

CLAIMS

1. A nucleic acid comprising an optimized nucleotide sequence encoding a full-length SARS-CoV-2 spike protein which has been modified relative to naturally occurring full-length SARS-CoV-2 spike protein of SEQ ID NO: 1 to remove the furin cleavage site and to mutate residues 986 and 987 to proline, wherein the optimized nucleotide sequence consists of codons associated with a usage frequency which is greater than or equal to 10%; wherein the optimized nucleotide sequence:

(i) does not contain a termination signal having one of the following nucleotide sequences:

5'-X₁ATCTX₂TX₃-3', wherein X₁, X₂ and X₃ are independently selected from A, C, T or G; and 5'-X₁AUCUX₂UX₃-3', wherein X₁, X₂ and X₃ are independently selected from A, C, U or G;

(ii) does not contain any negative cis-regulatory elements and negative repeat elements; and

(iii) has a codon adaptation index greater than 0.8;

wherein, when divided into non-overlapping 30 nucleotide-long portions, each portion of the optimized nucleotide sequence has a guanine cytosine content range of 30% - 70%.

2. The nucleic acid of claim 1, wherein:

(i) the optimized nucleotide sequence does not contain a termination signal having one of the following sequences: TATCTGTT; TTTTTT; AAGCTT; GAAGAGC; TCTAGA; UAUCUGUU; UUUUUU; AAGCUU; GAAGAGC; UCUAGA;

(ii) ~~3. The nucleic acid of claim 1 or 2, wherein~~ the full-length SARS-CoV-2 spike protein encoded by the optimized sequence further contains the L18F, D80A, D215G, L242-, A243-, L244-, K417N, E484K, N501Y, D614G and A701V mutations;

(iii) ~~4. The nucleic acid of any one of claims 1-3, wherein~~ the nucleic acid is mRNA or;

~~5. The nucleic acid of any one of claims 1-3, wherein~~ the nucleic acid is DNA; and/or;

(iv) ~~6. The nucleic acid of any one of claims 1-5, wherein~~ the optimized nucleotide sequences encodes;

(a) an amino acid sequence comprising SEQ ID NO:11, optionally wherein the optimized nucleotide sequence has the nucleic acid sequence of SEQ ID NO: 44 or SEQ ID NO: 148; or

~~(b) 7. The nucleic acid of any one of claims 1-5, wherein the optimized nucleotide sequences encodes~~ an amino acid sequence comprising SEQ ID NO:167, optionally wherein the optimized nucleotide sequence has the nucleic acid sequence of SEQ ID NO: 166 or SEQ ID NO: 173.

~~38.~~ The nucleic acid of ~~any one of claims 1~~ or ~~-27~~ for use in therapy.

~~49.~~ An immunogenic composition comprising the nucleic acid of ~~any one of claims 1 or 2-7~~ for use in (i) prophylaxis of an infection caused by a β -coronavirus; or

~~10. The immunogenic composition for use according to claim 9, wherein the β -coronavirus expresses a spike protein which binds to angiotensin-converting enzyme 2 (ACE2).~~

~~11. The immunogenic composition for use according to claim 9 or claim 10, wherein the β -coronavirus is SARS-CoV-2.~~

~~12. The immunogenic composition for use according to claims 9-11, wherein the β -coronavirus has a spike protein that is at least 75%, 80%, 90%, 95% or 99% identical to SEQ ID NO: 1.~~

~~13. A (ii) a method of treating or preventing an infection caused by a β -coronavirus, said method comprising administering to a subject an effective amount of ~~the~~ immunogenic composition comprising the nucleic acid of any one of claims 1-7.~~

~~14. The method according to claim 13,~~
optionally wherein:

(a) the β -coronavirus expresses a spike protein which binds to angiotensin-converting enzyme 2 (ACE2);

~~15. The method of claim 13 or claims 14, wherein (b) the β -coronavirus is SARS-CoV-2, and/or~~

~~16. The method according to claims 13-15, wherein (c) the β -coronavirus has a spike protein that is at least 75%, 80%, 90%, 95% or 99% identical to SEQ ID NO: 1.~~

~~517.~~ A pharmaceutical composition comprising i) the nucleic acid of ~~any one of~~ claims ~~1- or 27~~ and ii) a lipid nanoparticle, optionally -

~~18.~~ ~~The pharmaceutical composition of claim 17,~~ wherein:

