several constructs (Figure 2). As the naïve S protein is a trimer and this trimeric structure has most likely an effect on the stability of the protein and the antigenicity, we included a strategy based on a stabilized construct introducing the T4 bacteriophage fibritin domain which is also in use in HIV for generating stable gp140 trimers and functional for SARS RBD-constructs.

Summary

The present disclosure generally embraces the immunotherapeutic treatment of a subject comprising the administration of RNA, i.e., vaccine RNA, encoding an amino acid sequence, i.e., a vaccine antigen, comprising SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof, i.e., an antigenic peptide or protein. Thus, the vaccine antigen comprises an epitope of SARS-CoV-2 S protein for inducing an immune response against coronavirus S protein, in particular SARS-CoV-2 S protein, in the subject. RNA encoding vaccine antigen is administered to provide (following expression of the polynucleotide by appropriate target cells) antigen for induction, i.e., stimulation, priming and/or expansion, of an immune response, e.g., antibodies and/or immune effector cells, which is targeted to target antigen (coronavirus S protein, in particular SARS-CoV-2 S protein) or a procession product thereof. In one example, the immune response which is to be induced according to the present disclosure is a B cell-mediated immune response, i.e., an antibody-mediated immune response. Additionally or alternatively, in one example, the immune response which is to be induced according to the present disclosure is a T cell-mediated immune response. In one example, the immune response is an anti-coronavirus, in particular anti-SARS-CoV-2 immune response.

The vaccine described herein comprises as the active principle single-stranded RNA that may be translated into the respective protein upon entering cells of a recipient. In addition to wildtype or codon-optimized sequences encoding the antigen sequence, the RNA may contain one or more structural elements optimized for maximal efficacy of the RNA with respect to

stability and translational efficiency (5' cap, 5' UTR, 3' UTR, poly(A)-tail). In one example, the RNA contains all of these elements. In one example, beta-S-ARCA(D1) ($m_2^{7,2'\text{-}O}$GppSpG) or $m_2^{7,3'\text{-}O}$Gppp($m_1^{2'\text{-}O}$)ApG may be utilized as specific capping structure at the 5'-end of the RNA drug substances. As 5'-UTR sequence, the 5'-UTR sequence of the human alpha-globin mRNA, optionally with an optimized 'Kozak sequence' to increase translational efficiency may be used. As 3'-UTR sequence, a combination of two sequence elements (FI element) derived from the "amino terminal enhancer of split" (AES) mRNA (called F) and the mitochondrial encoded 12S ribosomal RNA (called I) placed between the coding sequence and the poly(A)-tail to assure higher maximum protein levels and prolonged persistence of the mRNA may be used. These were identified by an *ex vivo* selection process for sequences that confer RNA stability and augment total protein expression (see WO 2017/060314, herein incorporated by reference). Alternatively, the 3'-UTR may be two re-iterated 3'-UTRs of the human beta-globin mRNA. Furthermore, a poly(A)-tail measuring 110 nucleotides in length, consisting of a stretch of 30 adenosine residues, followed by a 10 nucleotide linker sequence (of random nucleotides) and another 70 adenosine residues may be used. This poly(A)-tail sequence was designed to enhance RNA stability and translational efficiency.

Furthermore, a secretory signal peptide (sec) may be fused to the antigen-encoding regions preferably in a way that the sec is translated as N terminal tag. In one example, sec corresponds to the secreotory signal peptide of the S protein. Sequences coding for short linker peptides predominantly consisting of the amino acids glycine (G) and serine (S), as commonly used for fusion proteins may be used as GS/Linkers.

The vaccine RNA described herein may be complexed with proteins and/or lipids, preferably lipids, to generate RNA-particles for administration. If a combination of different RNAs is used, the RNAs may be complexed together or complexed separately with proteins and/or lipids to generate RNA-particles for administration.

In one aspect, the present disclosure relates to a kit comprising: a) a primary container; b) a payload container; c) at least one tray placed within the payload container; and d) a dry ice container; wherein the at least one tray has dimensions A x B x H, where A is 228 to 233 mm, B is 228 to 233 mm, and H is 38 to 46 mm.

In one example, the disclosure relates to a composition or medical preparation comprising RNA encoding an amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof.

