IMMUNOGENIC COMPOSITIONS AND USES THEREOF

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 September 2, 2021, is named 51509-045WO2_Sequence_Listing_9_2_21_ST25 and is 52,787 bytes in size.

Background

Vaccination has made an enormous contribution to both human and animal health. Since the 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 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 polyribonucleotides (e.g., circular or linear polyribonucleotides) encoding one or more immunogens. In particular, the disclosure provides circular polyribonucleotides encoding multiple immunogens, circular polyribonucleotides encoding at least one immunogen and further encoding at least one adjuvant, 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 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~~example, provided herein is 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, and at least nine) of the polypeptide immunogens have at least 90% (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, and at least 99%) amino acid sequence identity. 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 have at least 90% amino acid sequence identity, optionally wherein the circular polyribonucleotide comprises 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 ~~embodiment~~examples, the at least two polypeptide immunogens have at least 95% (e.g., 96%, at least 97%, at least 98%, and at least 99%) amino acid sequence identity. In some ~~embodiment~~examples, the at least two polypeptide immunogens have less than 100% amino acid sequence identity. In some ~~embodiment~~examples, each of the polypeptide immunogens have at least 90% (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, and at least 99%) amino acid sequence identity. In some ~~embodiment~~examples, each of the polypeptide immunogens have at least 95% (e.g., 96%, at least 97%, at least 98%, and at least 99%) amino acid sequence identity. In some ~~embodiment~~examples, each of the polypeptide immunogens have less than 100% amino acid sequence identity.

In some ~~embodiment~~examples, each of the polypeptide immunogens includes one or more epitopes that identifies a target. In some ~~embodiment~~examples, the target is a pathogen. In some ~~embodiment~~examples, the pathogen is a virus, a bacterium, a fungus, or a parasite. In some ~~embodiment~~examples, the pathogen is a virus and each polypeptide immunogen includes one or more epitopes (e.g., two, three, four, five, six, seven, eight, nine, ten or more) corresponding to a viral immunogen. In some ~~embodiment~~examples, the pathogen is a bacterium and each polypeptide immunogen includes one or more (e.g., two, three, four, five, six, seven, eight, nine, ten or more) epitopes corresponding to a bacterial immunogen. In some ~~embodiment~~examples, the target is a cancer cell. In some ~~embodiment~~examples, polypeptide immunogen includes one or more (e.g., two, three, four, five, six, seven, eight, nine, and ten or more) epitopes corresponding to a tumor antigen. In some ~~embodiment~~examples, the target is a toxin or an allergen. In some ~~embodiment~~examples, the target is associated with a disease, disorder, or condition. In some ~~embodiment~~examples, the disease, disorder, or condition is a viral infection. In some ~~embodiment~~examples, the disease, disorder, or condition is a bacterial infection. In some ~~embodiment~~examples, the disease, disorder, or condition is a cancer.

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

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

In some ~~embodiment~~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 ~~embodiment~~examples, the circular polyribonucleotide includes between 500 and 5,000. In some ~~embodiment~~examples, the circular polyribonucleotide includes between 1,000 and 5,000 ribonucleotides. In some ~~embodiment~~examples, the circular polyribonucleotide includes between 5,000 and 10,000 ribonucleotides In some ~~embodiment~~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 ~~embodiment~~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 ~~embodiment~~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 ~~embodiment~~examples, at least one sequence encoding a polypeptide immunogen further encodes a signal sequence. In some ~~embodiment~~examples, each sequence encoding a polypeptide immunogen further encodes a signal sequence. In some ~~embodiment~~examples, each of the sequences encoding each of the polypeptide immunogens is operably linked to an internal ribosomal entry site (IRES). In some ~~embodiment~~examples, the circular polyribonucleotide includes a single IRES. In some ~~embodiment~~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 ~~embodiment~~examples, the polypeptide immunogens are each separated by a polypeptide linker. In some ~~embodiment~~examples, the polypeptide immunogens are each separated by a cleavage domain. In some ~~embodiment~~examples, each stagger element is a 2A self-cleaving peptide. In some ~~embodiment~~examples, the circular polyribonucleotide includes a plurality of IRESs. In some ~~embodiment~~examples, each IRES is operably linked to an open reading frame including a sequence encoding a polypeptide immunogen.

In some ~~embodiment~~examples, the circular polyribonucleotide further includes at least one sequence (e.g., at least two, at least three, at least four, at least five, at least six, at least seven, at least, eight, at least nine, and at least ten sequences) encoding an adjuvant. In some ~~embodiment~~examples, the at least one (e.g., at least two, at least three, at least four, at least five, at least six, at least seven, at least, eight, at least nine, and at least ten sequences) sequence encoding the adjuvant is operably linked to an IRES

In another ~~aspect~~example the disclosure provides a circular polyribonucleotide including a sequence encoding a polypeptide immunogen and a sequence encoding an adjuvant or a sequence that is an innate immune system stimulator. In one aspect, the present disclosure refers to a circular polyribonucleotide comprising (a) a sequence encoding a polypeptide immunogen and (b) a sequence encoding an adjuvant or a sequence that is an innate immune system stimulator. In some ~~embodiment~~examples, the sequence encoding the polypeptide immunogen is operably linked to an IRES. In some ~~embodiment~~examples, the sequence encoding the adjuvant is operably linked to an IRES. In some ~~embodiment~~examples, the polypeptide immunogen and the adjuvant polypeptide are 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 of the polypeptide immunogen and the adjuvant polypeptide. In some ~~embodiment~~examples, the polypeptide immunogen and the adjuvant polypeptide are each separated by a polypeptide linker. In some ~~embodiment~~examples, the polypeptide immunogen and the adjuvant polypeptide are each separated by a cleavage domain. In some ~~embodiment~~examples, each stagger element is a 2A self-cleaving peptide. In some ~~embodiment~~examples, the circular polyribonucleotide includes a plurality of IRESs. In some ~~embodiment~~examples, a first IRES is operably linked to the sequence encoding the polypeptide immunogen and a second IRES is operably linked to the sequence encoding the adjuvant polypeptide.

