

CORONAVIRUS ANTIGEN COMPOSITIONS AND THEIR USES

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on May 20, 2021 is named 51509-020WO6_Sequence_Listing_5.20.21_ST25 and is 207,385 bytes in size.

BACKGROUND

There is an urgent need for vaccines and therapeutics that are active against coronaviruses.

SUMMARY

The disclosure generally relates to circular polyribonucleotides comprising a sequence encoding a coronavirus antigen and to immunogenic compositions comprising the circular polyribonucleotide. This disclosure further relates to methods of using the circular polyribonucleotides comprising a sequence encoding a coronavirus antigen and the immunogenic composition. In some examples, the circular polyribonucleotides and immunogenic compositions of this disclosure are used in methods of generating polyclonal antibodies. The produced polyclonal antibodies can be used in methods of prophylaxis in subjects (e.g., human subjects) or methods of treatment for subjects (e.g., human subjects) having a coronavirus infection. The produced polyclonal antibodies can be administered to subjects at high risk for exposure to coronavirus infection.

The disclosure also relates to linear polyribonucleotides comprising a sequence encoding a sequence of SEQ ID NO selected from TABLE 3 and to immunogenic compositions comprising the linear polyribonucleotide. This disclosure further relates to methods of using the linear polyribonucleotide comprising a sequence encoding a coronavirus antigen and the immunogenic composition comprising the linear polyribonucleotide. In some examples, the linear polyribonucleotides and immunogenic compositions of this disclosure are used in methods of generating polyclonal antibodies. The produced polyclonal antibodies can be used in methods of prophylaxis in subjects (e.g., human subjects for treatment) or methods of treatment for subjects (e.g., human subjects for treatment) having a coronavirus infection. The produced polyclonal antibodies can be administered to subjects for treatment at high risk for exposure to coronavirus infection.

In one aspect, the present disclosure refers to an immunogenic composition comprising a circular polyribonucleotide comprising a sequence encoding a coronavirus antigen, wherein the coronavirus antigen is from severe acute respiratory syndrome (SARS)-related coronavirus or a fragment thereof.

In one aspect, the present disclosure refers to a immunogenic composition comprising a circular polyribonucleotide comprising a sequence encoding a coronavirus antigen, wherein the coronavirus antigen comprises a sequence having at least about 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% sequence identity to a coronavirus antigen selected from any one of SEQ ID NOs: 1-10, 13, 15, 17 19, 21, 23, 25-30, 48, and 49, or the circular polyribonucleotide comprises a sequence having at least about 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% sequence identity to a circular polyribonucleotide selected from SEQ ID NOs: 12, 14, 16, 18, 20, 22, and 24.

In one aspect, the present disclosure refers to use of any one of the immunogenic compositions disclosed herein in the manufacture of a medicament for delivering an immunogenic composition to a human subject.

In one aspect, the present disclosure refers to use of any one of the immunogenic compositions disclosed herein in the manufacture of a medicament for inducing an immune response against a coronavirus antigen in a non-human animal or human subject.

In one example, the disclosure features a composition (e.g., an immunogenic composition) comprising (a) a circular polyribonucleotide comprising a sequence encoding a coronavirus antigen, e.g., a sequence selected from a SEQ ID NO. in TABLE 1 or TABLE 2, or (b) a linear polyribonucleotide comprising a sequence selected from a SEQ ID NO. in TABLE 3.

In one example, the composition further comprises plasma from a non-human animal (e.g., a non-human animal comprising a humanized immune system) or a human subject (e.g., after immunization of a subject for immunization).

In one example, the composition further comprises plasma from a non-human animal (e.g., a non-human animal comprising a humanized immune system) and the coronavirus antigen (e.g., after immunization of a non-human animal subject for immunization). In one example, the composition further comprises plasma from a human subject (e.g., after immunization of a human subject for immunization) and the coronavirus antigen.

In some examples, the composition further comprises a non-human B cell comprising a humanized immunoglobulin gene locus and a humanized B cell receptor, wherein the humanized B cell receptor binds to the coronavirus antigen. In some examples, the composition or immunogenic composition further comprises a plurality of non-human B cells, wherein a non-human B cell of the plurality comprises a humanized immunoglobulin gene locus, wherein the plurality of non-human B cells comprises a first B cell that binds to a first epitope of the coronavirus antigen and a second B cell that binds to a second epitope of the coronavirus antigen.

In some examples, the coronavirus antigen is from a betacoronavirus or a fragment thereof or a sarbecovirus or a fragment thereof. In some examples, the coronavirus antigen is from severe acute respiratory syndrome (SARS)-related coronavirus or a fragment thereof. In some examples, the coronavirus antigen is from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or a fragment thereof, severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) or a fragment thereof, or Middle East respiratory syndrome coronavirus (MERS-CoV) or a fragment thereof.

