# IMMUNOGENIC COMPOSITIONS AND THEIR USES

## 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. However, there remains a need to develop new and improved immunogenic compositions and uses thereof.

## Summary

This disclosure provides compositions, pharmaceutical preparations, and methods relating to circular polyribonucleotides encoding one or more immunogens including a multimerization domain. This disclosure also provides methods of using circular polyribonucleotides encoding one or more immunogens including a multimerization domain. This disclosure also provides circular polyribonucleotides including a first expression sequence that encodes an immunogen including a multimerization domain and a second expression sequence that encodes an adjuvant. This disclosure also provides circular polyribonucleotides including an expression sequence that encodes an immunogen including a multimerization domain and a non-coding sequence that stimulates the innate immune system. 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 a first example, the disclosure provides a circular polyribonucleotide including an open reading frame including a sequence encoding an immunogen and a sequence encoding a multimerization domain. In one aspect, the present disclosure refers to a circular polyribonucleotide comprising an open reading frame comprising a sequence encoding an immunogen and a sequence encoding a multimerization domain.

In some examples, the open reading frame includes, arranged in the following 5'-to-3' order, a first sequence encoding an immunogen and second sequence encoding a multimerization domain.

In some examples, the open reading frame includes, arranged in the following 5'-to-3' order, a first sequence encoding an immunogen, a second sequence encoding a multimerization domain, and a third sequence encoding an immunogen.

In some examples, the open reading frame includes, arranged in the following 5'-to-3' order, a first sequence encoding an immunogen, a second sequence encoding a multimerization domain, a third sequence encoding an immunogen, and a fourth sequence encoding a multimerization domain.

In some examples, the open reading frame includes, arranged in the following 5'-to-3' order, a first sequence encoding an immunogen, a second sequence encoding a multimerization domain, and a third sequence encoding a multimerization domain.

In some examples, the open reading frame includes, arranged in the following 5'-to-3' order, a first sequence encoding a multimerization domain and second sequence encoding an immunogen.

In some examples, the open reading frame includes, arranged in the following 5'-to-3' order, a first sequence encoding a multimerization domain, a second sequence encoding an immunogen, and a third sequence encoding a multimerization domain.

In some examples, the open reading frame includes, arranged in the following 5'-to-3' order, a first sequence encoding a multimerization domain, a second sequence encoding an immunogen, a third sequence encoding a multimerization domain, and a fourth sequence encoding an immunogen.

In some examples, the open reading frame includes, arranged in the following 5'-to-3' order, a first sequence encoding a multimerization domain, a second sequence encoding a multimerization domain, and a third sequence encoding an immunogen.

In some examples, the multimerization domain or each multimerization domain includes a T4 foldon domain. In some examples, the multimerization domain or each multimerization domain includes a ferritin domain. In some examples, the multimerization domain or each multimerization domain includes a β-annulus peptide. In some examples, the multimerization domain or each multimerization domain includes an AaLS peptide. In some examples, the multimerization domain or each multimerization domain includes a lumazine synthase domain.

In some examples, the open reading frame includes, arranged in the following 5'-to-3' order, a first sequence encoding an immunogen and second sequence encoding a T4 foldon domain.

In some examples, the open reading frame includes, arranged in the following 5'-to-3' order, a first sequence encoding an immunogen and second sequence encoding a ferritin domain.

In some examples, the open reading frame includes, arranged in the following 5'-to-3' order, a first sequence encoding an immunogen and second sequence encoding a β-annulus peptide.

In some examples, the open reading frame includes, arranged in the following 5'-to-3' order, a first sequence encoding an immunogen and second sequence encoding an AaLS peptide.

In some examples, the open reading frame includes, arranged in the following 5'-to-3' order, a first sequence encoding an immunogen, a second sequence encoding a T4 foldon domain, and a third sequence encoding an immunogen.

In some examples, the open reading frame includes, arranged in the following 5'-to-3' order, a first sequence encoding an immunogen, a second sequence encoding a T4 foldon domain, and a third sequence encoding a ferritin domain.

In some examples, the open reading frame includes, arranged in the following 5'-to-3' order, a first sequence encoding an immunogen, a second sequence encoding a ferritin domain, and a third sequence encoding a T4 foldon domain.

In some examples, each immunogen is, independently, operably linked to a secretion signal sequence.

In some examples, the open reading frame is operably linked to an IRES.

In some examples, the circular polyribonucleotide further includes a second open reading frame encoding a second polypeptide operably linked to a second IRES.

In some examples, the second polypeptide is a polypeptide immunogen. In some examples, the second polypeptide 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, a viral component or a component of the innate immune system.

In some examples, the circular polyribonucleotide further includes a non-coding ribonucleic acid sequence that is an innate immune system stimulator. In some examples, the innate immune system stimulator is selected from a GU-rich motif, an AU-rich motif, a structured region including dsRNA, or an aptamer.

In another example, the disclosure provides an immunogenic composition including a circular polyribonucleotide described herein and a pharmaceutically acceptable excipient. In one aspect, the present disclosure refers to an immunogenic composition comprising the circular polyribonucleotide described herein and a pharmaceutically acceptable excipient. In some examples, the composition further includes a second circular polyribonucleotide. In some examples, the second circular polyribonucleotide includes an open reading frame encoding an immunogen. In some examples, the second circular polyribonucleotide includes an open reading frame encoding a polypeptide adjuvant. In some examples, the second circular polyribonucleotide includes a non-coding ribonucleic acid sequence that is an innate immune system stimulator.

In another example, the disclosure provides a method of inducing an immune response against an immunogen in a subject, the method including administering to the subject a circular polyribonucleotide or immunogenic composition described herein. In one aspect, the present disclosure refers to use of the circular polyribonucleotide or immunogenic composition described herein in the manufacture of a medicament for inducing an immune response against an immunogen in a subject.

In another example, the disclosure provides a method of treating or preventing a disease, condition, or disorder in a subject, the method including administering to the subject a circular polyribonucleotide or immunogenic composition described herein. In one aspect, the present disclosure refers to use of the circular polyribonucleotide or immunogenic composition described herein in the manufacture of a medicament for treating or preventing a disease, condition, or disorder in a subject.

In some examples, the subject is a human subject.

In some examples, the method further includes administering an adjuvant to the subject.

In some examples, the method further includes administering a polypeptide immunogen to the subject.

## Definitions

The present disclosure will be described with respect to particular embodiments and with reference to certain figures, but the disclosure is not limited thereto but only by the claims. Terms as set forth hereinafter are generally to be understood in their common sense unless indicated otherwise.

As used herein, the term “adaptive immune response” means either a humoral or cell-mediated immune response. For purposes of the present disclosure, a "humoral immune response" refers to an immune response mediated by antibody molecules, while a "cellular immune response" is one mediated by T-lymphocytes and/or other white blood cells.

As used herein, the term “adjuvant” refers to a composition (*e.g.,* a compound, polypeptide, nucleic acid, or lipid) that increases an immune response, for example, increases a specific immune response against an immunogen. Increasing an immune response includes intensification or broadening the specificity of either or both antibody and cellular immune responses.

As used herein, the term “carrier” means a compound, composition, reagent, or molecule that facilitates the transport or delivery of a composition (*e.g.,* a polyribonucleotide) into a subject, a tissue, or a cell. Non-limiting examples of carriers include carbohydrate carriers (*e.g.,* an anhydride-modified phytoglycogen or glycogen-type material), nanoparticles (*e.g.,* a nanoparticle that encapsulates or is covalently linked binds to the circular polyribonucleotide), liposomes, fusosomes, *ex vivo* differentiated