In some ~~embodiment~~examples, wherein the circular polyribonucleotide encodes at least one polypeptide immunogen and at least one adjuvant (e.g., a polypeptide adjuvant), the expression of the sequence encoding the polypeptide immunogen is at least 2-fold, 5-fold, 10-fold, 20-fold, 50-fold, or 100-fold greater

than the expression of the sequence encoding the adjuvant. In some ~~embodiment~~example, the expression of the sequence encoding the polypeptide immunogen is substantially the same as the expression of the sequence encoding the adjuvant.

In another ~~aspect~~example the disclosure provides a composition including a plurality of circular polyribonucleotides, wherein the composition includes at least a first circular polyribonucleotide including a sequence encoding a polypeptide immunogen and at least a second circular polyribonucleotide including a sequence encoding an adjuvant or a sequence that is an innate immune system stimulator. In one aspect, the present disclosure refers to an immunogenic composition comprising a plurality of circular polyribonucleotides, wherein the composition comprises (a) at least a first circular polyribonucleotide comprising a sequence encoding a polypeptide immunogen and (b) at least a second circular polyribonucleotide comprising a sequence encoding an adjuvant or a sequence that is an innate immune system stimulator, optionally wherein the adjuvant is a polypeptide. In some ~~embodiment~~examples, the polypeptide immunogen and the adjuvant are each operably linked to an IRES.

In some ~~embodiment~~examples, the adjuvant is a polypeptide. In some ~~embodiment~~examples, the 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 ~~embodiment~~examples, the cytokine is a pro-inflammatory cytokine. In some ~~embodiment~~examples, the pro-inflammatory cytokine is selected from GM-CSF, IL-1 alpha, IL-1 beta, TGF-beta, TNF-alpha, and TNF-beta. In some ~~embodiment~~examples, cytokine is a Th-1 inducing cytokine. In some ~~embodiment~~examples, the Th-1 inducing cytokine is selected from IFN-gamma, IL-2, IL-12, IL-15, and IL-18. In some ~~embodiment~~examples, the cytokine is a Th-2 inducing cytokine. In some ~~embodiment~~examples, the Th-2 inducing cytokine is selected from IFN-gamma, IL-4, IL-5, IL-6, IL-10, and IL-13. In some ~~embodiment~~examples, the chemokine is selected from MCP-1, MIP-1alpha, MIP-1beta, Rantes, and TCA-3. In some ~~embodiment~~examples, the costimulatory molecule is selected from CD80, CD86, CD40-L, CD70, and CD27. In some ~~embodiment~~examples, the innate immune stimulator is selected from STING (e.g., a constitutively active STING, such as $STING^{V155M}$, $STING^{R284M}$, $STING^{R284M/V147L/N154S/V155M}$, or $STING^{V147L/N154S/V155M}$), TLR3, TLR4, TLR9, TLR7, TLR8, TLR7, RIG-I/DDX58, and MDA-5/IFIH1. In some ~~embodiment~~examples, the innate immune stimulator is a constitutively active mutant. In some ~~embodiment~~examples, the signaling molecule is selected from STING (e.g., a constitutively active STING, such as $STING^{V155M}$, $STING^{R284M}$, $STING^{R284M/V147L/N154S/V155M}$, or $STING^{V147L/N154S/V155M}$), TRIF, TRAM, MyD88, IPS1, ASC, MAVS, MAPKs, IKK-alpha, IKK complex, TBK1, B-catenin, and caspase 1. In some ~~embodiment~~examples, the transcriptional activator is selected from AP1, NF-kappa B, IRF3, IRF7, IRF1, and IRF5. In some ~~embodiment~~examples, the cytokine receptor is selected from IL-2betaR, IFN-gammaR, and IL-6R. In some ~~embodiment~~examples, the bacterial component is selected from flagellin and MBL.

In some ~~embodiment~~examples of any one of the circular polyribonucleotides described herein, at least one sequence encoding the adjuvant further encodes a signal sequence. In some ~~embodiment~~examples, each sequence encoding an immunogen further encodes a signal sequence. In some ~~embodiment~~examples, at least one sequence encoding an immunogen does not encode a signal sequence. In some ~~embodiment~~examples, none of the sequences encoding an immunogen further encodes a signal sequence.

In some ~~embodiment~~examples, the circular polyribonucleotide further includes a sequence that is an innate immune system stimulator. In some ~~embodiment~~examples, the sequence that is an innate immune system stimulator is a GU-rich motif, an AU-rich motif, a structured region including dsRNA, or an aptamer.

In some ~~embodiment~~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 ~~embodiment~~examples, the circular polyribonucleotide is produced by splint ligation. In some ~~embodiment~~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 potion 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 ~~embodiment~~examples, the intron is a Group I self-splicing intron.

