

IMMUNOGENIC COMPOSITIONS AND USES THEREOF

Sequence Listing

The instant application contains a Sequence Listing which has been submitted electronically in
5 ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on May
18, 2021, is named 51509-024WO3_Sequence_Listing_05.18.21_ST25 and is 41,145 bytes in size.

Background

Vaccination has made an enormous contribution to both human and animal health. Since the
10 invention of the first vaccine in 1796, vaccines have come to be considered the most successful method
for preventing many infectious diseases by provoking an immune response in a subject. According to the
World Health Organization, immunization currently prevents 2-3 million deaths every year across all age
groups. Today, vaccines have been developed to prevent and control the spread of more than 20
infectious diseases, including diphtheria, tetanus, pertussis, influenza, and measles, and have led to the
15 complete eradication of smallpox. There remains a need to develop new and improved immunogenic
compositions and uses thereof.

Summary

This disclosure provides compositions, pharmaceutical preparations, and uses of
20 polyribonucleotides (e.g., circular or linear polyribonucleotides) encoding one or more immunogens. In
particular, the disclosure provides circular polyribonucleotides encoding multiple immunogens and
compositions including multiple circular polyribonucleotides. This disclosure further relates to methods of
using the circular polyribonucleotides encoding one or more polypeptide immunogens. Compositions and
pharmaceutical preparations of circular polyribonucleotides described herein may induce an immune
25 response in a subject upon administration. Compositions and pharmaceutical preparations of circular
polyribonucleotides described herein may be used to treat or prevent a disease, disorder, or condition in a
subject.

In one aspect, the present disclosure refers to a circular polyribonucleotide comprising a plurality
of sequences, each sequence encoding a polypeptide immunogen, wherein at least two of the
30 polypeptide immunogens identify different targets, wherein each target is a different pathogen.

In one aspect, the present disclosure refers to a circular polyribonucleotide comprising a plurality
of sequences, each sequence encoding a polypeptide immunogen, wherein at least two of the
polypeptide immunogens identify different proteins, wherein each of the different proteins identifies the
same target.

35 In one aspect, the present disclosure refers to an immunogenic composition comprising a
plurality of circular polyribonucleotides, each comprising a sequence encoding a polypeptide immunogen,
wherein the composition comprises (a) at least a first circular polyribonucleotide comprising a sequence
encoding a first polypeptide immunogen and (b) at least a second circular polyribonucleotide comprising a
sequence encoding a second polypeptide immunogen, wherein the first and second polypeptide
40 immunogens identify different proteins, wherein each different protein identifies the same target.

In one aspect, the present disclosure refers to an immunogenic composition comprising a plurality of circular polyribonucleotides, each comprising a sequence encoding a polypeptide immunogen, wherein the composition comprises (a) at least a first circular polyribonucleotide comprising a sequence encoding a first polypeptide immunogen and (b) at least a second circular polyribonucleotide comprising a sequence encoding a second polypeptide immunogen, wherein the first polypeptide immunogen identifies a first target and the second polypeptide immunogen identifies a second target.

In one aspect, the present disclosure refers to a pharmaceutical composition comprising any one of the circular polyribonucleotides disclosed herein or any one of the immunogenic compositions disclosed herein, and a pharmaceutically acceptable excipient.

In one aspect, the present disclosure refers to use of any one of the circular polyribonucleotides disclosed herein, any one of the immunogenic compositions disclosed herein, or any one of the pharmaceutical compositions disclosed herein, in the manufacture of a medicament for treating or preventing a disease, disorder, or condition in a subject.

In one aspect, the present disclosure refers to use of any one of the circular polyribonucleotides disclosed herein, any one of the immunogenic compositions disclosed herein, or any one of the pharmaceutical compositions disclosed herein, in the manufacture of a medicament for inducing an immune response in a subject.

In one example, the disclosure provides a circular polyribonucleotide including a plurality of sequences, each sequence encoding a polypeptide immunogen, wherein at least two (e.g., at least three, at least four, at least five, at least six, at least seven, at least eight, or at least nine) of the polypeptide immunogens identify different proteins, wherein each of the different proteins identifies the same target.