In some examples, the coronavirus antigen is a membrane protein or a variant or fragment thereof, an envelope protein of a virus or a variant or fragment thereof, a spike protein of a virus or a variant or fragment thereof, a nucleocapsid protein of a virus or a variant or fragment thereof, an accessory protein of a virus or a variant or fragment thereof. In some examples, the coronavirus antigen is a receptor binding domain of spike protein or a variant or fragment thereof. In some examples, the spike protein lacks a cleavage site. In some examples, an accessory protein of a coronavirus is selected from a group consisting of ORF3a, ORF7a, ORF7b, ORF8, ORF10, or any variant or fragment thereof. In some examples, the coronavirus antigen comprises a sequence having at least about 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% sequence identity to a sequence selected from TABLE 1 or a sequence of a SEQ ID NO. selected from TABLE 2. In some examples, the circular polyribonucleotide comprises a sequence having at least about 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% sequence identity to a sequence of a SEQ ID NO. selected from TABLE 2.

In some examples, the polyribonucleotide comprises a plurality of sequences, each encoding an antigen, and at least one sequence of the plurality encodes a coronavirus antigen. In some examples, the circular polyribonucleotide comprises two or more ORFs. In some examples, the circular polyribonucleotide comprises at least five sequences, each encoding an antigen and at least one of the antigens is a coronavirus antigen. In some examples, the circular polyribonucleotide comprises at least two ORFs, e.g., at least 2, 3, 4 or 5 ORFs. In some examples, the circular polyribonucleotide comprises between 5 and 20 sequences, each encoding an antigen and at least one of the antigens is a

coronavirus antigen. In some examples, the circular polyribonucleotide comprises between 5 and 10 sequences, each encoding an antigen and at least one of the antigens is a coronavirus antigen. In some examples, the circular polyribonucleotide comprises sequences encoding antigens from at least two different microorganisms, and at least one microorganism is a coronavirus. In some examples, the linear
5 polyribonucleotide comprises sequences encoding two or more antigens and at least one antigen is a coronavirus antigen encoded by a sequence of a SEQ ID NO. in TABLE 3. In some examples, the linear polyribonucleotide comprises sequences encoding at least 2, 3, 4 or 5 antigens and at least one antigen is a coronavirus antigen encoded by a sequence of a SEQ ID NO. in TABLE 3. In some examples, the coronavirus antigen comprises an epitope. In some examples, the coronavirus antigen comprises an
10 epitope recognized by a B cell. In some examples, the coronavirus antigen comprises at least two epitopes.

In some examples, the composition or immunogenic composition comprising a circular polyribonucleotide further comprises a second circular polyribonucleotide comprising a sequence encoding a second antigen. In some examples, the composition or immunogenic composition further
15 comprises a second circular polyribonucleotide comprising a second ORF. In some examples, the composition or immunogenic composition further comprises a third, fourth, or fifth circular polyribonucleotide comprising a sequence encoding a third, fourth, or fifth antigen. In some examples, the composition or immunogenic composition further comprises a second linear polyribonucleotide comprising a sequence encoding a second antigen. In some examples, the composition or immunogenic
20 composition comprising the linear polyribonucleotide further comprises a second linear polyribonucleotide comprising a second ORF. In some examples, the composition or immunogenic composition further comprises a third, fourth, or fifth linear polyribonucleotide comprising a sequence encoding a third, fourth, or fifth antigen. In some examples, the first antigen, second antigen, third antigen, fourth antigen, and fifth antigen are different antigens.

25 In some examples, the composition or immunogenic composition further comprises a pharmaceutically acceptable carrier or excipient. In some examples, the polyribonucleotide is administered without a carrier (“naked”). In other examples, the polyribonucleotide is formulated with a carrier, e.g., an LNP, VLP, liposome, or the like.

30 In some examples, the composition further comprises an adjuvant. In some examples, the composition or immunogenic composition further comprises a diluent. In some examples, the composition or immunogenic composition further comprises protamine.

In another example, the disclosure features methods including (a) administering a composition described herein (e.g., a composition comprising (i) a circular polyribonucleotide comprising a sequence encoding a coronavirus antigen, e.g., a sequence selected from a SEQ ID NO. in TABLE 1, 2 or 3, or (ii)
35 a linear polyribonucleotide comprising a sequence selected from a SEQ ID NO. in TABLE 3) to a non-human animal or to a human subject (e.g., to induce an immune response against the antigen or to produce polyclonal antibodies against the antigen in the non-human animal or human subject for

immunization) and (b) optionally, collecting antibodies against the antigen from the non-human animal or the human subject (e.g., the non-human animal or human subject for immunization).

In some examples, the method further comprises administering an adjuvant (e.g., AddavaxTM adjuvant, MF59, AS03, complete Freund's adjuvant) to the non-human animal or to the human subject (e.g., the non-human animal or human subject for immunization). The adjuvant may be co-formulated and co-administered with the polyribonucleotide, or it may be formulated and administered separately.

In some examples, the method further comprises pre-administering (priming) the non-human animal or human subject (e.g., the non-human animal or human subject for immunization) with an agent, e.g., antigen, to improve immunogenic response. For example, the method includes administering the protein antigen to the non-human animal or human subject (e.g., the non-human animal or human subject for immunization) prior (e.g., from 1-7 days, e.g., 1, 2, 3, 4, 5, 6, 7 days prior) to administration of the polyribonucleotide comprising a sequence encoding the antigen. The protein antigen may be administered as a protein preparation, or encoded in a plasmid (pDNA), or presented in a virus-like-particle (VLP), formulated in a lipid nanoparticle (LNP), or the like.

