VARICELLA-ZOSTER VIRUS IMMUNOGEN COMPOSITIONS AND THEIR USES

Background

Varicella-zoster virus (VZV) is a virus that belongs to the α-herpesvirus family. VZV is present worldwide and is highly infectious. Primary infection leads to acute varicella, also referred to as “chickenpox.” After initial infection, VZV establishes lifelong latency in cranial nerve and dorsal root ganglia and can reactivate years to decades later as herpes zoster (HZ), referred to as “shingles.” VZV can also cause a number of neurologic conditions ranging from aseptic meningitis to encephalitis. Other serious complications of VZV infection include postherpetic neuralgia, Mollaret's meningitis, zoster multiplex, thrombocytopenia, myocarditis, arthritis, and inflammation of arteries in the brain leading to stroke, myelitis, herpes ophthalmicus, and zoster sine herpete. There is a need for vaccines and therapeutics that are active against varicella-zoster virus.

Summary

This disclosure provides compositions, pharmaceutical preparations, and methods relating to circular polyribonucleotides encoding one or more VZV immunogens. This disclosure also provides methods of using circular polyribonucleotides encoding one or more VZV 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 (*e.g.,* chickenpox or shingles).

In a first example, the disclosure provides a circular polyribonucleotide including an open reading frame encoding a varicella-zoster virus (VZV) polypeptide immunogen. In one aspect, the present disclosure refers to a circular polyribonucleotide comprising an open reading frame encoding a varicella-zoster virus (VZV) polypeptide immunogen.

In some examples, the VZV polypeptide immunogen is a VZV glycoprotein or an immunogenic fragment thereof. In some examples, the VZV glycoprotein is selected from VZV gE, gl, gB, gH, gK, gL, gC, gN, and gM, or an immunogenic fragment thereof.

In some examples, the VZV glycoprotein is VZV gE, or an immunogenic fragment thereof. In some examples, the VZV glycoprotein is a mutational variant of VZV gE, or an immunogenic fragment thereof, including no more than 10 amino acid substitutions, deletions, or insertions relative to wild-type VZV gE. In some examples, the VZV gE polypeptide is a truncated polypeptide lacking an anchor domain (ER retention domain). In some examples, the VZV gE polypeptide is a truncated polypeptide lacking a carboxy terminal tail domain. In some examples, the VZV gE polypeptide includes amino acids 1-524, 1-546, 1-561, 1-573, or 1-623 of VZV gE. In some examples, the VZV gE polypeptide includes a Y569A mutation, a Y582G mutation, or a Y569A/Y582G double mutation. In some examples, the VZV gE polypeptide includes amino acids 1-573 of VZV gE and a Y569A mutation. In some examples, the VZV gE polypeptide includes amino acids 1-623 of VZV gE and a Y569A mutation, a Y582G mutation, or a Y569A/Y582G double mutation.

In some examples, the VZV gE polypeptide includes an amino acid sequence having at least 85% (*e.g.,* at least 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity with the amino acid sequence of any one of SEQ ID NOs: 29-33 and 65-68. In some examples, the VZV gE polypeptide includes the amino acid sequence of any one of SEQ ID NOs: 29-33 and 65-68. In some examples, the VZV

immunogen is an immunogenic fragment including a contiguous stretch of at least 100, 150, 200, 250, 300, 350, 400, 450, 500, or 550 amino acids of the amino acid sequence of any one of SEQ ID NOs: 29-33 and 65-68. In some examples, the VZV immunogen is an immunogenic fragment including a contiguous stretch of at least 50%, 60%, 70%, 80%, 90%, or 95% of the amino acids of the amino acid sequence of any one of SEQ ID NOs: 29-33 and 65-68. In some examples, the VZV immunogen is a variant of an amino acid sequence of any one of SEQ ID NOs: 29-33 and 65-68 that includes no more than one, two, three, four, five, six, seven, eight, nine, or ten mutations (*e.g.,* point mutations, deletions, or insertions).