(a) the nucleic acid is encapsulated in the lipid nanoparticle~~;~~

~~25.~~ ~~The pharmaceutical composition of any one of claims 17-24,~~
~~wherein~~ (b) the lipid nanoparticle has an average size of less than 150 nm, e.g., less than 100 nm-

~~26.~~ ~~The pharmaceutical composition of claim 25, wherein the lipid nanoparticle has an average size of,~~ about 50-70 nm, or e.g., about 55-65 nm~~;~~ and/or

~~27.~~ ~~The pharmaceutical composition any one of claims 17-26,~~
~~wherein~~ (c) the nucleic acid is mRNA at a concentration of between about 0.5 mg/mL to about 1.0 mg/mL.

~~619.~~ The pharmaceutical composition of claim ~~517 or claim 18~~, wherein the lipid nanoparticle comprises a cationic lipid, a non-cationic lipid, a cholesterol-based lipid, and a PEG-modified lipid-

~~20.~~ ~~The pharmaceutical composition of claim 19,~~ optionally wherein:

(i) a. ~~the~~ the cationic lipid is selected from cKK-E12, cKK-E10, OF-Deg-Lin and OF-02;

~~b.~~ the non-cationic lipid is selected from DOPE and DEPE;

~~e.~~ the cholesterol-based lipid is cholesterol; and

~~d.~~ the PEG-modified lipid is DMG-PEG-2K~~;~~

(ii) 21. ~~The pharmaceutical composition of claim 19 or 20, wherein~~
cationic lipid constitutes about 30-60% of the lipid nanoparticle by molar ratio, e.g., about 35-40%~~;~~

(iii) 22. ~~The pharmaceutical composition of any one of claims 19-21,~~
~~wherein~~ the ratio of cationic lipid to non-cationic lipid to cholesterol-based lipid to PEG-modified lipid is approximately 30-60:25-35:20-30:1-15 by molar ratio~~;~~

(iv) 23. ~~The pharmaceutical composition of any one of claims 19-22,~~
~~wherein~~ the lipid nanoparticle comprises cKK-E12, DOPE, cholesterol and DMG-PEG2K; cKK-E10, DOPE, cholesterol and DMG-PEG2K; OF-Deg-Lin, DOPE,

cholesterol and DMG-PEG2K; or OF-02, DOPE, cholesterol and DMG-PEG2K;
and/or

~~(v) 24. The pharmaceutical composition of any one of claims 19-23,~~
~~wherein~~ the lipid nanoparticle comprises cKK-E10, DOPE, cholesterol and DMG-PEG2K at the molar ratios 40:30:28.5:1.5.

~~728.~~ The pharmaceutical composition ~~of any one of claims 5 or 617-27~~ for use in treating or preventing an infection caused by a β -coronavirus, optionally wherein:-

~~(i) 29. The pharmaceutical for use according to claim 28, wherein~~ the β -coronavirus expresses a spike protein which binds to angiotensin-converting enzyme 2 (ACE2);

~~(ii) 30. The pharmaceutical composition for use according to claim 28 or claim 29, wherein~~ the β -coronavirus is SARS-CoV-2;

~~(iii) 31. The pharmaceutical composition for use according to claims 28 to 30, wherein~~ the β -coronavirus has a spike protein that is at least 75%, 80%, 90%, 95% or 99% identical to SEQ ID NO: 1.

~~832.~~ The pharmaceutical composition for use according to claims ~~728-31~~, wherein the pharmaceutical composition is administered intramuscularly:

~~33. The pharmaceutical composition for use according to claim 32, optionally~~ wherein:

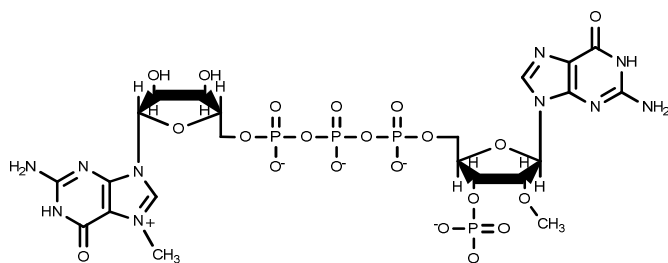
~~(i) the pharmaceutical composition is administered at least once;~~ or

~~34. The pharmaceutical composition for use according to claim 33, wherein~~ (ii) the pharmaceutical composition is administered at least twice:

~~35. The pharmaceutical composition for use according to claim 34, optionally~~ wherein the period between administrations is at least 2 weeks, e.g. 3 weeks, or 1 month.