In some examples, the method further comprises administering or immunizing the subject (e.g., the subject for immunization) with protamine.

In some examples, the polyribonucleotide is administered without a carrier ("naked"). In other examples, the polyribonucleotide is formulated with a carrier, e.g., an LNP, VLP, liposome, or the like.

In some examples, the method further comprises administering or immunizing the subject (e.g., the subject for immunization) with the polyribonucleotide (e.g., the circular or linear polyribonucleotide) at least two times, e.g., 2, 3, 4, 5 times.

In some examples, the method further comprises collecting plasma from the subject (e.g., after immunization of the subject for immunization). In some examples, the method further comprises purifying polyclonal antibodies from the subject (e.g., after immunization of the subject for immunization). In some examples, the method further comprises administering or immunizing the subject (e.g., the subject for immunization) with a vaccine. In some examples, the vaccine is pneumococcal polysaccharide vaccine (e.g., PCV13 or PPSV23). In some examples, the vaccine is for a bacterial infection. In some examples, the subject (e.g., the subject for immunization) is immunized with the circular RNA by injection. In some examples, the subject (e.g., the subject for immunization) is immunized with the linear RNA by injection.

In examples, the subject is a human subject (e.g., the human subject for immunization). In some examples, the human subject (e.g., the human subject for immunization) is a subject at risk for a coronavirus-related disease, e.g., a human over 50 years old; an immune-compromised human; a human with a chronic health condition such as obesity, diabetes, cancer; a health care worker.

In examples, the subject is a non-human animal (e.g., the non-human animal for immunization). In some examples, the non-human animal (e.g., the non-human animal for

immunization) is an agricultural animal, e.g., a cow, pig, sheep, horse, goat; a pet, e.g., a cat or dog; or a zoo animal, e.g., a feline.

In some examples, the non-human animal (e.g., the non-human animal for immunization) is a mammal, e.g., a rodent (e.g., a rabbit, rat or mouse), or an ungulate, e.g., a pig, cow, goat, or sheep. In some examples, the non-human animal (e.g., the non-human animal for immunization) is a transchromosomal non-human animal comprising a humanized immunoglobulin gene locus. In some examples, the non-human animal is a transchromosomal cow comprising a human artificial chromosome (HAC) vector that comprises the humanized immunoglobulin gene locus. In some examples, the humanized immunoglobulin gene locus encodes an immunoglobulin heavy chain. In some examples, the humanized immunoglobulin heavy chain comprises an IgG isotype heavy chain. In some examples, the humanized immunoglobulin heavy chain comprises an IgG1, IgG2, IgG3, or IgG4 isotype heavy chain.

In some examples, the non-human animal (e.g., the non-human animal for immunization) comprises a B cell having a B cell receptor, the B cell receptor binds to the coronavirus antigen. In some examples, the non-human animal comprises a plurality of B cells comprising a first B cell that binds to a first epitope of the coronavirus antigen and a second B cell that binds to a second epitope of the coronavirus antigen.

In some examples, the non-human animal (e.g., the non-human animal for immunization) comprises a T cell, wherein the T cell comprises a T Cell Receptor that binds to the coronavirus antigen. In some examples, upon activation, the T cell enhances production of an antibody that binds to the antigen. In some examples, upon activation, the T cell enhances antibody production by a B cell that binds to the coronavirus antigen. In some examples, upon activation, the T cell enhances survival, proliferation, plasma cell differentiation, somatic hypermutation, immunoglobulin class switching, or a combination thereof of a B cell that binds to the coronavirus antigen.

In some examples, the non-human animal or human subject (e.g., the non-human animal or human subject for immunization) produces an antibody that specifically binds to the coronavirus antigen. In some examples, the antibody is a humanized antibody or a fully human antibody. In some examples, the antibody is an IgG, IgA, or IgM isotype antibody. In some examples, the antibody is an IgG1, IgG2, IgG3, or IgG4 isotype antibody. In some examples, the non-human animal (e.g., the non-human animal for immunization) comprises plurality of polyclonal antibodies that specifically bind at least two epitopes that are encoded by the circular polyribonucleotide. In some examples, the non-human animal or human subject (e.g., the non-human animal or human subject for immunization) comprises plurality of polyclonal antibodies that specifically bind at least two epitopes that are encoded by the linear RNA. In some examples, the plurality of antibodies comprises humanized antibodies. In some examples, the plurality of polyclonal antibodies comprises fully human antibodies. In some examples, the plurality of polyclonal antibodies comprise IgG antibodies, IgG1 antibodies, IgG2 antibodies, IgG3 antibodies, IgG4 antibodies, IgM antibodies, IgA antibodies, or a

combination thereof. In some examples, the immunoglobulin heavy chain comprises an IgM or IgA isotype heavy chain. In some examples, the humanized immunoglobulin gene locus encodes an immunoglobulin light chain. In some examples, the immunoglobulin light chain comprises a kappa light chain or a lambda light chain.