In some examples, each of the polypeptide immunogens identifies a different protein. In some examples, the target is a pathogen. In some examples, the pathogen is a virus, a bacterium, a fungus, or a parasite. In some examples, the pathogen is a virus and each of the different proteins is a viral protein associated with the virus. In some examples, the pathogen is a bacterium and each of the different proteins is a bacterial protein associated with the bacteria. In some examples the target is a cancer cell. In some examples, each of the different proteins is a different tumor antigen associated with the cancer cell. In some examples, the target is an allergen or a toxin.

In another example, the disclosure provides a circular polyribonucleotide including a plurality of sequences, each sequence encoding a polypeptide immunogen, wherein at least two (e.g., at least three, at least four, at least five, at least six, at least seven, at least eight, or at least nine) of the polypeptide

immunogens identify different targets. In some examples, each of the polypeptide immunogens identifies a different target. In some examples, each target is a different pathogen. In some examples, each target is, independently, a cancer cell, a virus, a bacterium, a fungus, or a parasite. In some examples, each target is a different virus. In some examples, each target is a different bacterium. In some examples, the targets include a virus and a bacterium.

In some examples, each of the plurality of immunogens encoded by the circular polyribonucleotide share less than 90% sequence identity.

In some examples of any one of the circular polyribonucleotides described herein, the circular polyribonucleotide includes between 500 and 20,000 ribonucleotides (e.g., between 500 and 10,000, 500 and 9,000, 500 and 8,000, 500 and 5,000, 500 and 4,000, 500 and 3,000, 1000 and 10,000, 1,000 and 8,000, 1,000 and 5,000, 3,000 and 8,000, 4,000 and 9,000, or 10,000 and 20,000)). In some examples, the circular polyribonucleotide includes between 500 and 5,000. In some examples, the circular polyribonucleotide includes between 1,000 and 5,000 ribonucleotides. In some examples, the circular polyribonucleotide includes between 5,000 and 10,000 ribonucleotides. In some examples, the circular polyribonucleotide includes at least 500 ribonucleotides (e.g. at least 600, at least 1000, at least 1500, at least 2000, at least 2500, at least 3000, at least 3500, at least 4000, at least 4500, at least 5000, at least 5500, at least 6000, at least 6500, at least 7000, at least 7500, at least 8000, at least 8500, at least 9000, or at least 9500 ribonucleotides).

In some examples, the circular polyribonucleotide includes at least three, at least four, at least five, at least six, at least seven, at least eight, or at least nine sequences, each sequence encoding a polypeptide immunogen. In some examples, the circular polyribonucleotide includes two or three, between two and five (e.g., two, three or four), or between five and ten sequences (e.g., five, six, seven, eight, nine, or sequences), each sequence encoding a polypeptide immunogen.

In some examples, at least one sequence encoding a polypeptide immunogen further encodes a signal sequence. In some examples, each sequence encoding a polypeptide immunogen further encodes a signal sequence. In some examples, each of the sequences encoding each of the polypeptide immunogens is operably linked to an internal ribosomal entry site (IRES). In some examples, the circular polyribonucleotide includes a single IRES. In some examples, each of the polypeptide immunogens is encoded by a single open-reading frame operably linked to the single IRES, wherein the expression of the open reading frame produces a polypeptide including the amino acid sequence of each the polypeptide immunogens.

In some examples, the polypeptide immunogens are each separated by a polypeptide linker. In some examples, the polypeptide immunogens are each separated by a cleavage domain. In some examples, each stagger element is a 2A self-cleaving peptide. In some examples, the circular polyribonucleotide includes a plurality of IRESs. In some examples, each IRES is operably linked to an open reading frame including a sequence encoding a polypeptide immunogen.

In some examples, at least once sequence encoding an immunogen further encodes a signal sequence. In some examples, each sequence encoding an immunogen further encodes a signal sequence. In some examples, at least one sequence encoding an immunogen does not encode a signal sequence. In some examples, none of the sequences encoding an immunogen further encodes a signal sequence.

In some examples of any one of the circular polyribonucleotides described herein, wherein the circular polyribonucleotide includes a first polyribonucleotide with a 5' end and a 3' end, wherein 5' end and 3' end are each hybridized to a second polynucleotide, there by linking the 5' end and the 3' end of the first polyribonucleotide to form a circular polyribonucleotide. In some examples, the circular polyribonucleotide is produced by splint ligation. In some examples, the circular polyribonucleotide is produced by providing a linear polyribonucleotide having a 3' end and a 5' end, wherein the 3' end and the 5' end each include a portion of an intron, and wherein the intron portion of the 3' end and the intron portion of the 5' end catalyze a splicing reaction thereby covalently conjugating the 5' end and the 3' end to produce a circular polyribonucleotide. In some examples, the intron is a Group I self-splicing intron.