In some examples, the VZV gE polypeptide further includes signal sequence and the VZV gE polypeptide and signal sequence together include an amino acid sequence having at least 85% (*e.g.,* at least 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity with the amino acid sequence of any one of SEQ ID NOs: 34-38 and 69-70. In some examples, the VZV gE polypeptide further includes signal sequence and the VZV gE polypeptide and signal sequence together include the amino acid sequence of any one of SEQ ID NOs: 34-38 and 69-70. In some examples, the VZV immunogen is an immunogenic fragment including a contiguous stretch of at least 100, 150, 200, 250, 300, 350, 400, 450, 500, or 550 amino acids of the amino acid sequence of any one of SEQ ID NOs: 34-38 and 69-70. In some examples, the VZV immunogen is an immunogenic fragment including a contiguous stretch of at least 50%, 60%, 70%, 80%, 90%, or 95% of the amino acids of the amino acid sequence of any one of SEQ ID NOs: 34-38 and 69-70. In some examples, the VZV immunogen is a variant of an amino acid sequence of any one of SEQ ID NOs: 34-38 and 69-70 that includes no more than one, two, three, four, five, six, seven, eight, nine, or ten mutations (*e.g.,* point mutations, deletions, or insertions).

In some examples, VZV gE polypeptide, optionally further including a signal sequence, is encoded by a nucleic acid sequence having at least 85% (*e.g.,* at least 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity with the nucleic acid sequence of any one of SEQ ID NOs: 39-47 and 71-83. In some examples, the VZV gE polypeptide, optionally further including a signal sequence, is encoded by a nucleic acid sequence of any one of SEQ ID NOs: 39-47 and 71-83. In some examples, the VZV polypeptide immunogen is a VZV immediate early protein or an immunogenic fragment thereof. In some examples, the VZV immediate early protein is an IE63 polypeptide. In some examples, the VZV IE63 polypeptide comprises an amino acid sequence having at least 85% (*e.g.,* at least 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity with the amino acid sequence of SEQ ID NO: 84. In some examples, the VZV IE63 polypeptide comprises the amino acid sequence of SEQ ID NO: 84. In some examples, the VZV IE63 polypeptide, optionally further comprising a signal sequence, is encoded by a nucleic acid sequence having at least 85% (*e.g.,* at least 90%, 95%, 96%, 97%, 98%, or 99%) sequence identity with the nucleic acid sequence of SEQ ID NO: 85. In some examples, the VZV IE63 polypeptide, optionally further comprising a signal sequence, is encoded by a nucleic acid sequence of SEQ ID NO: 85.

In some examples, the polyribonucleotide sequence encoding the VZV immunogen is a fragment including a contiguous stretch of at least 300, 400, 500, 600, 700, 800, 900, 1,000, 1100, 1200, 1300, 1400, or 1500 nucleotides of any one of SEQ ID NOs: 39-47. In some examples, the polyribonucleotide sequence encoding the VZV immunogen is a fragment including a contiguous stretch of at least 50%, 60%, 70%, 80%, 90%, or 95% of any one of SEQ ID NOs: 39-47.

In some examples, the nucleic acid sequence encoding the VZV polypeptide immunogen has a GC content of at least 51% (*e.g.,* at least 52%, 53%, 54%, 55%, 56%, 57%, 58%, 59%, or 60%). In some examples, the GC content of a nucleic acid sequence encoding a VZV immunogen is at most 52%, 53%, 54%, 55%, 56%, 57%, 58% or 59%, or 60%. In some examples, the GC content of a nucleic acid sequence encoding a VZV immunogen is 51% to 60%, 52% to 60%, 53% to 60%, 54% to 60%, 55% to 60%, 52% to 58%, 53% to 58%. In some examples, the nucleic acid sequence encoding the VZV polypeptide immunogen has a GC content of 51% to 60%.