5 In some examples, the method further includes collecting blood from the non-human animal or human subject (e.g., after immunizing the non-human animal or human subject for immunization), and purifying antibodies against the antigen from the blood.

 In another example, the disclosure features an anti-coronavirus antibody preparation (e.g., a polyclonal antibody preparation) produced by (a) administering a composition comprising a
10 polyribonucleotide described herein to a non-human animal described herein (e.g., a cow having a humanized immune system as described herein) or to a human subject (e.g., the non-human animal or human subject for immunization), and (b) collecting antibodies against the antigen from the non-human animal or human subject (e.g., after immunizing the non-human animal or human subject for immunization).

15 In examples, the polyribonucleotide is (a) a circular polyribonucleotide comprising a sequence encoding a coronavirus antigen, e.g., a sequence selected from a SEQ ID NO. in TABLE 1, 2 or 3, or (b) a linear polyribonucleotide comprising a sequence selected from a SEQ ID NO. in TABLE 3.

 In examples, the antibody preparation is formulated as a pharmaceutical composition.

 In another example, the disclosure features a method of delivering antibodies against a
20 coronavirus to a subject (e.g., the subject for treatment) having a coronavirus infection, at risk of exposure to a coronavirus infection, or in need thereof, e.g., a method of preventing or treating the subject (e.g., the subject for treatment) for a coronavirus infection. The method includes administering to the subject (e.g., the subject for treatment) having a coronavirus infection, at risk of exposure to a coronavirus infection, or in need thereof polyclonal antibodies produced from an animal (e.g., a mammal) having a human or
25 humanized immune system, that has been immunized with a polyribonucleotide described herein, e.g., (a) a circular polyribonucleotide comprising a sequence encoding a coronavirus antigen, e.g., a sequence selected from a SEQ ID NO. in TABLE 1, 2 or 3, or (b) a linear polyribonucleotide comprising a sequence selected from a SEQ ID NO. in TABLE 3.

30 In certain examples, the method further includes one or more of: immunizing a non-human animal (e.g., a non-human animal for immunization) that has been genetically modified to produce human antibodies with a polyribonucleotide disclosed herein, collecting blood from the non-human animal, purifying antibodies from the non-human animal, formulating the antibodies for pharmaceutical use, and administering the formulated antibodies to the human subject (e.g., the human subject for treatment).

35 In some examples, the mammal having a human or humanized immune system is a human (e.g., a human subject for immunization).

 In some examples, the mammal having a human or humanized immune system is a non-human animal that has been genetically modified to produce human antibodies, e.g. a non-human animal

comprising a humanized immunoglobulin gene locus, e.g., a transchromosomal cow comprising a human artificial chromosome (HAC) vector that comprises a human immunoglobulin gene locus.

In examples, the subject (e.g., the subject for treatment) having a coronavirus infection or in need thereof is a human subject diagnosed with a coronavirus-related disease, e.g., Covid-19, SARS, MERS.

5 In some examples, the subject (e.g., the subject for treatment) at risk of exposure to a coronavirus infection or in need thereof is a subject at risk for a coronavirus-related disease, e.g., a human over 50 years old; an immune-compromised human; a human with a chronic health condition such as obesity, diabetes, cancer; a health care worker.

10 In some examples, the administration or immunization is before, after, or simultaneously with risk of exposure to the coronavirus.

In some examples, the method further comprises monitoring the human subject (e.g., the subject for treatment) for the presence of antibodies to coronavirus, e.g., before and/or after administration.

Exemplary examples of the disclosure are described in the enumerated paragraphs below. E1. An immunogenic composition comprising:

15 a) a circular polyribonucleotide comprising a sequence encoding a coronavirus antigen; or
b) a linear polyribonucleotide comprising a sequence selected from any one of SEQ ID NOs: 13, 15, and 12.

E2. A immunogenic composition comprising a circular polyribonucleotide comprising a sequence encoding a coronavirus antigen, wherein the coronavirus antigen comprises a sequence having
20 at least about 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% sequence identity to a coronavirus antigen selected from any one of SEQ ID NOs: 1-10, 13, 15, 17 19, 21, 23, 25-30, 48, and 49, or the circular polyribonucleotide comprises a sequence having at least about 80%, 85%, 90%, 95%, 97%, 98%, 99%, or 100% sequence identity to a circular polyribonucleotide selected from SEQ ID NOs: 12, 14, 16, 18, 20, 22, and 24.

25 E3. The immunogenic composition of any one of the preceding examples, further comprising plasma from a non-human animal (e.g., a non-human animal comprising a humanized immune system; e.g., a non-human animal for immunization) or a human subject (e.g., a human subject for immunization).

E4. The immunogenic composition of any one of the preceding examples, further comprising the coronavirus antigen.