~~936.~~ An mRNA construct consisting of the following structural elements:

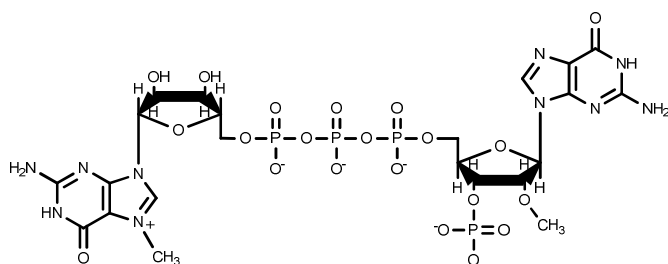
(i) a 5' cap with the following structure:



- (ii) a 5' untranslated region (5' UTR) having the nucleic acid sequence of SEQ ID NO: 144;
- (iii) a protein coding region having the nucleic acid sequence of SEQ ID NO: 148;
- (iv) a 3' untranslated region (3' UTR) having the nucleic acid sequence of SEQ ID NO: 145; and
- (v) a polyA tail.

1037. An mRNA construct consisting of the following structural elements:

- (i) a 5' cap with the following structure:



- (ii) a 5' untranslated region (5' UTR) having the nucleic acid sequence of SEQ ID NO: 144;
- (iii) a protein coding region having the nucleic acid sequence of SEQ ID NO: 173;
- (iv) a 3' untranslated region (3' UTR) having the nucleic acid sequence of SEQ ID NO: 145; and
- (v) a polyA tail.

1138. A lipid nanoparticle encapsulating the mRNA construct of claim 936.

39. ~~A lipid nanoparticle encapsulating and/or~~ the mRNA construct of claim 1037.

40. ~~A lipid nanoparticle encapsulating the mRNA construct of claim 36 and the mRNA construct of claim 37.~~

41. ~~The lipid nanoparticle of any one of claims 38-40,~~ optionally wherein the lipid nanoparticle comprises a cationic lipid, a non-cationic lipid, a cholesterol-based lipid and a PEG-modified lipid.

~~42. The lipid nanoparticle of claim 41, further optionally~~ wherein the cationic lipid is selected from cKK-E12, cKK-E10, OF-Deg-Lin and OF-02; the non-cationic lipid is selected from DOPE and DEPE; the cholesterol-based lipid is cholesterol; and the PEG-modified lipid is DMG-PEG-2K-

~~43. The lipid nanoparticle of claim 42, for example~~ wherein the lipid nanoparticle comprises cKK-E10, DOPE, cholesterol and DMG-PEG2K at the molar ratios 40:30:28.5:1.5.

~~1244.~~ An immunogenic composition comprising the mRNA construct of claim ~~936~~ and/or the mRNA construct of claim ~~1037~~, or the lipid nanoparticle ~~of any of claim 11s 38-43.~~

~~45. The immunogenic composition according to claim 44, optionally~~ comprising between 5 µg and 200 µg of the mRNA construct(s)-₁

~~46. The immunogenic composition according to claim 45 further optionally~~ comprising between 7 µg and 135 µg of the mRNA construct(s)-₁, ~~optionally wherein~~

~~47. The immunogenic composition according to claim 46 comprising~~ at least 10 µg of the mRNA construct(s)-₁

~~48. The immunogenic composition according to claim 46 comprising~~ at least 15 µg of the mRNA construct(s)-₁

~~49. The immunogenic composition according to claim 46 comprising~~ at least 20 µg of the mRNA construct(s)-₁

~~50. The immunogenic composition according to claim 46 comprising~~ at least 25 µg of the mRNA construct(s)-₁

~~51. The immunogenic composition according to claim 46 comprising~~ at least 35 µg of the mRNA construct(s)-₁

~~52. The immunogenic composition according to claim 46 comprising~~ at least 40 µg of the mRNA construct(s)-₁ ~~or~~

~~53. The immunogenic composition according to claim 46 comprising~~ at least 45 µg of the mRNA construct(s)-₁ ~~or~~

~~54. The immunogenic composition according to claim 46 comprising~~ 7.5 µg, 15 µg, 45 µg or 135 µg of the mRNA construct(s).