In one example, an immunogenic fragment of the SARS-CoV-2 S protein comprises the S1 subunit of the SARS-CoV-2 S protein, or the receptor binding domain (RBD) of the S1 subunit of the SARS-CoV-2 S protein.

In one example, the amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof is able to form a multimeric complex, in particular a trimeric complex. To this end, the amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof may comprise a domain allowing the formation of a multimeric complex, in particular a trimeric complex of the amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof. In one example, the domain allowing the formation of a multimeric complex comprises a trimerization domain, for example, a trimerization domain as described herein.

In one example, the amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof is encoded by a coding sequence which is codon-optimized and/or the G/C content of which is increased compared to wild type coding sequence, wherein the codon-optimization and/or the increase in the G/C content preferably does not change the sequence of the encoded amino acid sequence.

In one example,

(i) the RNA encoding a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the nucleotide sequence of nucleotides 979 to 1584 of SEQ ID NO: 2, 8 or 9, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 979 to 1584 of SEQ ID NO: 2, 8 or 9, or a fragment of the nucleotide sequence of nucleotides 979 to 1584 of SEQ ID NO: 2, 8 or 9, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 979 to 1584 of SEQ ID NO: 2, 8 or 9; and/or

(ii) a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the amino acid sequence of amino acids 327 to 528 of SEQ ID NO: 1, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 327 to 528 of SEQ ID NO: 1, or an immunogenic fragment of the amino acid sequence of amino acids 327 to 528 of SEQ ID NO: 1, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 327 to 528 of SEQ ID NO: 1.

In one example,

(i) the RNA encoding a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the nucleotide sequence of nucleotides 49 to 2055 of SEQ ID NO: 2, 8 or 9, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 49 to 2055 of SEQ ID NO: 2, 8 or 9, or a fragment of the nucleotide sequence of nucleotides 49 to 2055 of SEQ ID NO: 2, 8 or 9, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 49 to 2055 of SEQ ID NO: 2, 8 or 9; and/or

(ii) a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the amino acid sequence of amino acids 17 to 685 of SEQ ID NO: 1, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 17 to 685 of SEQ ID NO: 1, or an immunogenic fragment of the amino acid sequence of amino acids 17 to 685 of SEQ ID NO: 1, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 17 to 685 of SEQ ID NO: 1.

In one example,

(i) the RNA encoding a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the nucleotide sequence of nucleotides 49 to 3819 of SEQ ID NO: 2, 8 or 9, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 49 to 3819 of SEQ ID NO: 2, 8 or 9, or a fragment of the nucleotide sequence of nucleotides 49 to 3819 of SEQ ID NO: 2, 8 or 9, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 49 to 3819 of SEQ ID NO: 2, 8 or 9; and/or

(ii) a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the amino acid sequence of amino acids 17 to 1273 of SEQ ID NO: 1 or 7, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 17 to 1273 of SEQ ID NO: 1 or 7, or an immunogenic fragment of the amino acid sequence of amino acids 17 to 1273 of SEQ ID NO: 1 or 7, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 17 to 1273 of SEQ ID NO: 1 or 7.

In one example, the amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises a secretory signal peptide.

In one example, the secretory signal peptide is fused, preferably N-terminally, to a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof.

In one example,

(i) the RNA encoding the secretory signal peptide comprises the nucleotide sequence of nucleotides 1 to 48 of SEQ ID NO: 2, 8 or 9, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 1 to 48 of SEQ ID NO: 2, 8 or 9, or a fragment of the nucleotide sequence of nucleotides 1 to 48 of SEQ ID NO: 2, 8 or 9, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 1 to 48 of SEQ ID NO: 2, 8 or 9; and/or

(ii) the secretory signal peptide comprises the amino acid sequence of amino acids 1 to 16 of SEQ ID NO: 1, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 16 of SEQ ID NO: 1, or a functional fragment of the amino acid sequence of amino acids 1 to 16 of SEQ ID NO: 1, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 16 of SEQ ID NO: 1.

In one example,

(i) the RNA encoding a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the nucleotide sequence of SEQ ID NO: 6, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 6, or a fragment of the nucleotide sequence of SEQ ID NO: 6, or the nucleotide sequence

having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 6; and/or

(ii) a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the amino acid sequence of SEQ ID NO: 5, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 5, or an immunogenic fragment of the amino acid sequence of SEQ ID NO: 5, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 5.