In another ~~aspect~~example the disclosure provides, a composition including a plurality of circular polyribonucleotides, each including a sequence encoding a polypeptide immunogen. In some ~~embodiment~~examples, each of the plurality of circular polyribonucleotides is a circular polyribonucleotide described herein. In some ~~embodiment~~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 have at least 90% (e.g., at least 91%, at least 92%, at least 93%, at last 94%, at least 95%, at least 96%, at least 97%, at least 98%, and at least 99%) amino acid sequence identity. In some ~~embodiment~~examples, the first and second polypeptide immunogens have at least 95% (at least 96%, at least 97%, at least 98%, and at least 99%) amino acid sequence identity. In some ~~embodiment~~examples, the first and second polypeptide immunogens have less than 100% amino acid sequence identity. In one aspect, the present disclosure refers to an immunogenic composition comprising (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 immunogens have at least 90% amino acid sequence identity and less than 100% amino acid sequence identity. In some ~~embodiment~~examples, each of the polypeptide immunogens includes one or more epitopes that identifies a target. In some ~~embodiment~~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 ~~embodiment~~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 ~~embodiment~~examples, each target is, independently, a cancer cell, a virus, a bacterium, a fungus, a parasite, a toxin, or an allergen. In some ~~embodiment~~examples, the target is a pathogen. In some ~~embodiment~~examples, the pathogen is a virus, a bacterium, a fungus, or a parasite. In some ~~embodiment~~examples, the target is a cancer cell, an allergen, or a toxin. In some ~~embodiment~~examples, each polypeptide immunogen is operably linked to an IRES.

In another ~~aspect~~example, the disclosure provides a pharmaceutical preparation of a circular polyribonucleotide, wherein the circular polyribonucleotide includes a sequence encoding a polypeptide immunogen, and wherein the circular polyribonucleotide constitutes at least 25% (w/w) (e.g., at least 30%(w/w), 40%(w/w), 50% (w/w), 60% (w/w), 70% (w/w), 75% (w/w), 80% (w/w), 85% (w/w), 90% (w/w), 91% (w/w), 92% (w/w), 93% (w/w), 94% (w/w), 95% (w/w), 96% (w/w), 97% (w/w), 98% (w/w) or 99% (w/w)) of total ribonucleotide molecules in the pharmaceutical preparation. In some ~~embodiment~~examples, the circular polyribonucleotide constitutes at least 70% (w/w), 75% (w/w), 80% (w/w), 85% (w/w), 90% (w/w), 91% (w/w), 92% (w/w), 93% (w/w), 94% (w/w), 95% (w/w), 96% (w/w), 97% (w/w), 98% (w/w) or 99% (w/w) of total ribonucleotide molecules in the pharmaceutical preparation. In some ~~embodiment~~examples, the pharmaceutical preparation includes no more than 75% (w/w), 60% (w/w), 50% (w/w), 40% (w/w), 30% (w/w), 20% (w/w), 15% (w/w), 10% (w/w), 5% (w/w), or 1% (w/w) linear polyribonucleotide molecules of the total ribonucleotide molecules in the pharmaceutical preparation. In some ~~embodiment~~examples, the

pharmaceutical preparation includes no more than 0.5% (w/w) (e.g., 0.4% (w/w), 0.3% (w/w), 0.2% (w/w), 0.1 (w/w), and 0.05% (w/w) linear polyribonucleotide molecules of the total ribonucleotide molecules in the pharmaceutical preparation. In some ~~embodiment~~examples, the pharmaceutical preparation includes at least 1% (w/w) linear polyribonucleotide. In some ~~embodiment~~examples, the pharmaceutical preparation includes between 30%-40% (w/w) circular polyribonucleotide and between 1%-10% (w/w), 10%-20% (w/w), 20%-30% (w/w), 30%-40% (w/w), 40%-50% (w/w), 50%-60% (w/w), or 60%-70% (w/w) linear polyribonucleotide. In some ~~embodiment~~examples, the pharmaceutical preparation includes between 40%-60% (w/w) circular polyribonucleotide and between 1%-10% (w/w), 10%-20% (w/w), 20%-30% (w/w), 30%-40% (w/w), 40%-50% (w/w), 50%-60% (w/w) linear polyribonucleotide. In some ~~embodiment~~examples, the pharmaceutical preparation includes between 60%-80% (w/w) circular polyribonucleotide and between 1%-10% (w/w), 10%-20% (w/w), 20%-30% (w/w), 30%-40% (w/w) linear polyribonucleotide.

In another ~~aspect~~example, the disclosure provides a pharmaceutical preparation of a circular polyribonucleotide, wherein the circular polyribonucleotide includes a sequence encoding a polypeptide immunogen, and wherein the pharmaceutical preparation includes no more than 75% (w/w), 60% (w/w), 50% (w/w), 40% (w/w), 30% (w/w), 20% (w/w), 15% (w/w), 10% (w/w), 5% (w/w), or 1% (w/w) linear polyribonucleotide molecules of the total ribonucleotide molecules in the pharmaceutical preparation. In some ~~embodiment~~examples, the pharmaceutical preparation includes no more than 20% (w/w), 15% (w/w), 10% (w/w), 5% (w/w), 1% (w/w), or 0.5% (w/w) linear polyribonucleotide molecules of the total ribonucleotide molecules in the pharmaceutical preparation.