~~1355.~~ An immunogenic composition of claim 12 for use in a method of treating or preventing an infection caused by a β-coronavirus, said method comprising administering to a

subject an effective amount of the immunogenic composition ~~of any one of claims 44 to 54,~~
optionally wherein:-

(i)

~~56. The method according to claim 55, wherein~~ the β -coronavirus expresses a spike protein which binds to angiotensin-converting enzyme 2 (ACE2);~~:-~~

(ii) 57.

~~The method of claim 55 or claim 56, wherein~~ the β -coronavirus is SARS-CoV-2;~~:-~~

(iii) 58.

~~The method according to any one of claims 55 to 57, wherein~~ the β -coronavirus has a spike protein that is at least 75%, 80%, 90%, 95% or 99% identical to SEQ ID NO: 1; and/or-

(iv) 59.

~~The method of any one of claims 55 58, wherein~~ the immunogenic composition is administered to the subject at least twice, optionally -

~~60.~~

~~The method of claim 59,~~ wherein the period between administrations is at least 2 weeks, e.g., 3 weeks, or 1 month.

1461. An immunogenic composition comprising at least two nucleic acids, wherein

1. the first nucleic acid comprises an optimized nucleotide sequence encoding a full-length SARS-CoV-2 spike protein which has been modified relative to naturally occurring full-length SARS-CoV-2 spike protein of SEQ ID NO: 1 to remove the furin cleavage site and to mutate residues 986 and 987 to proline; and
2. the second nucleic acid comprises an optimized nucleotide sequence encoding a full-length SARS-CoV-2 spike protein which has been modified relative to naturally occurring full-length SARS-CoV-2 spike protein of SEQ ID NO: 1 to remove the furin cleavage site and to mutate residues 986 and 987 to proline and further contains the L18F, D80A, D215G, L242-, A243-, L244-, K417N, E484K, N501Y, D614G and A701V mutations;

~~62. The immunogenic composition according to claim 61;:-~~

optionally wherein:-

(i) the first nucleic acid comprises an optimized nucleotide sequence which encodes an amino acid sequence comprising SEQ ID NO:11, optionally wherein the optimized nucleotide sequence has the nucleic acid sequence of SEQ ID NO: 44 or SEQ ID NO: 148;~~:-~~

~~(ii) 63. The immunogenic composition according to claim 61 or 62, wherein~~ the second nucleic acid comprises an optimized nucleotide sequence that encodes an amino acid sequence comprising SEQ ID NO: 167, optionally wherein the optimized nucleotide sequence has the nucleic acid sequence of SEQ ID NO: 166 or SEQ ID NO: 173;

~~(iii) 64. The immunogenic composition according to any one of claims 61-63, wherein~~ the at least two nucleic acids are mRNA constructs, optionally;

~~65. The immunogenic composition according to claim 64,~~ wherein the optimized nucleotide sequence of the first nucleic acid has the nucleic acid sequence of SEQ ID NO: 148, wherein the optimized nucleotide sequence of the second nucleic acid has the nucleic acid sequence of SEQ ID NO: 173, further optionally;

~~66. The immunogenic composition according to claim 65,~~ wherein the first nucleic acid is the mRNA construct of claim ~~1036~~, wherein the second nucleic acid is the mRNA construct of claim ~~1137~~, and/or

~~73. (iv) The immunogenic composition according to any one of claims 61-72 comprising~~ at total of 7.5 µg, 15 µg, 45 µg or 135 µg of the at least two nucleic acids.

1567. The immunogenic composition according to ~~any one of~~ claim ~~1461-66~~, wherein:

(i) the at least two nucleic acids are encapsulated in lipid nanoparticles, optionally wherein;

~~68. The immunogenic composition according to claim 67, wherein~~ the at least two nucleic acids are encapsulated in the same lipid nanoparticle or;

~~69. The immunogenic composition according to claim 67, wherein~~ the at least two nucleic acids are encapsulated in separate lipid nanoparticles; and/or

(ii) 70. The immunogenic composition according to any one of claims 67-69, wherein the lipid nanoparticle comprises a cationic lipid, a non-cationic lipid, a cholesterol-based lipid and a PEG-modified lipid, optionally wherein;

~~71. The immunogenic composition according to claim 70, wherein~~ the cationic lipid is selected from cKK-E12, cKK-E10, OF-Deg-Lin and OF-02; the non-cationic lipid is selected from DOPE and DEPE; the cholesterol-based lipid is cholesterol; and the PEG-modified lipid is DMG-PEG-2K, further optionally;