In one example, the RNA is a modified RNA, in particular a stabilized mRNA. In one example, the RNA comprises a modified nucleoside in place of at least one uridine. In one example, the RNA comprises a modified nucleoside in place of each uridine. In one example, the modified nucleoside is independently selected from pseudouridine (ψ), N1-methyl-pseudouridine (m1ψ), and 5-methyl-uridine (m5U).

In one example, the RNA comprises a modified nucleoside in place of uridine.

In one example, the modified nucleoside is selected from pseudouridine (ψ), N1-methyl-pseudouridine (m1ψ), and 5-methyl-uridine (m5U).

In one example, the RNA comprises a 5’ cap.

In one example, the RNA encoding an amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises a 5’ UTR comprising the nucleotide sequence of SEQ ID NO: 12, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 12.

In one example, the RNA encoding an amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises a 3’ UTR comprising the nucleotide

sequence of SEQ ID NO: 13, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 13.

In one example, the RNA encoding an amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises a poly-A sequence.

In one example, the poly-A sequence comprises at least 100 nucleotides.

In one example, the poly-A sequence comprises or consists of the nucleotide sequence of SEQ ID NO: 14.

In one example, the RNA is formulated or is to be formulated as a liquid, a solid, or a combination thereof.

In one example, the RNA is formulated or is to be formulated for injection.

In one example, the RNA is formulated or is to be formulated for intramuscular administration.

In one example, the RNA is formulated or is to be formulated as particles.

In one example, the particles are lipid nanoparticles (LNP) or lipoplex (LPX) particles.

In one example, the LNP particles comprise ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate), 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide, 1,2-Distearoyl-sn-glycero-3-phosphocholine, and cholesterol.

In one example, the RNA lipoplex particles are obtainable by mixing the RNA with liposomes.

In one example, the RNA lipoplex particles are obtainable by mixing the RNA with lipids.

In one example, the RNA is formulated or is to be formulated as colloid. In one example, the RNA is formulated or is to be formulated as particles, forming the dispersed phase of a colloid. In one example, 50% or more, 75% or more, or 85% or more of the RNA are present in the dispersed phase. In one example, the RNA is formulated or is to be formulated as particles comprising RNA and lipids. In one example, the particles are

formed by exposing RNA, dissolved in an aqueous phase, with lipids, dissolved in an organic phase. In one example, the organic phase comprises ethanol. In one example, the particles are formed by exposing RNA, dissolved in an aqueous phase, with lipids, dispersed in an aqueous phase. In one example, the lipids dispersed in an aqueous phase form liposomes.

In one example, the RNA is mRNA or saRNA.

In one example, the composition or medical preparation is a pharmaceutical composition.

In one example, the composition or medical preparation is a vaccine.

In one example, the pharmaceutical composition further comprises one or more pharmaceutically acceptable carriers, diluents and/or excipients.

In one example, the composition or medical preparation is a kit.

In one example, the RNA and optionally the particle forming components are in separate vials.

In one example, the kit further comprises instructions for use of the composition or medical preparation for inducing an immune response against coronavirus in a subject.

In one example, the disclosure relates to the composition or medical preparation described herein for pharmaceutical use.

In one example, the pharmaceutical use comprises inducing an immune response against coronavirus in a subject.

In one example, the pharmaceutical use comprises a therapeutic or prophylactic treatment of a coronavirus infection.

In one example, the composition or medical preparation described herein is for administration to a human.

In one example, the coronavirus is a betacoronavirus.

In one example, the coronavirus is a sarbecovirus.

In one example, the coronavirus is SARS-CoV-2.

In one example, the disclosure relates to a method of inducing an immune response against coronavirus in a subject comprising administering to the subject a composition comprising RNA encoding an amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof.

In one example, an immunogenic fragment of the SARS-CoV-2 S protein comprises the S1 subunit of the SARS-CoV-2 S protein, or the receptor binding domain (RBD) of the S1 subunit of the SARS-CoV-2 S protein.