In some ~~embodiment~~examples, the preparation includes no more than 15% (w/w) (e.g., 15% (w/w), 10% (w/w), 9% (w/w), 8% (w/w), 7% (w/w), 6% (w/w), 5% (w/w), 4% (w/w), 3% (w/w), 2% (w/w), 1% (w/w), and 0.5% (w/w)) nicked polyribonucleotide molecules of the total ribonucleotide molecules in the pharmaceutical preparation. In some ~~embodiment~~examples, the preparation includes no more than 8% (w/w), 7% (w/w), 6% (w/w), 5% (w/w), 4% (w/w), 3% (w/w), 2% (w/w), 1% (w/w), or 0.5% (w/w) nicked polyribonucleotide molecules of the total ribonucleotide molecules in the pharmaceutical preparation.

In another ~~aspect~~example, the disclosure provides a pharmaceutical preparation of a circular polyribonucleotide, wherein the circular polyribonucleotide includes a sequence encoding a polypeptide immunogen, and wherein the pharmaceutical preparation includes no more than 15% (w/w) (e.g., 15% (w/w), 10% (w/w), 9% (w/w), 8% (w/w), 7% (w/w), 6% (w/w), 5% (w/w), 4% (w/w), 3% (w/w), 2% (w/w), 1% (w/w), and 0.5% (w/w)) nicked polyribonucleotide molecules of the total ribonucleotide molecules in the pharmaceutical preparation. In some ~~embodiment~~examples, the preparation includes no more than 8% (w/w), 7% (w/w), 6% (w/w), 5% (w/w), 4% (w/w), 3% (w/w), 2% (w/w), 1% (w/w), or 0.5% (w/w) nicked polyribonucleotide molecules of the total ribonucleotide molecules in the pharmaceutical preparation.

In some ~~embodiment~~examples of an one of the pharmaceutical preparations described herein the pharmaceutical preparation includes less than 10 EU/kg (e.g., 9 EU/kg, 8 EU/kg, 7 EU/kg, 6 EU/kg, 5 EU/kg, 4 EU/kg, 3 EU/KG, 2 EU/kg, and 1 EU/kg) or lacks endotoxin as measured by a Limulus amebocyte lysate test. In some ~~embodiment~~examples, the pharmaceutical preparation includes a bioburden of less than 100 CFU/100 ml or less than 10 CFU/100 ml before sterilization. In some ~~embodiment~~examples, the pharmaceutical preparation is a sterile pharmaceutical preparation, e.g., supports growth of fewer than 100 viable microorganisms as tested under aseptic conditions. In some ~~embodiment~~examples, the pharmaceutical preparation meets the standard of USP <71>. In some ~~embodiment~~examples, the pharmaceutical

preparation meets the standard of USP <85>. In some ~~embodiment~~examples, the pharmaceutical preparation is an intermediate pharmaceutical preparation of a final drug product.

In some ~~embodiment~~examples, the pharmaceutical preparation is a final drug product for administration to a subject. In some ~~embodiment~~examples, the pharmaceutical preparation includes a concentration of at least 0.1 ng/mL, 0.5 ng/mL, 1 ng/mL, 5 ng/mL, 10 ng/mL, 50 ng/mL, 0.1 µg/mL, 0.5 µg/mL,1 µg/mL, 2 µg/mL, 5 µg/mL, 10 µg/mL, 20 µg/mL, 30 µg/mL, 40 µg/mL, 50 µg/mL, 60 µg/mL, 70 µg/mL, 80 µg/mL, 100 µg/mL, 200 µg/mL, 300 µg/mL, 500 µg/mL, 1 mg/mL, 2 mg/mL, 3 mg/mL, 5 mg/mL, 10 mg/mL, 100 mg/mL, 200 mg/mL, or 500 mg/mL circular polyribonucleotide molecules. In some ~~embodiment~~examples, the pharmaceutical preparation includes no more than 1 ng/ml, 5 ng/ml, 10 ng/ml, 15 ng/ml, 20 ng/ml, 25 ng/ml, 30 ng/ml, 35 ng/ml, 40 ng/ml, 50 ng/ml, 60 ng/ml, 70 ng/ml, 80 ng/ml, 90 ng/ml, 100 ng/ml, 200 ng/ml, 300 ng/ml, 400 ng/ml, 500 ng/ml, 1000 µg/mL, 5000 µg/mL, 10,000 µg/mL, or 100,000 µg/mL of deoxyribonucleotide molecules. In some ~~embodiment~~examples, the pharmaceutical preparation includes a protein contamination (e.g. an enzyme) of less than 0.1 ng, 1 ng, 5 ng, 10 ng, 15 ng, 20 ng, 25 ng, 30 ng, 35 ng, 40 ng, 50 ng, 60 ng, 70 ng, 80 ng, 90 ng, 100 ng, 200 ng, 300 ng, 400 ng, or 500 ng of the protein contamination per milligram (mg) of the circular polyribonucleotide molecules. In some ~~embodiment~~examples, the pharmaceutical preparation includes an A260/A280 absorbance ratio of from about 1.6 to 2.3 (e.g., 1.7, 1.8, 1.9, 2.0, 2.1, and 2.2) as measured by a spectrophotometer.