In another example the disclosure provides a composition including a plurality of circular polyribonucleotides, each including a sequence encoding a polypeptide immunogen. In some examples, each of the plurality of circular polyribonucleotides is a circular polyribonucleotide described herein. In some examples, each of the polypeptide immunogens includes one or more epitopes that identifies a target. In some examples, the composition includes at least a first circular polyribonucleotide including a sequence encoding a first polypeptide immunogen and at least a second circular polyribonucleotide including a sequence encoding a second polypeptide immunogen, wherein the first and second polypeptide immunogens identify different proteins, wherein each different protein identifies the same target. In some examples, the composition includes at least a first circular polyribonucleotide including a sequence encoding a first polypeptide immunogen and at least a second circular polyribonucleotide including a sequence encoding a second polypeptide immunogen, wherein the first polypeptide immunogens identifies a first target and the second polypeptide immunogen identifies a second target. In some examples, each target is, independently, a cancer cell, a virus, a bacterium, a fungus, a parasite, a toxin, or an allergen. In some examples, the target is a pathogen. In some examples, the pathogen is a virus, a bacterium, a fungus, or a parasite. In some examples, the target is a cancer cell, an allergen, or a toxin. In some examples, each polypeptide immunogen is operably linked to an IRES.

In another example the disclosure provides a pharmaceutical composition including any one of the circular polyribonucleotides, compositions, or pharmaceutical preparations described herein and a pharmaceutically acceptable excipient. In some examples, the pharmaceutical composition includes any one of the circular polyribonucleotides, compositions, or pharmaceutical preparations described herein and protamine or a protamine salt (e.g., protamine sulfate). In some examples, the pharmaceutical composition further includes an adjuvant. In some examples, the adjuvant is an inorganic adjuvant, a small molecule adjuvant, and oil in water emulsion, a lipid or polymer, a peptide or peptidoglycan, a carbohydrate or polysaccharide, a saponin, an RNA-based adjuvant, a DNA-based adjuvant, a viral particle, a bacterial adjuvant, a hybrid molecule, a fungal or oocyte microbe-associated molecular pattern (MAMP), an inorganic nanoparticle, or a multi-component adjuvant. In some examples, the adjuvant is an inorganic adjuvant. In some examples, the inorganic adjuvant is an aluminum salt or a calcium salt. In some examples, the adjuvant is a small molecule. In some examples, the small molecule is imiquimod, resiquimod, or gardiquimod. In some examples, the adjuvant is an oil in water emulsion. In some examples, the oil in water emulsion is Squalene, MF59, AS03, a Montanide formulation, optionally Montanide ISA 51 or Montanide ISA 720, or