In one example, the amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof is able to form a multimeric complex, in particular a trimeric complex. To this end, the amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof may comprise a domain allowing the formation of a multimeric complex, in particular a trimeric complex of the amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof. In one example, the domain allowing the formation of a multimeric complex comprises a trimerization domain, for example, a trimerization domain as described herein.

In one example, the amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof is encoded by a coding sequence which is codon-optimized and/or the G/C content of which is increased compared to wild type coding sequence, wherein the codon-optimization and/or the increase in the G/C content preferably does not change the sequence of the encoded amino acid sequence.

In one example,

(i) the RNA encoding a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the nucleotide sequence of nucleotides 979 to 1584 of SEQ ID NO: 2, 8 or 9, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 979 to 1584 of SEQ ID NO: 2, 8 or 9, or a fragment of the nucleotide sequence of nucleotides 979 to 1584 of SEQ ID NO: 2, 8 or 9, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 979 to 1584 of SEQ ID NO: 2, 8 or 9; and/or

(ii) a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the amino acid sequence of amino acids 327 to 528 of SEQ ID NO: 1, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 327 to 528 of SEQ ID NO: 1, or an immunogenic fragment of the amino acid sequence of amino acids 327 to 528 of SEQ ID NO: 1, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 327 to 528 of SEQ ID NO: 1.

In one example,

(i) the RNA encoding a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the nucleotide sequence of nucleotides 49 to 2055 of SEQ ID NO: 2, 8 or 9, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 49 to 2055 of SEQ ID NO: 2, 8 or 9, or a fragment of the nucleotide sequence of nucleotides 49 to 2055 of SEQ ID NO: 2, 8 or 9, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 49 to 2055 of SEQ ID NO: 2, 8 or 9; and/or

(ii) a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the amino acid sequence of amino acids 17 to 685 of SEQ ID NO: 1, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 17 to 685 of SEQ ID NO: 1, or an immunogenic fragment of the amino acid sequence of amino acids 17 to 685 of SEQ ID NO: 1, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 17 to 685 of SEQ ID NO: 1.

In one example,

(i) the RNA encoding a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the nucleotide sequence of nucleotides 49 to 3819 of SEQ ID NO: 2, 8 or 9, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 49 to 3819 of SEQ ID NO: 2, 8 or 9, or a fragment of the nucleotide sequence of nucleotides 49 to 3819 of SEQ ID NO: 2, 8 or 9, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 49 to 3819 of SEQ ID NO: 2, 8 or 9; and/or

(ii) a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the amino acid sequence of amino acids 17 to 1273 of SEQ ID NO: 1 or 7, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 17 to 1273 of SEQ ID NO: 1 or 7, or an immunogenic fragment of the amino acid sequence of amino acids 17 to 1273 of SEQ ID NO: 1 or 7, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 17 to 1273 of SEQ ID NO: 1 or 7.

In one example, the amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises a secretory signal peptide.

In one example, the secretory signal peptide is fused, preferably N-terminally, to a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof.

In one example,

(i) the RNA encoding the secretory signal peptide comprises the nucleotide sequence of nucleotides 1 to 48 of SEQ ID NO: 2, 8 or 9, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 1 to 48 of SEQ ID NO: 2, 8 or 9, or a fragment of the nucleotide sequence of nucleotides 1 to 48 of SEQ ID NO: 2, 8 or 9, or the nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of nucleotides 1 to 48 of SEQ ID NO: 2, 8 or 9; and/or

(ii) the secretory signal peptide comprises the amino acid sequence of amino acids 1 to 16 of SEQ ID NO: 1, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 16 of SEQ ID NO: 1, or a functional fragment of the amino acid sequence of amino acids 1 to 16 of SEQ ID NO: 1, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of amino acids 1 to 16 of SEQ ID NO: 1.

In one example,

(i) the RNA encoding a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the nucleotide sequence of SEQ ID NO: 6, a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 6, or a fragment of the nucleotide sequence of SEQ ID NO: 6, or the nucleotide sequence

having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 6; and/or

(ii) a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises the amino acid sequence of SEQ ID NO: 5, an amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 5, or an immunogenic fragment of the amino acid sequence of SEQ ID NO: 5, or the amino acid sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the amino acid sequence of SEQ ID NO: 5.