In some examples, the nucleic acid sequence encoding the VZV polypeptide immunogen has a uridine content of more than 20%. In some examples, the uridine content of a nucleic acid sequence encoding a VZV immunogen is more than 10% (*e.g.,* more than 11%, 12%, 13%, 14%, 15%, 16%, 17%, 18%, 19%, 20%, 21%, 22%, 23%, 24%, or 25%). In some examples, the uridine content of a nucleic acid sequence encoding a VZV immunogen is at most 30% (*e.g.,* at most 29%, 28%, 27%, 26%, 25%, 24%, 23%, 22%, 21%, or 20%). In some examples, the uridine content of a nucleic acid sequence encoding a VZV immunogen is 20% to 28%, 21% to 26%, 10% to 24%, 15% to 24%, 20% to 24%, 21% to 24%, 22% to 24%, 23% to 24%, 10% to 23%, 15% to 23%, 20% to 23%, 21% to 23%, or 22% to 23%. In some examples, the nucleic acid sequence encoding the VZV polypeptide immunogen has a uridine content of 20% to 28%.

In some examples, the VZV polypeptide immunogen further includes a sequence encoding a multimerization domain. In some examples, the multimerization domain is selected from a T4 foldon domain, a ferritin domain, a β-annulus peptide, an AaLS peptide, or a lumazine synthase domain. In some examples, the multimerization domain is at the N-terminus of the VZV polypeptide immunogen. In some examples, the multimerization domain is at the C-terminus of the VZV polypeptide immunogen.

In some examples, the open reading frame encoding the VZV polypeptide immunogen is operably linked to an IRES.

In some examples, the open reading frame encoding the VZV polypeptide immunogen encodes a second polypeptide. In some examples, the VZV polypeptide immunogen and the second polypeptide are separated by a polypeptide linker, a 2A self-cleaving peptide, a protease cleavage site, or 2A self-cleaving peptide in tandem with a protease cleavage site. In some examples, the protease cleavage site is a furin cleavage site. 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 VZV polypeptide immunogen. In some examples, the second polypeptide is a VZV glycoprotein selected from VZV gE, gl, gB, gH, gK, gL, gC, gN, and gM, a VZV immediate early protein, or an immunogenic fragment thereof. In some examples, the second polypeptide is VZV gE, or an immunogenic fragment thereof. In some examples, the second polypeptide is VZV IE63, or an immunogenic fragment thereof.

In some examples, the second polypeptide is a polypeptide adjuvant. In some 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 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 some examples, the open reading frame encodes a concatemeric VZV immunogen. In some examples, the open reading frame comprises between 2-100 VZV immunogens connected directly to one another or interspersed by linkers. In other examples the immunogen is a concatemeric peptide immunogen composed of multiple peptide epitopes. In some examples, the circular polyribonucleotide encodes 2-10 VZV immunogens. In some examples, the circular polyribonucleotide encodes at least 10, 20, 30, 40, 50, 60, 70, 80, 90 or 100 VZV immunogens. In some examples, the VZV immunogens are separated by a polypeptide linker, a 2A self-cleaving peptide, a protease cleavage site, or 2A self-cleaving peptide in tandem with a protease cleavage site. In some examples, the protease cleavage site is a furin cleavage site.

In another example, the disclosure provides an immunogenic composition including any circular polyribonucleotide described herein and a pharmaceutically acceptable excipient. In one aspect, the present disclosure refers to an immunogenic composition comprising the circular polyribonucleotide as 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 a second polypeptide 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 in a subject against VZV, 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 as described herein in the manufacture of a medicament for inducing an immune response in a subject against VZV.

In another example, the disclosure provides a method of preventing a VZV infection 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 as described herein in the manufacture of a medicament for preventing a VZV infection in a subject.

In another example, the disclosure provides a method of treating a subject who has or is suspected to have a VZV infection, 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 as described herein in the manufacture of a medicament for treating a subject who has or is suspected to have a VZV infection.

In some examples, the subject has been previously diagnosed with a VZV infection or a disorder associated with a VZV infection. In some examples, the VZV infection is asymptomatic or the VZV infection is dormant. In some examples, the subject has been diagnosed with Shingles. In some examples, administering the circular polyribonucleotide or immunogenic composition decreases the frequency or severity of symptoms associated with Shingles. 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 VZV polypeptide immunogen to the subject.