30 E5. The immunogenic composition of any one of the preceding examples, wherein the composition further comprises a non-human B cell comprising a humanized immunoglobulin gene locus and a humanized B cell receptor, wherein the humanized B cell receptor binds to the coronavirus antigen.

E6. The immunogenic composition of any one of the preceding examples, wherein the composition further comprises a plurality of non-human B cells, wherein a non-human B cell of the plurality
35 comprises a humanized immunoglobulin gene locus, wherein the plurality of B cells comprises a

first B cell that binds to a first epitope of the coronavirus antigen and a second B cell that binds to a second epitope of the coronavirus antigen.

E7. The immunogenic composition of any one of the preceding examples, wherein the coronavirus antigen is from a betacoronavirus or a fragment thereof or a sarbecovirus or a fragment thereof.

5 E8. The immunogenic composition of any one of the preceding examples, wherein the coronavirus antigen is from severe acute respiratory syndrome-related coronavirus or a fragment thereof.

E9. The immunogenic composition of any one of the preceding examples, wherein the coronavirus antigen is from severe acute respiratory syndrome (SARS)-related coronavirus or a fragment thereof.

10 E10. The immunogenic composition of any one of the preceding examples, wherein the coronavirus antigen is from severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) or a fragment thereof, severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) or a fragment thereof, or Middle East respiratory syndrome coronavirus (MERS-CoV) or a fragment thereof.

15 E11. The immunogenic composition of any one of the preceding examples, wherein the coronavirus antigen is a membrane protein or a variant or fragment thereof, an envelope protein of a virus or a variant or fragment thereof, a spike protein of a virus or a variant or fragment thereof, a nucleocapsid protein of a virus or a variant or fragment thereof, an accessory protein of a virus or a variant or fragment thereof.

E12. The immunogenic composition of any one of the preceding examples wherein the coronavirus antigen is a receptor binding domain of spike protein or a variant or fragment thereof.

20 E13. The immunogenic composition of example 8, wherein the spike protein lacks a cleavage site.

E14. The immunogenic composition of any one of the preceding examples, wherein an accessory protein of a coronavirus is selected from a group consisting of ORF3a, ORF7a, ORF7b, ORF8, ORF10, or any variant or fragment thereof.

25 E15. The immunogenic composition of any one of the preceding examples, wherein the circular polyribonucleotide comprises a plurality of sequences, each encoding an antigen, and at least one sequence encodes a coronavirus antigen.

E16. The immunogenic composition of any one of the preceding examples, wherein the circular polyribonucleotide comprises two or more ORFs.

30 E17. The immunogenic composition of any one of the preceding examples, wherein the circular polyribonucleotide comprises at least five sequences, each encoding an antigen, and at least one antigen is a coronavirus antigen.

E18. The immunogenic composition of any one of the preceding examples, wherein the circular polyribonucleotide comprises at least two ORFs (e.g., at least 2, 3, 4, or 5).

- E19. The immunogenic composition of any one of the preceding examples, wherein the circular polyribonucleotide comprises sequences encoding antigens from at least two different microorganisms, and at least one microorganism is a coronavirus.
- 5 E20. The immunogenic composition of any one of the preceding examples, wherein the linear polyribonucleotide comprises sequences encoding two or more antigens and at least one antigen is the coronavirus antigen.
- E21. The immunogenic composition of any one of the preceding examples, wherein the linear polyribonucleotide comprises sequences encoding at least 2, 3, 4, or 5 antigens and at least one antigen is a coronavirus antigen encoded by a sequence of SEQ ID NO. in TABLE 3.
- 10 E22. The immunogenic composition of any one of the preceding examples, wherein the coronavirus antigen comprises an epitope.
- E23. The immunogenic composition of any one of the preceding examples, wherein the coronavirus antigen comprises an epitope recognized by a B cell.
- E24. The immunogenic composition of any one of the preceding examples, wherein the coronavirus antigen comprises at least two epitopes.
- 15 E25. The immunogenic composition of any one of the preceding examples, further comprising a second circular polyribonucleotide comprising a sequence encoding a second antigen.
- E26. The immunogenic composition of any one of the preceding examples, further comprising a second circular polyribonucleotide comprising a second ORF.
- 20 E27. The immunogenic composition of any one of the preceding examples, further comprising a third, fourth, or fifth circular polyribonucleotide comprising a sequence encoding a third, fourth, or fifth antigen.
- E28. The immunogenic composition of any one of the preceding examples, further comprising a second linear polyribonucleotide comprising a sequence encoding a second antigen.
- 25 E29. The immunogenic composition of any one of the preceding examples, further comprising a second linear polyribonucleotide comprising a second ORF.
- E30. The immunogenic composition of any one of the preceding examples, further comprising a third, fourth, or fifth linear polyribonucleotide comprising a sequence encoding a third, fourth, or fifth antigen.
- 30 E31. The immunogenic composition of any one of the preceding examples, wherein the first antigen, second antigen, third antigen, fourth antigen, and fifth antigen are different antigens.
- E32. The immunogenic composition of any one of the preceding examples, wherein the immunogenic composition further comprises a pharmaceutically acceptable carrier or excipient.
- E33. The immunogenic composition of any one of the preceding examples, wherein the immunogenic composition further comprises a pharmaceutically acceptable excipient and is free of any carrier.
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E34. The immunogenic composition of any one of the preceding examples, wherein the circular polyribonucleotide, linear polyribonucleotide, or immunogenic composition is formulated with a carrier (e.g., a lipid nanoparticle, virus-like particle, or a liposome).