In some ~~embodiment~~examples, the pharmaceutical preparation is substantially free of a process-related impurity selected from a cell protein, a cell deoxyribonucleic acid, an enzyme, a reagent component, a gel component, or a chromatographic material. In some ~~embodiment~~examples, the pharmaceutical preparation has a reduced level of one or more markers of an immune or inflammatory response after purification compared to prior to purification. In some ~~embodiment~~examples, the circular polyribonucleotide is a circular polyribonucleotide described herein.

In some ~~embodiment~~examples, the circular polyribonucleotide is produced according to a method including the steps of: providing a plurality of linear polyribonucleotide molecules; circularizing the linear polyribonucleotide molecules to provide a preparation of circular polyribonucleotide molecules; and processing the preparation to remove linear polyribonucleotide molecules from the preparation. In some ~~embodiment~~examples, the method further includes evaluating the amount of linear polyribonucleotide molecules in the preparation remaining after the processing step. In some ~~embodiment~~examples, the method further includes further processing the preparation, wherein further processing includes: processing the preparation to remove nicked polyribonucleotide molecules from the preparation; and/or processing the preparation to remove deoxyribonucleotide molecules from the preparation; and/or processing the preparation to remove protein contaminants from the preparation; and/or processing the preparation to remove endotoxin from the preparation.

In another ~~aspect~~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 one aspect, the present disclosure refers to a pharmaceutical composition comprising the circular polyribonucleotide or immunogenic composition as disclosed herein, and a pharmaceutically acceptable excipient. In some ~~embodiment~~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 ~~embodiment~~examples, the pharmaceutical composition further includes an adjuvant. In some ~~embodiment~~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 ~~embodiment~~examples, the adjuvant is an inorganic adjuvant. In some ~~embodiment~~examples, the inorganic adjuvant is an aluminum salt or a calcium salt. In some ~~embodiment~~examples, the adjuvant is a small molecule. In some ~~embodiment~~examples, the small molecule is imiquimod, resiquimod, or gardiquimod. In some ~~embodiment~~examples, the adjuvant is an oil in water emulsion. In some ~~embodiment~~examples, the oil in water emulsion is Squalene, MF59, AS03, a Montanide formulation, optionally Montanide ISA 51 or Montanide ISA 720, or Incomplete Freunds Adjuvant an oil in water emulsion. In some ~~embodiment~~examples, the adjuvant is a lipid or polymer. In some ~~embodiment~~examples, the lipid or polymer is a polymeric nanoparticle, optionally PLGA, PLG, PLA, PGA, or PHB, a liposome, optionally a Virosomes 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, trehalose dimycolate. In some ~~embodiment~~examples, the adjuvant is a peptide or peptidoglycan. In some ~~embodiment~~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 cytokines, or a chemokine. In some ~~embodiment~~examples, the adjuvant is a carbohydrate or polysaccharide. In some ~~embodiment~~examples, the carbohydrate or polysaccharide is dextran (branched microbial polysaccharide), dextran-sulfate, Lentinan, zymosan, Beta-glucan, Deltin, Mannan, or Chitin. In some ~~embodiment~~examples, the adjuvant is a saponin. In some ~~embodiment~~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 ~~embodiment~~examples, the adjuvant is an RNA-based adjuvant. In some ~~embodiment~~examples, the RNA-based adjuvant is Poly IC, Poly IC:LC, a hairpin RNAs, 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 analogs, optionally cyclic GMP-AMP or a cyclic dinucleotide such as cyclic di-GMP, or an immunostimulatory base analog, optionally a C8-substitued or an N7,C8-disubstituted guanine ribonucleotide. In some ~~embodiment~~examples, the adjuvant is a DNA-based adjuvant. In some ~~embodiment~~examples, the DNA-based adjuvant is a CpG, dsDNA, or a natural or synthetic immunostimulatory DNA sequence. In some ~~embodiment~~examples, the adjuvant is a viral particle. In some ~~embodiment~~examples, the viral particle is a virosome, optionally, a phospholipid cell membrane bilayer. In some ~~embodiment~~examples, the adjuvant is a bacterial adjuvant. In some ~~embodiment~~examples, the bacterial adjuvant is flagellin, LPS, or a bacterial toxin, optionally an enterotoxin, a heat-labile toxin, or a Cholera toxin. In some ~~embodiment~~examples, the adjuvant is a hybrid molecule. In some ~~embodiment~~examples, the adjuvant is CpG conjugated to Imiquimod. In some ~~embodiment~~examples, the adjuvant is a fungal or oocyte microbe-associated molecular pattern (MAMP). In some ~~embodiment~~examples, the fungal or oocyte MAMP is chitin or beta-glucan. In some ~~embodiment~~examples, the adjuvant is an inorganic nanoparticle. In some ~~embodiment~~examples, the inorganic nanoparticle is a gold nanorod, a silica-based nanoparticle, optionally a mesoporous silica nanoparticle (MSN). In some ~~embodiment~~examples, the adjuvant is a multi-component adjuvant. In some ~~embodiment~~examples, the multi-component adjuvant is AS01, AS03, AS04, Complete Freunds Adjuvant, or CAF01.

In another ~~aspect~~example, the disclosure provides a pharmaceutical composition including a mixture including any one of the circular polyribonucleotides, the compositions, or the pharmaceutical preparations described herein, and an alcohol, wherein the alcohol constitutes about 0.3% v/v to about

75% v/v (e.g., 0.5% v/v, 1% v/v, 5% v/v, 10% v/v, 15% v/v, 20% v/v, 25% v/v, 30% v/v, 35% v/v, 40% v/v, 45% v/v, 50% v/v, 55% v/v, 60% v/v, 65% v/v, and 70% v/v) of the mixture.

