according to any one of claims 35 to 37, wherein said prophylactically or therapeutically effective amount of the SARS-CoV-2 vaccine per dose is defined as about 0.05

to 50 μg total protein, about 0.1 to 25 μg, about 0.25 to 12.5 μg, preferably about 0.5 to 5 μg total protein, more preferably at least 2.5 μg total protein, at least 3.5 μg total protein or at least 2.5 μg total protein, even more preferably 2.5 μg to 25 μg, 3.5 μg to 10 μg or 4 μg to 6 μg total protein/dose, most preferably about 5 μg total protein/dose, e.g. as measured by (μ)BCA.

39. The method according to any one of claims 35 to 38, wherein said prophylactically or therapeutically effective amount of the SARS-CoV-2 vaccine is defined as about 0.025 to 25 μg S-protein, about 0.05 to 12.5 μg, about 0.125 to 6.25 μg, preferably about 0.25 to 2.5 μg S-protein, as measured by ELISA.

40. The method according to any one of claims 35 to 39, wherein the second dose of the SARS-CoV-2 vaccine is administered about 7 days, about 14 days, about 21 days or about 28 days after a first dose of the SARS-CoV-2 vaccine, preferably about 21 days.

41. The method according to any one of claims 35 to 40, wherein the administering results in production of SARS-CoV-2 neutralizing antibodies.

42. A method of producing a SARS-CoV-2 vaccine, comprising:

(a) producing native SARS-CoV-2 particles;

(b) inactivating the native SARS-CoV-2 particles to obtain inactivated SARS-CoV-2 particles;

(c) incorporating the inactivated SARS-CoV-2 particles in a vaccine composition comprising a CpG-containing oligodeoxynucleotide (CpG ODN) and an alum adjuvant.

43. The method according to claim 42, wherein the CpG-ODN is CpG 1018 and the alum adjuvant is aluminium hydroxide.

44. The method according to claim 43, wherein the SARS-CoV-2 vaccine comprising aluminium hydroxide contains less than 1.25 ppb Cu.

45. The method according to any of claims 42 to 44, wherein a native surface conformation of the SARS-CoV-2 particle is preserved in the inactivation step, such that the vaccine is capable of generating neutralizing antibodies against native SARS-CoV-2 particles in a human subject.

46. The method according to claim 45, wherein the inactivation step preferentially targets viral RNA in the inactivated SARS-CoV-2 particle.

47. The method according to any one of claims 42 to 4046 wherein the inactivation step comprises (i) alkylating and/or acylating viral RNA (ii) modifying purine (preferably guanine) residues or introducing strand breaks into viral RNA and/or (iii) cross-linking viral RNA with one or more viral proteins.

48. The method according to any of claims 42 to 47, wherein the inactivation step comprises treating the native SARS-CoV-2 particles with beta-propiolactone.

49. The method according to claim 48, wherein the concentration of beta-propiolactone in the inactivation step is 0.01 to 1% by weight, preferably 0.05 to 0.5% by weight, more preferably about 0.1% by weight, even more preferably between 300 to 700ppm, most preferred around 500ppm at 2°C to 8°C for at least 5 hours, at least 10 hours, at least 24 hours, followed optionally by a hydrolyzation for 2.5 hours ± 0.5 hours at 35°C to 39°C, preferably around 37°C.

50. The method according to claim 48 or 49, wherein the native SARS-CoV-2 particles are contacted with beta-propiolactone for at least 5 hours, at least 10 hours, at least 24 hours.

51. The method according to any one of claims 42 to 50, wherein the inactivation step is performed at about 0°C to about 25°C, preferably about 4°C or about 8°C.

52. The method according to any of claims 42 to 51, wherein the inactivation step comprises treating the native SARS-CoV-2 particles with ultraviolet (UV) light.

53. The method according to any of claims 42 to 52, wherein step (a) comprises one or more of the following steps:

(i) passaging a SARS-CoV-2 on Vero cells, thereby producing a culture medium comprising the SARS-CoV-2;

(ii) harvesting the culture medium of (i);

(iii) precipitating the harvested culture medium of (ii), thereby producing native SARS-CoV-2 particles in a supernatant.