5 E35. The immunogenic composition of any one of the preceding examples, wherein the immunogenic composition further comprises an adjuvant.

E36. The immunogenic composition of example 35, wherein the adjuvant is a saponin or an oil emulsion.

E37. The immunogenic composition of example 36, wherein the oil emulsion is a squalene-water emulsion (e.g., Addavax™ adjuvant, MF59 or AS03).

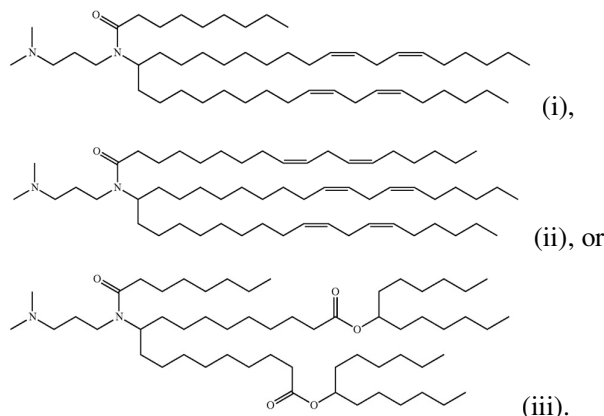
10 E38. The immunogenic composition of any one of the preceding examples, wherein the immunogenic composition further comprises a diluent.

E40. A lipid nanoparticle (LNP) comprising the immunogenic composition of any one of the preceding examples.

E41. The LNP of example 40, comprising an ionizable lipid.

15 E42. The LNP of example 40, comprising a cationic lipid.

E43. The LNP of example 42, wherein the cationic lipid has a structure according to:



20 E44. The LNP of any one of examples 40 to 43, further comprising one or more neutral lipids, e.g., DSPC, DPPC, DMPC, DOPC, POPC, DOPE, SM, a steroid, e.g., cholesterol, and/or one or more polymer conjugated lipid, e.g., a pegylated lipid, e.g., PEG-DAG, PEG-PE, PEG-S-DAG, PEG-cer or a PEG dialkoxypylpropylcarbamate.

25 E45. A method of delivering an immunogenic composition to a non-human animal or human subject (e.g., a non-human animal or human subject for immunization) comprising: a) administering the immunogenic composition of any one of the preceding examples to the non-human animal or human subject, and b) optionally, collecting antibodies against the coronavirus antigen from the non-human animal or human subject.

30 E46. A method of inducing an immune response against a coronavirus antigen in a non-human animal or human subject (e.g., a non-human animal or human subject for immunization) comprising: a) administering the immunogenic composition of any one of the preceding examples to the non-human

animal or human subject, and b) optionally, collecting antibodies against the coronavirus antigen from the non-human animal or human subject.

5 E47. The method of any one of the preceding examples, further comprising administering an adjuvant to the non-human animal or human subject (e.g., a non-human animal or human subject for immunization).

E48. The method of example 47, wherein the adjuvant is co-formulated and co-administered with the immunogenic composition, or is formulated and administered separately from the immunogenic composition.

10 E49. The method of any one of the preceding examples, further comprising administering (e.g., pre-administering or priming) the non-human animal or human subject (e.g., a non-human animal or human subject for immunization) with the coronavirus antigen prior to administration of the immunogenic composition.

15 E50. The method of any one of the preceding examples, further comprising administering the coronavirus antigen to the non-human animal or human subject (e.g., a non-human animal or human subject for immunization) from 1 to 7 days prior (e.g., 1, 2, 3, 4, 5, 6, or 7 days prior) to administering the immunogenic composition.

E51. The method of any one of the preceding examples, wherein the coronavirus antigen is administered as a protein preparation, encoded in a plasmid (pDNA), presented in a virus-like particle (VLP), or formulated in a lipid nanoparticle (LNP).

20 E52. The method of any one of the preceding examples, further comprising administering the circular polyribonucleotide or linear polyribonucleotide without a carrier.

E53. The method of any one of the preceding examples, further comprising formulating the immunogenic composition with a carrier (e.g., lipid nanoparticle, virus-like particle, or liposome).

25 E54. The method of any one of the preceding examples, further comprising administering or immunizing the circular polyribonucleotide or linear polyribonucleotide at least two times, (e.g., 2, 3, 4, or 5 times) to the non-human animal or human subject (e.g., a non-human animal or human subject for immunization).

E55. The method of any one of the preceding examples, further comprising collecting plasma from the non-human animal or human subject (e.g., a non-human animal or human subject for immunization).

30 E56. The method of any one of the preceding examples, further comprising purifying polyclonal antibodies from the plasma of a non-human animal or human subject (e.g., a non-human animal or human subject for immunization).