In another ~~aspect~~example, the disclosure provides a pharmaceutical composition including a mixture including a linear polyribonucleotide that includes at least one sequence encoding a polypeptide immunogen and an alcohol, wherein the alcohol constitutes about 0.3% v/v to about 75% v/v (e.g., 0.5% v/v, 1% v/v, 5% v/v, 10% v/v, 15% v/v, 20% v/v, 25% v/v, 30% v/v, 35% v/v, 40% v/v, 45% v/v, 50% v/v, 55% v/v, 60% v/v, 65% v/v, and 70% v/v) of the mixture.

In some ~~embodiment~~examples, the linear polyribonucleotide includes 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, and at least nine) of the polypeptide immunogens have at least 90% (e.g., at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98%, and at least 99%) amino acid sequence identity. In some ~~embodiment~~examples, the at least two polypeptide immunogens have at least 95% (e.g., 96%, at least 97%, at least 98%, and at least 99%) amino acid sequence identity. In some ~~embodiment~~examples, the at least two polypeptide immunogens have less than 100% amino acid sequence identity. In some ~~embodiment~~examples, each of the polypeptide immunogens includes one or more epitopes that identifies a target. In some ~~embodiment~~examples, the target is a pathogen. In some ~~embodiment~~examples, the pathogen is a virus, a bacterium, a fungus, or a parasite. In some ~~embodiment~~examples, the pathogen is a virus and each polypeptide immunogen includes one or more epitopes (e.g., two, three, four, five, six, seven, eight, nine, ten, or more) corresponding to a viral immunogen. In some ~~embodiment~~examples, the pathogen is a bacterium and each polypeptide immunogen includes one or more (e.g., two, three, four, five, six, seven, eight, nine, ten, or more) epitopes corresponding to a bacterial immunogen. In some ~~embodiment~~examples, the target is a cancer cell. In some ~~embodiment~~examples, polypeptide immunogen includes one or more (e.g., two, three, four, five, six, seven, eight, nine, ten, or more) epitopes corresponding to a tumor antigen. In some ~~embodiment~~examples, the target is a toxin or an allergen. In some ~~embodiment~~examples, the target is associated with a disease, disorder, or condition. In some ~~embodiment~~examples, the disease, disorder, or condition is a viral infection. In some ~~embodiment~~examples, the disease, disorder, or condition is a bacterial infection. In some ~~embodiment~~examples, the disease, disorder, or condition is a cancer.

In some ~~embodiment~~examples, the linear polyribonucleotide includes 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, and at least nine) of the polypeptide immunogens identify different proteins, wherein each of the different proteins identifies the same target. In some ~~embodiment~~examples, each of the polypeptide immunogens identifies a different protein. In some ~~embodiment~~examples, the target is a pathogen. In some ~~embodiment~~examples, the pathogen is a virus, a bacterium, a fungus, or a parasite. In some ~~embodiment~~examples, the pathogen is a virus and each of the different proteins is a viral protein associated with the virus. In some ~~embodiment~~examples, the pathogen is a bacterium and each of the different proteins is a bacterial protein associated with the bacteria. In some ~~embodiment~~examples the target is a cancer cell. In some ~~embodiment~~examples, each of the different proteins is a different tumor antigen associate with the cancer cell. In some ~~embodiment~~examples, the target is an allergen or a toxin.

In some ~~embodiment~~examples, the linear polyribonucleotide includes 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, ant at least nine) of the polypeptide immunogens

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

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

In some ~~embodiment~~examples, the linear polyribonucleotide includes further includes at least one sequence (e.g., at least two, at least three, at least four, at least five, at least six, at least seven, at least, eight, at least nine, or at least ten sequences) encoding an adjuvant. In some ~~embodiment~~examples, the at least one (e.g., at least two, at least three, at least four, at least five, at least six, at least seven, at least, eight, at least nine, or at least ten sequences) sequence encoding the adjuvant is operably linked to an IRES.