Incomplete Freund's Adjuvant an oil in water emulsion. In some examples, the adjuvant is a lipid or polymer. In some examples, the lipid or polymer is a polymeric nanoparticle, optionally PLGA, PLG, PLA, PGA, or PHB, a liposome, optionally a virosome or CAF01, a lipid nanoparticle or a component thereof, a lipopolysaccharide (LPS), optionally monophosphoryl lipid A (MPLA) or glucopyranosyl Lipid A (GLA), a lipopeptide, optionally Pam2 (Pam2CSK4) or Pam3 (Pam3CSK4), or a glycolipid, optionally, 5 trehalose dimycolate. In some examples, the adjuvant is a peptide or peptidoglycan. In some examples, the peptide or peptidoglycan corresponds to all or a portion of a synthetic or purified gram negative or gram positive bacteria, optionally N-acetyl-muramyl-L-alanyl-D-isoglutamine (MDP), a flagellin-fusion protein, Mannose-binding Lectin (MBL), a cytokine, or a chemokine. In some examples, 10 the adjuvant is a carbohydrate or polysaccharide. In some examples, the carbohydrate or polysaccharide is dextran (branched microbial polysaccharide), dextran-sulfate, Lentinan, zymosan, Beta-glucan, Deltin, Mannan, or Chitin. In some examples, the adjuvant is a saponin. In some examples, the saponin is a glycoside or a polycyclic aglycones attached to one or more sugar side chains, optionally ISCOMS, ISCOMS matrix, or QS-21. In some examples, the adjuvant is an RNA-based adjuvant. In some examples, the RNA-based adjuvant is Poly IC, Poly IC:LC, a hairpin RNAs, 15 optionally with a 5'PPP containing sequence, a viral sequence, a polyU containing sequences, dsRNA, a natural or synthetic immunostimulatory RNA sequence, a nucleic acid analog, optionally cyclic GMP-AMP or a cyclic dinucleotide such as cyclic di-GMP, or an immunostimulatory base analog, optionally a C8-substituted or an N7,C8-disubstituted guanine ribonucleotide. In some examples, the adjuvant is a 20 DNA-based adjuvant. In some examples, the DNA-based adjuvant is a CpG, dsDNA, or a natural or synthetic immunostimulatory DNA sequence. In some examples, the adjuvant is a viral particle. In some examples, the viral particle is a virosome, optionally, a phospholipid cell membrane bilayer. In some examples, the adjuvant is a bacterial adjuvant. In some examples, the bacterial adjuvant is flagellin, LPS, or a bacterial toxin, optionally an enterotoxin, a heat-labile toxin, or a Cholera toxin. In 25 some examples, the adjuvant is a hybrid molecule. In some examples, the adjuvant is CpG conjugated to Imiquimod. In some examples, the adjuvant is a fungal or oocyte microbe-associated molecular pattern (MAMP). In some examples, the fungal or oocyte MAMP is chitin or beta-glucan. In some examples, the adjuvant is an inorganic nanoparticle. In some examples, the inorganic nanoparticle is a gold nanorod, a silica-based nanoparticle, optionally a mesoporous silica nanoparticle (MSN). In some 30 examples, the adjuvant is a multi-component adjuvant. In some examples, the multi-component adjuvant is AS01, AS03, AS04, Complete Freund's Adjuvant, or CAF01.

In another example the disclosure provides a method of treating or preventing a disease, disorder, or condition in a subject, the method including administering to the subject any one of the circular polyribonucleotides, the compositions, pharmaceutical preparations, or the pharmaceutical 35 compositions described herein. In some examples, the disease, disorder, or condition is a viral infection, a bacterial infection, or a fungal infection. In some examples, the disease, disorder, or condition is a cancer. In some examples, the disease, disorder, or condition is associated with exposure to an allergen. In some examples, disease, disorder, or condition is associated with exposure to a toxin.

In another example, the disclosure provides a method of inducing an immune response in a 40 subject, the method including administering to the subject any one of the circular polyribonucleotides, the compositions, pharmaceutical preparations, or pharmaceutical compositions described herein. In some

examples, the subject is a mammal. In some examples, the subject is a human. In some examples, the method is a non-human mammal. In some examples, the non-human mammal is a cow, a sheep, a goat, a pig, a dog, a horse, or a cat. In some examples, the subject is a bird. In some examples, the bird is a hen, a rooster, a turkey, or a parrot. In some examples, the method further includes administering an adjuvant to the subject. In some examples, the adjuvant is a separate molecular entity from the circular polyribonucleotide, linear polyribonucleotide, or preparation or composition thereof. In some examples, the adjuvant is an inorganic adjuvant, a small molecule adjuvant, and oil in water emulsion, a lipid or polymer, a peptide or peptidoglycan, a carbohydrate or polysaccharide, a saponin, an RNA-based adjuvant, a DNA-based adjuvant, a viral particle, a bacterial adjuvant, a hybrid molecule, a fungal or oocyte microbe-associated molecular pattern (MAMP), an inorganic nanoparticle, or a multi-component adjuvant. In some examples, the adjuvant is a polypeptide adjuvant. In some examples, the polypeptide adjuvant is a cytokine, a chemokine, a costimulatory molecule, an innate immune stimulator, a signaling molecule, a transcriptional activator, a cytokine receptor, a bacterial component, or a component of the innate immune system. In some examples, the adjuvant is an innate immune system stimulator. In some examples, the innate immune system stimulator is selected from an RNA including a GU-rich motif, an AU-rich motif, a structured region including dsRNA, or an aptamer.

In some examples, any one of the circular polyribonucleotides, compositions, pharmaceutical preparations, or pharmaceutical compositions described herein is administered to the subject as a single dose. In some examples, any one of the circular polyribonucleotides, compositions, pharmaceutical preparations, or pharmaceutical compositions described herein is administered to the subject two or more times, three or more times, four or more times, or five or more times. In some examples, administration of any one of the circular polyribonucleotides, compositions, pharmaceutical preparations, or pharmaceutical compositions described herein occurs about weekly, about every two weeks, about every three weeks, about every month, about every two months, about every three months, about every four months, about every five months, about every six months, about every year, about every two years, about every three years, about every four years, about every five years, or about every ten years.