In one example, the RNA is a modified RNA, in particular a stabilized mRNA. In one example, the RNA comprises a modified nucleoside in place of at least one uridine. In one example, the RNA comprises a modified nucleoside in place of each uridine. In one example, the modified nucleoside is independently selected from pseudouridine (ψ), N1-methyl-pseudouridine (m1ψ), and 5-methyl-uridine (m5U).

In one example, the RNA comprises a modified nucleoside in place of uridine.

In one example, the modified nucleoside is selected from pseudouridine (ψ), N1-methyl-pseudouridine (m1ψ), and 5-methyl-uridine (m5U).

In one example, the RNA comprises a cap.

In one example, the RNA encoding an amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises a 5’ UTR comprising the nucleotide sequence of SEQ ID NO: 12, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 12.

In one example, the RNA encoding an amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises a 3’ UTR comprising the nucleotide

sequence of SEQ ID NO: 13, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 13.

In one example, the RNA encoding an amino acid sequence comprising a SARS-CoV-2 S protein, an immunogenic variant thereof, or an immunogenic fragment of the SARS-CoV-2 S protein or the immunogenic variant thereof comprises a poly-A sequence.

In one example, the poly-A sequence comprises at least 100 nucleotides.

In one example, the poly-A sequence comprises or consists of the nucleotide sequence of SEQ ID NO: 14.

In one example, the RNA is formulated as a liquid, a solid, or a combination thereof.

In one example, the RNA is administered by injection.

In one example, the RNA is administered by intramuscular administration.

In one example, the RNA is formulated as particles.

In one example, the particles are lipid nanoparticles (LNP) or lipoplex (LPX) particles.

In one example, the LNP particles comprise ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis(2-hexyldecanoate), 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide, 1,2-Distearoyl-sn-glycero-3-phosphocholine, and cholesterol.

In one example, the RNA lipoplex particles are obtainable by mixing the RNA with liposomes.

In one example, the RNA lipoplex particles are obtainable by mixing the RNA with lipids.

In one example, the RNA is formulated as colloid. In one example, the RNA is formulated as particles, forming the dispersed phase of a colloid. In one example, 50% or more, 75% or more, or 85% or more of the RNA are present in the dispersed phase. In one example, the RNA is formulated as particles comprising RNA and lipids. In one example, the particles are formed by exposing RNA, dissolved in an aqueous phase, with lipids, dissolved in an organic phase. In one example, the organic phase comprises ethanol. In one example, the particles are formed by exposing RNA, dissolved in an

aqueous phase, with lipids, dispersed in an aqueous phase. In one example, the lipids dispersed in an aqueous phase form liposomes.

In one example, the RNA is mRNA or saRNA.

In one example, the method is a method for vaccination against coronavirus.

In one example, the method is a method for therapeutic or prophylactic treatment of a coronavirus infection.

In one example, the subject is a human.

In one example, the coronavirus is a betacoronavirus.

In one example, the coronavirus is a sarbecovirus.

In one example, the coronavirus is SARS-CoV-2.

In one example of the method described herein, the composition is a composition described herein.

In one example, the disclosure relates to a composition or medical preparation described herein for use in a method described herein.

Among other things, the present disclosure demonstrates that a composition comprising a lipid nanoparticle encapsulated mRNA encoding at least a portion (e.g., that is or comprises an epitope) of a SARS-CoV-2-encoded polypeptide (e.g., of a SARS-CoV-2-encoded S protein) can achieve detectable antibody titer against the epitope in serum within 7 days after administration to a population of adult human subjects according to a regimen that includes administration of at least one dose of the vaccine composition. Moreover, the present disclosure demonstrates persistence of such antibody titer. In some examples, the present disclosure demonstrates increased such antibody titer when a modified mRNA is used, as compared with that achieved with a corresponding unmodified mRNA.

In some examples, a provided regimen includes at least one dose. In some examples, a provided regimen includes a first dose and at least one subsequent dose. In some examples, the first dose is the same amount as at least one subsequent dose. In some examples, the first dose is the same amount as all subsequent doses. In some examples, the first dose is a different amount as at least one subsequent dose. In some examples, the first dose is a different amount than all subsequent doses. In some examples, a provided regimen comprises two doses. In some examples, a provided regimen consists of two doses.