In some ~~embodiment~~examples, the linear polyribonucleotide includes a sequence encoding a polypeptide immunogen and a sequence encoding an adjuvant or a sequence that is an innate immune system stimulator. In some ~~embodiment~~examples, the sequence encoding the polypeptide immunogen is operably linked to an IRES. In some ~~embodiment~~examples, the sequence encoding the adjuvant is operably linked to an IRES. In some ~~embodiment~~examples, the adjuvant is a polypeptide. In some ~~embodiment~~examples, the 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 ~~embodiment~~examples, the cytokine is a pro-inflammatory cytokine. In some ~~embodiment~~examples, the pro-inflammatory cytokine is selected from GM-CSF, IL-1 alpha, IL-1 beta, TGF-beta, TNF-alpha, and TNF-beta. In some ~~embodiment~~examples, cytokine is a Th-1 inducing cytokine. In some ~~embodiment~~examples, the Th-1 inducing cytokine is selected from IFN-gamma, IL-2, IL-12, IL-15, and IL-18. In some ~~embodiment~~examples, the cytokine is a Th-2 inducing cytokine. In some ~~embodiment~~examples, the Th-2 inducing cytokine is selected from IFN-gamma, IL-4, IL-5, IL-6, IL-10, and IL-13. In some ~~embodiment~~examples, the chemokine is selected from MCP-1, MIP-1alpha, MIP-1beta, Rantes, and TCA-3. In some ~~embodiment~~examples, the costimulatory molecule is selected from CD80, CD86, CD40-L, CD70, and CD27. In some ~~embodiment~~examples, the innate immune stimulator is selected from STING (e.g., constitutively active STING (caSTING), such as $STING^{V155M}$, $STING^{R284M}$, $STING^{R284M/V147L/N154S/V155M}$, or $STING^{V147L/N154S/V155M}$), TLR3, TLR4, TLR9, TLR7, TLR8, TLR7, RIG-I/DDX58, and MDA-5/IFIH1. In some ~~embodiment~~examples, the innate immune stimulator is a constitutively active mutant. In some ~~embodiment~~examples, the signaling molecule is selected from STING (e.g., caSTING, such as $STING^{V155M}$, $STING^{R284M}$, $STING^{R284M/V147L/N154S/V155M}$, or $STING^{V147L/N154S/V155M}$), TRIF, TRAM, MyD88, IPS1, ASC, MAVS, MAPKs, IKK-alpha, IKK complex, TBK1, B-catenin, and caspase 1. In some ~~embodiment~~examples, the transcriptional activator is selected from AP1, NF-kappa B, IRF3, IRF7, IRF1, and IRF5. In some ~~embodiment~~examples, the cytokine receptor is selected from IL-2betaR, IFN-gammaR, and IL-6R. In some ~~embodiment~~examples, the bacterial component is selected from flagellin and MBL.

In some ~~embodiment~~examples, the linear polyribonucleotide includes a sequence that is an innate immune system stimulator. In some ~~embodiment~~examples, the sequence that is an innate immune system stimulator is a GU-rich motif, an AU-rich motif, a structured region including dsRNA, or an aptamer.

In some ~~embodiment~~examples, the alcohol constitutes about 0.3% v/v to about 60% v/v (e.g., 0.5% v/v, 1% v/v, 5% v/v, 10% v/v, 15% v/v, 20% v/v, 25% v/v, 30% v/v, 35% v/v, 40% v/v, 45% v/v, 50% v/v, and 55% v/v) , about 0.3% v/v to about 50% v/v (e.g., 0.5% v/v, 1% v/v, 5% v/v, 10% v/v, 15% v/v, 20% v/v, 25% v/v, 30% v/v, 35% v/v, 40% v/v, and 45% v/v), about 0.3% v/v to about 40% v/v (e.g., 0.5% v/v, 1% v/v, 5% v/v, 10% v/v, 15% v/v, 20% v/v, 25% v/v, 30% v/v, and 35% v/v), about 0.3% v/v to about 20% v/v, about 0.3% v/v to about 15% v/v (e.g., 0.5% v/v, 1% v/v, 5% v/v, 10% v/v, and 15% v/v), about 0.3% v/v to about 10% v/v (e.g., 0.5% v/v, 1% v/v, 5% v/v, and 10% v/v), about 0.3% v/v to about 5% v/v (e.g., 0.5% v/v, 1% v/v, 2% v/v, 3% v/v, and 4% v/v), about 0.3% v/v to about 1% v/v (e.g., 0.4% v/v, 0.5% v/v, 0.6% v/v, 0.7% v/v, 0.8% v/v, and 0.9% v/v) or about 0.3% v/v to about 0.5% v/v of the mixture. In some ~~embodiment~~examples, the alcohol constitutes about 0.5% v/v to about 75% v/v, about 1% v/v to about 75% v/v, about 5% v/v to about 75% v/v, about 10% v/v to about 75% v/v, about 15% v/v to about 75% v/v, about 20% v/v to about 75% v/v, about 30% v/v to about 75% v/v, about 40% v/v to about 75% v/v, about 50% v/v to about 75% v/v, about 60% v/v to about 75% v/v, or about 70% v/v to about 75% v/v of the mixture.

In some ~~embodiment~~examples, the alcohol is selected from methanol, ethanol, isopropanol, phenoxyethanol, triethanolamine, phenethyl alcohol, butanol, pentanol, cetyl alcohol, ethylene glycol, propylene glycol, denatured alcohol, benzyl alcohol, specially denatured alcohol, glycol, stearyl alcohol, cetearyl alcohol, menthol, polyethylene glycols (PEG)-400, ethoxylated fatty acids, and hydroxyethylcellulose. In some ~~embodiment~~examples, the alcohol is ethanol.

In some ~~embodiment~~examples, the pharmaceutical composition is formulated for topical administration. In some ~~embodiment~~examples, the pharmaceutical composition is a liquid, gel, lotion, paste, cream, foam, or stick. In some ~~embodiment~~examples, the pharmaceutical composition has a pH of about 6-8 (e.g., of about 6.5-7.5, or of about 7). In some ~~embodiment~~examples, the pharmaceutical composition has a viscosity that is about the same as water.

In some ~~embodiment~~examples, the pharmaceutical composition is substantially free of hydrophobic or lipophilic groups. In some ~~embodiment~~examples, the pharmaceutical composition is substantially free of hydrocarbons. In some ~~embodiment~~examples, the pharmaceutical composition is substantially free of cationic liposomes. In some ~~embodiment~~examples, the pharmaceutical composition is substantially free of fatty acids, lipids, liposomes, cholesterol, or any combination thereof. In some ~~embodiment~~examples, the composition further including an adjuvant. In some ~~embodiment~~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 ~~embodiment~~examples, the composition further includes a cell-penetrating agent.