35 E57. The method of any one of the preceding examples, further comprising administering or immunizing the non-human animal or human subject (e.g., a non-human animal or human subject for immunization) with a vaccine.

E58. The method of example 51, wherein the vaccine is pneumococcal polysaccharide vaccine (e.g., PCV13 or PPSV23).

E59. The method of example 57, wherein the vaccine is for a bacterial infection.

5 E60. The method of any one of the preceding examples, wherein the non-human animal or human subject (e.g., a non-human animal or human subject for immunization) is immunized with the circular polyribonucleotide or linear polyribonucleotide by injection.

E61. The method of any one of the preceding examples, wherein the human subject (e.g., the human subject for immunization) at risk for a coronavirus-related disease.

10 E62. The method of any one of the preceding examples, wherein the human subject (e.g., the human subject for immunization) is a human over 50 years old, an immune-compromised human, a human with a chronic health condition (e.g., obesity, diabetes, cancer), or a health care worker.

E63. The method of any one of the preceding examples, wherein the non-human animal (e.g., the non-human animal subject for immunization) is an agricultural animal (e.g., a pig, cow, goat, chicken, sheep).

15 E64. The method of any one of the preceding examples, wherein the non-human animal (e.g., the non-human animal subject for immunization) is a pet (e.g., a dog or cat), a zoo animal (e.g., a feline), a mammal (e.g., an ungulate (e.g., a pig, a cow, a goat, a sheep), a rodent (e.g., a rabbit, a rat, a mouse).

E65. The method of any one of the preceding examples, wherein the non-human animal is a transchromosomal non-human animal comprising a humanized immunoglobulin gene locus.

20 E66. The method of example 65, wherein the non-human animal is a transchromosomal cow comprising a human artificial chromosome (HAC) vector that comprises the humanized immunoglobulin gene locus.

E67. The method of any one of examples 65 or 66, wherein the humanized immunoglobulin gene locus encodes an immunoglobulin heavy chain.

25 E68. The method of example 67, wherein the humanized immunoglobulin heavy chain comprises an IgG isotype heavy chain.

E69. The method of any one of examples 67 or 68, wherein the humanized immunoglobulin heavy chain comprises an IgG1, IgG2, IgG3, or IgG4 isotype heavy chain.

30 E70. The method of any one of examples 65-69, wherein the humanized immunoglobulin gene locus encodes an immunoglobulin light chain.

E71. The method of example 70, wherein the immunoglobulin light chain comprises a kappa light chain or a lambda light chain.

E72. The method of any one of the preceding examples, wherein the non-human animal comprises a B cell having a B cell receptor, and wherein the B cell receptor binds to the antigen.

35 E73. The method of any one of the preceding examples, wherein the non-human animal comprises a plurality of B cells comprising a first B cell that binds to a first epitope of the coronavirus antigen and a second B cell that binds to a second epitope of the coronavirus antigen.

- E74. The method of any one of the preceding examples, wherein the non-human animal comprises a T cell, and wherein the T cell comprises a T cell receptor that binds to the coronavirus antigen.
- E75. The method of any one of the preceding examples, wherein upon activation, the T cell enhances production of an antibody that binds to the coronavirus antigen.
- 5 E76. The method of any one of the preceding examples, wherein upon activation, the T cell enhances antibody production by a B cell that binds to the coronavirus antigen.
- E77. The method of any one of the preceding examples, wherein upon activation, the T cell enhances survival, proliferation, plasma cell differentiation, somatic hypermutation, immunoglobulin class switching, or a combination thereof of a B cell that binds to the coronavirus antigen.
- 10 E78. The method of any one of the preceding examples, further comprising purifying polyclonal antibodies against the coronavirus antigen from the plasma of the non-human animal or human subject (e.g., the non-human animal or human subject for immunization).
- E79. The method of any one of the preceding examples, wherein an antibody of the polyclonal antibodies specifically binds to the coronavirus antigen.
- 15 E80. The method of any one of the preceding examples, wherein an antibody of the polyclonal antibodies is a humanized antibody or a fully human antibody.
- E81. The method of any one of the preceding examples, wherein an antibody of the polyclonal antibodies is an IgG, IgA, or IgM isotype antibody.
- E82. The method of any one of the preceding examples, wherein an antibody of the polyclonal antibodies is an IgG1, IgG2, IgG3, or IgG4 isotype antibody.
- 20 E83. The method of any one of the preceding examples, wherein the non-human animal comprises a plurality of polyclonal antibodies that specifically bind at least two epitopes that are encoded by the circular polyribonucleotide.
- E84. The method of any one of the preceding examples, wherein the non-human animal comprises a plurality of polyclonal antibodies that specifically bind at least two epitopes that are encoded by the linear polyribonucleotide.
- 25 E85. The method of any one of the examples 81 or 82, wherein the plurality of polyclonal antibodies comprises humanized antibodies.
- E86. The method of any one of the examples 83 or 84, wherein the plurality of polyclonal antibodies comprises fully human antibodies.
- 30 E87. The method of any one of the examples 83-86, wherein the plurality of polyclonal antibodies comprise IgG antibodies, IgG1 antibodies, IgG2 antibodies, IgG3 antibodies, IgG4 antibodies, IgM antibodies, IgA antibodies, or a combination thereof.
- E88. The method of any one of examples 83-86, wherein the plurality of polyclonal antibodies comprise humanized immunoglobulin gene loci comprising an IgM or IgA isotype heavy chains.
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E89. The method of any one of examples 83-88, wherein the plurality of polyclonal antibodies comprise humanized immunoglobulin gene loci encoding immunoglobulin light chains.