In some examples, the method further comprises administering to the subject a polypeptide immunogen (e.g., a protein subunit comprising a polypeptide immunogen). In some examples the polypeptide immunogen corresponds to (e.g., shares 90%, 95%, 96%, 97%, 98%, or 100% amino acid sequence identity with a polypeptide immunogen encoded by a sequence of the circular polyribonucleotide. In some examples, the polypeptide immunogen is administered to the subject after administering any one of the circular polyribonucleotides, immunogenic compositions, pharmaceutical preparations, or pharmaceutical compositions described herein. In some examples, the polypeptide immunogen maintains or enhances an immune response in the subject against the polypeptide immunogen.

In another example, the disclosure provides a method of maintaining or enhancing an immune response in a subject comprising (i) administering to the subject a circular polyribonucleotide encoding a polypeptide immunogen and (ii) administering to the subject the polypeptide immunogen, wherein step (ii) occurs between 1 week and 6 months (e.g., between 1 month and 5 months, 2 months and 3 months, 2 weeks and 3 months, or 3 months and 6 months) after step (i), and wherein administration of the polypeptide immunogen of step (ii) maintains or enhances the immune response in the subject against

the polypeptide immunogen. In some examples, the polypeptide immunogen comprises one or more epitopes that identifies a target. In some examples, the target is a pathogen. In some examples, the target is a cancer cell, an allergen, or a toxin.

5 *Numbered Examples:*

[1] A circular polyribonucleotide comprising a plurality of sequences, each sequence encoding a polypeptide immunogen, wherein at least two of the polypeptide immunogens identify different targets.

[2] The circular polyribonucleotide of paragraph [1], wherein each of the polypeptide immunogens identifies a different target.

10 [3] The circular polyribonucleotide of paragraph [1] or [2], wherein each target is a different pathogen.

[4] The circular polyribonucleotide of paragraph [3], wherein each target is, independently a virus, a bacterium, a fungus, or a parasite.

[5] The circular polyribonucleotide of paragraph [4], wherein each target is a different virus.

15 [6] The circular polyribonucleotide of paragraph [4], wherein each target is a different bacterium.

[7] The circular polyribonucleotide of paragraph [4], wherein the targets include a virus and a bacterium.

[8] A circular polyribonucleotide comprising a plurality of sequences, each sequence encoding a polypeptide immunogen, wherein at least two of the polypeptide immunogens identify different proteins, wherein each of the different proteins identifies the same target.

[9] The circular polyribonucleotide of paragraph [8], wherein each of the polypeptide immunogens identifies a different protein.

[10] The circular polyribonucleotide of paragraph [8] or [9], wherein the target is a pathogen.

25 [11] The circular polyribonucleotide of paragraph [10], wherein the pathogen a virus, a bacterium, a fungus, or a parasite.

[12] The circular polyribonucleotide of paragraph [11], wherein the pathogen is a virus and each of the different proteins is a viral protein associated with the virus.

[13] The circular polyribonucleotide of paragraph [11], wherein the pathogen is a bacterium and each of the different proteins is a bacterial protein associated with the bacteria.

30 [14] The circular polyribonucleotide of paragraph [8] or [9], wherein the target is a cancer cell.

[15] The circular polyribonucleotide of paragraph [14], wherein and each of the different proteins is a different tumor antigen associate with the cancer cell.

[16] The circular polyribonucleotide of paragraph [8] or [9], wherein the target is an allergen or a toxin.

35 [17] The circular polyribonucleotide of any one of paragraphs [1] to [16], wherein the circular polyribonucleotide comprises between 500 and 20,000 ribonucleotides.

[18] The circular polyribonucleotide of paragraph [17], wherein the circular polyribonucleotide comprises between 500 and 10,000 ribonucleotides.

40 [19] The circular polyribonucleotide of paragraph [18], wherein the circular polyribonucleotide comprises between 500 and 5,000 ribonucleotides.

[20] The circular polyribonucleotide of any one of paragraphs [1] to [16], wherein the circular polyribonucleotide comprises at least 500 ribonucleotides.