~~72. The immunogenic composition according to claim 71, wherein the lipid nanoparticle comprises cKK-E10, DOPE, cholesterol and DMG-PEG2K at the molar ratios 40:30:28.5:1.5.~~

~~1674.~~ The immunogenic composition according to ~~any one of claims 14 or 1561-73~~ for use in (i) the prophylaxis of an infection caused by a β -coronavirus, or

~~75. The immunogenic composition according to claim 74, wherein the β -coronavirus expresses a spike protein which binds to angiotensin-converting enzyme 2 (ACE2)~~

~~76. The immunogenic composition for use according to claim 74 or claim 75, wherein the β -coronavirus is SARS-CoV-2.~~

~~77. The immunogenic composition for use according to claims 74 to 76, wherein the β -coronavirus has a spike protein that is at least 75%, 80%, 90%, 95% or 99% identical to SEQ ID NO: 1.~~

~~78. A (ii) a method of treating or preventing an infection caused by a β -coronavirus, said method comprising administering to a subject an effective amount of the immunogenic composition, of any one of claims 61-73.~~

optionally wherein:

~~(a) 79. The method according to claim 78, wherein the β -coronavirus expresses a spike protein which binds to angiotensin-converting enzyme 2 (ACE2).~~

~~(b) 80. The method of claim 78 or claim 79, wherein the β -coronavirus is SARS-CoV-2; and/or:~~

~~(c) 81. The method according to claims 78 to 80, wherein the β -coronavirus has a spike protein that is at least 75%, 80%, 90%, 95% or 99% identical to the SEQ ID NO:1.~~

~~1782.~~ The immunogenic composition for use according to method of any one of claim 1678-81, wherein:

(i) the subject has not previously been administered an immunogenic composition for the prophylaxis of said infection, or

(ii)

~~83. The method of any one of claims 78-81, wherein the subject has previously been administered with one or more immunogenic composition(s) for the prophylaxis of said infection, optionally wherein: -~~

~~(a) 84. The method of claim 83, wherein~~ the subject has previously been administered with two immunogenic compositions at least two weeks apart for the prophylaxis of said infection;

~~(b) 85. The method of claim 83 or 84, wherein~~ the one or more immunogenic composition(s) is/are different from the immunogenic composition of ~~any one of claims 14 or 15~~⁶¹⁻⁷³;

~~(c) 86. The method of any one of claims 83-85, wherein~~ the one or more immunogenic composition(s) is/are selected from:

~~Ia.~~ —the immunogenic composition according to claims ~~49-12~~;

~~IIb.~~ —the pharmaceutical composition according to any one of claims ~~5-8~~¹⁷⁻³⁵;

~~IIIe.~~ —the immunogenic composition according to ~~any one of claim 12~~^{s 44-54}; and

~~IVd.~~ —the Moderna (COVID-19 Vaccine Moderna, such as for example, mRNA-1273 or mRNA-1283), CureVac (CVnCoV), Johnson & Johnson (COVID-19 Vaccine Janssen), AstraZeneca (Vaxzevria), Pfizer/BioNTech (Comirnaty), Sputnik (Gam-COVID-Vac), Sinovac (COVID-19 Vaccine (Vero Cell) Inactivated) and Novavax (NVX-CoV2373);

~~(d) 87. The method of any one of claims 83 to 86, wherein~~ the immunogenic composition of ~~any one of claims 14 or 15~~⁶¹⁻⁷³ is administered 3-18 months after administration of the one or more immunogenic composition(s), ~~optionally -~~

~~88. —The method of claim 87, wherein~~ the immunogenic composition of ~~any one of claims 14 or 15~~⁵³⁻⁶⁵ is administered at least 9 months or at least 12 months after administration of the one or more immunogenic composition(s); ~~and/or~~

~~89. —The method of any one of claims 83 to 88, wherein~~(e) the immunogenic composition of ~~any one of claims 14 or 15~~⁶¹⁻⁷³ is administered at least once, e.g., at least twice.