E90. The method of example 89, wherein the immunoglobulin light chains comprises kappa light chains or lambda light chains.

5 E91. The method of any one of the preceding examples, further comprising collecting blood from the non-human animal or human subject (e.g., the non-human animal or human subject for immunization) and purifying antibodies against the coronavirus antigen from the blood.

E92. A method of producing a polyclonal antibody preparation against a coronavirus antigen (e.g., an anti-coronavirus antibody preparation), comprising:

- 10 a) administering to the immunogenic composition of any one of the preceding examples to a non-human animal or human subject (e.g., the non-human animal or human subject for immunization); and
b) collecting blood or plasma from the non-human animal or human subject.

E93. The method of example 92, wherein the polyclonal antibody preparation is formulated as a pharmaceutical composition or veterinary composition.

15 E94. A method of delivering a polyclonal antibody preparation against a coronavirus to a subject (e.g. a subject for treatment) having a coronavirus infection, comprising administering the polyclonal antibody preparation of any one of the preceding examples to the subject having a coronavirus infection.

E95. A method of delivering a polyclonal antibody preparation to a subject (e.g. a subject for treatment) at risk for exposure to a coronavirus infection, comprising administering the polyclonal
20 antibodies preparation of any one of the preceding examples to the subject at risk for exposure to a coronavirus infection.

E96. A method of preventing or treating a subject (e.g. a subject for treatment) in need thereof for coronavirus infection comprising administering the polyclonal antibodies preparation of any one of the preceding examples to the subject in need thereof.

25 E97. The method of any one for the preceding examples, further comprising:

- a) immunizing a non-human animal that has been genetically modified to produce human antibodies with the circular polyribonucleotide of any one of the preceding examples or the linear polyribonucleotide of any one of the preceding examples;
b) collecting blood from the non-human animal;
30 c) purifying antibodies from the non-human animal;
d) formulating the antibodies for pharmaceutical use; and
e) administering the formulated antibodies to a human subject.

E98. The method of example 97, wherein the non-human animal has a humanized immune system.

E99. The method of example 97, wherein the non-human animal has a humanized immunoglobulin
35 gene locus.

E100. The method of example 97, wherein the non-human animal is a transchromosomal cow comprising a human artificial chromosome (HAC) vector that comprises a human immunoglobulin gene locus

5 E101. The method of any one of the preceding examples, wherein the administration or immunization is before, after, or simultaneously with the subject in need thereof's risk of exposure to the coronavirus.

E102. The method of any one of the preceding examples, wherein the subject (e.g. a subject for treatment) having a coronavirus infection, the subject at risk for exposure to a coronavirus infection, or the subject in need thereof is a human subject .

10 E103. The method of example 102, wherein the human subject (e.g. a human subject for treatment) is a human over 50 years old, an immune-compromised human, a human with a chronic health condition (e.g., obesity, diabetes, or cancer), or a health care worker.

15 E104. The method of any one of the preceding examples, wherein the subject (e.g. a subject for treatment) at risk for exposure to a coronavirus infection, or the subject in need thereof is a human subject at risk for a coronavirus related disease.

E105. The method of any one of the preceding examples, wherein the subject (e.g. a subject for treatment) having a coronavirus infection, the subject at risk for exposure to a coronavirus infection, or the subject in need thereof is a human subject diagnosed with a coronavirus-related disease (e.g., Covid-19, SARS, MERS).

20 E106. The method of any one of the preceding examples, wherein the subject (e.g. a subject for treatment) having a coronavirus infection, the subject (e.g. a subject for treatment) at risk for exposure to a coronavirus infection, or the subject (e.g. a subject for treatment) in need thereof is a non-human animal subject.

25 E107. The method of any one of the preceding examples, wherein the subject(e.g. a subject for treatment) having a coronavirus infection, the subject (e.g. a subject for treatment) at risk for exposure to a coronavirus infection, or the subject (e.g. a subject for treatment) in need thereof is an agricultural animal (e.g., cow, pig, sheep, horse, goat), pet (e.g., a cat or dog), or zoo animal (e.g., a feline).

30 E108. The method of any one of the preceding examples, further comprising monitoring the subject (e.g. a subject for treatment) having a coronavirus infection, the subject (e.g. a subject for treatment) at risk for exposure to a coronavirus infection, or the subject in need thereof for the presence of the polyclonal antibodies.

E109. The method of any one of the preceding examples, wherein the monitoring is prior to administration of the polyclonal antibodies and/or after the administration of the polyclonal antibodies.