54. The method according to claim 53, further comprising concentrating the culture medium of step (ii) prior to step (iii).

55. The method according to claim 53 or 54, wherein precipitation step (iii) comprises contacting the culture medium of step (ii) with protamine sulfate and/or benzonase.

56. The method according to any of claims 42 to 55, further comprising dialyzing the inactivated SARS-CoV-2 particles, thereby producing a dialyzed SARS-CoV-2.

57. The method according to claim 56, further comprising filtering the dialyzed SARS-CoV-2.

58. The method according to any of claims 42 to 57, wherein the inactivation step comprises contacting a liquid composition comprising native SARS-CoV-2 particles with a chemical viral inactivating agent in a container, mixing the chemical viral inactivating agent and the liquid composition comprising SARS-CoV-2 particles under conditions of laminar flow but not turbulent flow, and incubating the chemical viral inactivating agent and the liquid composition comprising SARS-CoV-2 particles for a time sufficient to inactivate the viral particles.

59. The method according to claim 58, wherein the inactivation step is performed in a flexible bioreactor bag.

60. The method according to claim 58 or 59, wherein the inactivation step comprises five or less container inversions during the period of inactivation.

61. The method according to any one of claims 58 to 60, wherein the mixing of the chemical viral inactivating agent and the composition comprising native SARS-CoV-2 particles comprises subjecting the container to rocking, rotation, orbital shaking, or oscillation for not more than 10 minutes at not more than 10 rpm during the period of incubation.

62. The method according to any one of claims 42 to 61, further comprising purifying the inactivated SARS-CoV-2 particles by one or more methods selected from (i) batch chromatography and/or (ii) sucrose density gradient centrifugation.

63. The method according to any one of claims 42 to 62, wherein said SARS-CoV-2 particle comprises an RNA sequence corresponding to a DNA sequence (i) as defined by SEQ ID NO: 9; or (ii) having at least 80%, at least 85%, at least 90%, at least 95% or at least 99% sequence identity to SEQ ID NO: 9; preferably wherein a native (non-inactivated) SARS-CoV-2 particle comprising the RNA sequence is able to pack a virulent SARS-CoV-2.

64. The method according to any one of claims 42 to 63, wherein said SARS-CoV-2 virus comprises an S protein comprising or consisting of (i) an amino acid sequence as defined by SEQ ID NO: 11, or (ii) an amino acid sequence having at least 95%, at least 97% or at least 99% identity to SEQ ID NO: 11.

65. The method according to any one of claims 42 to 64, wherein said SARS-CoV-2 virus comprises a polyprotein comprising or consisting of (i) an amino acid sequence as defined by SEQ ID NO: 10, or (ii) an amino acid sequence having at least 95%, at least 97% or at least 99% identity to SEQ ID NO: 10.

66. A SARS-CoV-2 vaccine obtained or obtainable by the method of any one of claims 42 to 65.

67. Use of a SARS-CoV-2 vaccine of any one of claims 1 to 34 or 66 for the treatment or prevention of a SARS-CoV-2 infection in a subject.

68. The SARS-CoV-2 vaccine of any one of claims 1 to 34 or 66 for use as a medicament.

69. A pharmaceutical composition for use in the prevention or treatment of a SARS-CoV-2 infection in a subject, wherein said pharmaceutical composition is a vaccine of any one of claims 1 to 34 or 66, optionally in combination with one or more pharmaceutically acceptable excipients.

70. A vaccine, method, use or pharmaceutical composition according to any preceding claim, wherein the subject is (i) an elderly subject, preferably a subject over 65, over 70 or over 80 years of age; (ii) an immunocompromised subject; or (iii) a pregnant subject.

71. A vaccine, method, use or pharmaceutical composition according to any preceding claim, for use in prevention or treatment of a SARS-CoV-2 infection without induction of (i) antibody-dependent enhancement (ADE) of SARS-CoV-2-associated disease (COVID-19); and/or (ii) immunopathology in the subject.