~~1890.~~ An immunogenic composition for use in a A—method of treating or preventing an infection caused by a β -coronavirus, said method comprising administering to a subject an effective amount of ~~thean~~ immunogenic composition, wherein the immunogenic composition

comprising an mRNA construct, wherein said mRNA construct comprises an optimized nucleotide sequence encoding a full-length SARS-CoV-2 spike protein which has been modified relative to naturally occurring full-length SARS-CoV-2 spike protein of SEQ ID NO: 1 to remove the furin cleavage site and to mutate residues 986 and 987 to proline and further contains the L18F, D80A, D215G, L242-, A243-, L244-, K417N, E484K, N501Y, D614G and A701V mutations, optionally wherein:

~~(i) 91. The method of claim 90, wherein the~~ optimized nucleotide sequence encodes an amino acid sequence comprising SEQ ID NO: 167, optionally wherein the optimized nucleotide sequence has the nucleic acid sequence of SEQ ID NO: 173;

~~92. The method of claim 90 or 91, wherein the mRNA construct is the mRNA construct of claim 31.~~

~~(ii) 93. The method of any one of claims 90-92, wherein the~~ mRNA construct is encapsulated in a lipid nanoparticle.

~~94. The method of claim 93, optionally~~ wherein the lipid nanoparticle comprises a cationic lipid, a non-cationic lipid, a cholesterol-based lipid and a PEG-modified lipid;

~~95. The method of claim 94, further optionally~~ wherein the cationic lipid is selected from cKK-E12, cKK-E10, OF-Deg-Lin and OF-02; the non-cationic lipid is selected from DOPE and DEPE; the cholesterol-based lipid is cholesterol; and the PEG-modified lipid is DMG-PEG-2K;

~~96. The method of claim 95, for example~~ wherein the lipid nanoparticle comprises cKK-E10, DOPE, cholesterol and DMG-PEG2K at the molar ratios 40:30:28.5:1.5.

~~(iii) 97. The method of any one of claims 90-96, wherein the~~ immunogenic composition comprises 7.5 µg, 15 µg, 45 µg or 135 µg of the mRNA construct;

~~(iv) 98. The method of claims 90-97, wherein the~~ β-coronavirus expresses a spike protein which binds to angiotensin-converting enzyme 2 (ACE2);

~~(v) 99. The method of claim 90-98, wherein the~~ β-coronavirus is SARS-CoV-2;

~~(vi) 100. The method according to claims 90-99, wherein the β -coronavirus has a spike protein that is at least 75%, 80%, 90%, 95% or 99% identical to SEQ ID NO:1.~~

~~19101. The immunogenic composition for use according to method of claim 1890-100, wherein:~~

~~(i) the subject has not previously been administered an immunogenic composition for the prophylaxis of said infection, or~~

~~102. The method of claim 90-101, wherein (ii) the subject has previously been administered with one or more immunogenic composition(s) for the prophylaxis of said infection, optionally wherein:-~~

~~(a) 103. The method of claim 102, wherein the subject has previously been administered with two immunogenic compositions at least two weeks apart for the prophylaxis of said infection:-~~

~~(b) 104. The method of claim 102 or 103, wherein the one or more immunogenic composition(s) is/are different from the immunogenic composition of claim 14 or 15:-~~

~~(c) 105. The method of any one of claims 102-104, wherein the first and/or second immunogenic composition(s) is/are selected from:~~

~~Ia. the immunogenic composition according to claim 4s-9-12;~~

~~IIb. the pharmaceutical composition according to any one of claims 5-8+7-35; or~~

~~IIIe. the immunogenic composition according to any one of claim 12s-44-54; and~~

~~IVd. the Moderna (COVID-19 Vaccine Moderna, such as for example, mRNA-1273 or mRNA-1283), CureVac (CVnCoV), Johnson & Johnson (COVID-19 Vaccine Janssen), AstraZeneca (Vaxzevria), Pfizer/BioNTech (Comirnaty), Sputnik (Gam-COVID-Vac), Sinovac (COVID-19 Vaccine (Vero Cell) Inactivated) and Novavax (NVX-CoV2373):-~~

~~(d) 106. The method of any one of claims 102 to 105, wherein the immunogenic composition of claim 14 or 1561-73 is administered 3-18 months after administration of the one or more immunogenic composition(s), optionally -~~

~~107. The method of claim 106, wherein the immunogenic composition of any one of claims 14 or 1561-73 is administered at least 9 months or at~~

least 12 months after administration of the one or more immunogenic composition(s);
and/or-

(e) 108. ~~The method of any one of claims 90-107, wherein~~ the immunogenic composition of ~~any one of claims 14 or 15~~⁶¹⁻⁷³ is administered at least once, e.g., at least twice.