In another ~~aspect~~example, the disclosure provides, a method of delivering a polyribonucleotide to a subject including topically applying a circular polyribonucleotide described herein, an immunogenic composition described herein, a pharmaceutical preparation descried herein to a surface area of the subject.

In another ~~aspect~~example, the disclosure provides, a method of delivering a polyribonucleotide to a subject including: (a) applying a sterilizing agent to a surface area of the subject; and (b) applying a composition including a circular polyribonucleotide described herein, a immunogenic composition described herein, a pharmaceutical preparation descried herein, or the pharmaceutical composition

described herein and a diluent to the surface area of the subject. In some ~~embodiment~~examples, the composition of (b) does not comprise a carrier. In some ~~embodiment~~examples, the sterilizing agent is an alcohol, UV light, laser light, or heat.

In another ~~aspect~~example, the disclosure provides a method of delivering a polyribonucleotide to a subject including: (a) applying an alcohol to a surface area of the subject; and (b) applying a composition including a circular polyribonucleotide described herein, a immunogenic composition described herein, a pharmaceutical preparation descried herein, or the pharmaceutical composition described herein and a diluent to the surface area of the subject. In some ~~embodiment~~examples, the alcohol is selected from the group consisting of: methanol, ethanol, isopropanol, phenoxyethanol, triethanolamine, phenethyl alcohol, butanol, pentanol, cetyl alcohol, ethylene glycol, propylene glycol, denatured alcohol, benzyl alcohol, specially denatured alcohol, glycol, stearyl alcohol, cetearyl alcohol, menthol, polyethylene glycols (PEG)-400, ethoxylated fatty acids, and hydroxyethylcellulose. In some ~~embodiment~~examples, the alcohol is ethanol.

In some ~~embodiment~~examples (e.g., in some ~~embodiment~~examples of topical delivery described herein), the surface area of the subject is selected from a surface area of the skin, oral cavity, nasal cavity, gastrointestinal tract, respiratory tract, or any combination thereof.

In another ~~aspect~~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 compositions described herein. In some ~~embodiment~~examples, the disease, disorder, or condition is a viral infection, a bacterial infection, or a fungal infection. In some ~~embodiment~~examples, the disease, disorder, or condition is a cancer. In some ~~embodiment~~examples, the disease, disorder, or condition is associated with exposure to an allergen. In some ~~embodiment~~examples, disease, disorder, or condition is associated with exposure to a toxin.

In another ~~aspect~~example, the disclosure provides a method of inducing an immune response in a subject, the method including administering to the subject any one of the circular polyribonucleotides, the compositions, pharmaceutical preparations, or pharmaceutical compositions described herein. In one aspect, the present disclosure refers to the circular polyribonucleotide or immunogenic composition, or pharmaceutical composition as disclosed herein for use in inducing an immune response in a subject. In one aspect, the present disclosure refers to use of the circular polyribonucleotide or immunogenic composition, or pharmaceutical composition as disclosed herein in the manufacture of a medicament for inducing an immune response in a subject. In some ~~embodiment~~examples, the subject is a mammal. In some ~~embodiment~~examples, the subject is a human. In some ~~embodiment~~examples, the method is a non-human mammal. In some ~~embodiment~~examples, the non-human mammal is a cow, a sheep, a goat, a pig, a dog, a horse, or a cat. In some ~~embodiment~~examples, the subject is a bird. In some ~~embodiment~~examples, the bird is a hen, a rooster, a turkey, or a parrot. In some ~~embodiment~~examples, the method further includes administering an adjuvant to the subject. In some ~~embodiment~~examples, the adjuvant is a separate molecular entity from the circular polyribonucleotide, linear polyribonucleotide, or preparation or composition thereof. In some ~~embodiment~~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 ~~embodiment~~examples, the adjuvant is a polypeptide adjuvant. In some ~~embodiment~~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 ~~embodiment~~examples, the adjuvant is an innate immune system stimulator. In some ~~embodiment~~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 ~~embodiment~~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 ~~embodiment~~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 ~~embodiment~~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 ~~embodiment~~examples, the method further comprises administering to the subject a polypeptide immunogen (e.g., a protein subunit comprising a polypeptide immunogen). In some ~~embodiment~~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 ~~embodiment~~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 ~~embodiment~~examples, the polypeptide immunogen maintains or enhances an immune response in the subject against the polypeptide immunogen.

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

Numbered Example~~Embodiment~~s:

[1] A circular polyribonucleotide comprising a plurality of sequences, each sequence encoding a polypeptide immunogen, wherein at least two of the polypeptide immunogens have at least 90% amino acid sequence identity.

[2] The circular polyribonucleotide of paragraph [1], wherein the at least two polypeptide immunogens have at least 95% amino acid sequence identity.

[3] The circular polyribonucleotide of paragraph [1] or [2], wherein the at least two polypeptide immunogens have less than 100% amino acid sequence identity.

[4] The circular polyribonucleotide of paragraph [1], wherein each of the polypeptide immunogens have at least 90% amino acid sequence identity.

[5] The circular polyribonucleotide of paragraph [4], wherein each of the polypeptide immunogens have at least 95% amino acid sequence identity.