In particular examples, the immunogenic composition is formulated as a single-dose in a container, e.g., a vial. In some examples, the immunogenic composition is formulated as a multi-dose formulation in a vial. In some examples, the multi-dose formulation includes at least 2 doses per vial. In some examples, the multi-dose formulation includes a total of 2-20 doses per vial, such as, for example, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 doses per vial. In some examples, each dose in the vial is equal in volume. In some examples, a first dose is a different volume than a subsequent dose.

A "stable" multi-dose formulation exhibits no unacceptable levels of microbial growth, and substantially no or no breakdown or degradation of the active biological molecule component(s). As used herein, a "stable" immunogenic composition includes a formulation that remains capable of eliciting a desired immunologic response when administered to a subject.

In some examples, the multi-dose formulation remains stable for a specified time with multiple or repeated inoculations/insertions into the multi-dose container. For example, in some examples the multi-dose formulation may be stable for at least three days with up to ten usages, when contained within a multi-dose container. In some examples, the multi-dose formulations remain stable with 2-20 inoculations/insertions.

In some examples, administration of a composition comprising a lipid nanoparticle encapsulated mRNA encoding at least a portion (e.g., that is or comprises an epitope) of a SARS-CoV-2-encoded polypeptide (e.g., of a SARS-CoV-2-encoded S protein), e.g., according to a regimen as described herein, may result in lymphopenia in some subjects (e.g., in all subjects, in most subjects, in about 50% or fewer, in about 40% or fewer, in about 40% or fewer, in about 25% or fewer, in about 20% or fewer, in about 15% or fewer, in about 10% or fewer, in about 5% or fewer, etc). Among other things, the present disclosure demonstrates that such lymphopenia can resolve over time. For example, in some examples, lymphopenia resolves within about 14, about 10, about 9, about 8, about 7 days or less. In some examples, lymphopenia is Grade 3, Grade 2, or less.

Thus, among other things, the present disclosure provides compositions comprising a lipid nanoparticle encapsulated mRNA encoding at least a portion (e.g., that is or comprises an epitope) of a SARS-CoV-2-encoded polypeptide (e.g., of a SARS-CoV-2-encoded S protein) that are characterized, when administered to a relevant population of adults, to display certain characteristics (e.g., achieve certain effects) as described herein. In some examples, provided compositions may have been prepared, stored, transported, characterized, and/or used under conditions where temperature does not exceed a particular threshold. Alternatively or additionally, in some examples, provided compositions may have been protected from light (e.g., from certain wavelengths) during some or all of their preparation, storage, transport, characterization, and/or use. In some examples, one or more features of provided compositions (e.g., mRNA stability, as may be assessed, for example, by one or more of size, presence of particular moiety or modification, etc; lipid nanoparticle stability or aggregation, pH, etc) may be or have been assessed at one or more points during preparation, storage, transport, and/or use prior to administration.

Among other things, the present disclosure documents that certain provided compositions in which nucleotides within an mRNA are not modified (e.g., are naturally-occurring A, U, C, G),

and/or provided methods relating to such compositions, are characterized (e.g., when administered to a relevant population, which may in some examples be or comprise an adult population), by an intrinsic adjuvant effect. In some examples, such composition and/or method can induce an antibody and/or a T cell response. In some examples, such a composition and/or method can induce a higher T cell response, as compared to conventional vaccines (e.g., non-mRNA vaccines such as protein vaccines).

Alternatively or additionally, the present disclosure documents that provided compositions (e.g., compositions comprising a lipid nanoparticle encapsulated mRNA encoding at least a portion (e.g., that is or comprises an epitope) of a SARS-CoV-2-encoded polypeptide (e.g., of a SARS-CoV-2-encoded S protein)) in which nucleotides within an mRNA are modified, and/or provided methods relating to such compositions, are characterized (e.g., when administered to a relevant population, which may in some examples be or comprise an adult population), by absence of an intrinsic adjuvant effect, or by a reduced intrinsic adjuvant effect as compared with an otherwise comparable composition (or method) with unmodified results. Alternatively or additionally, in some examples, such compositions (or methods) are characterized in that they (e.g., when administered to a relevant population, which may in some examples be or comprise an adult population) induce an antibody response and/or a CD4+ T cell response. Still further alternatively or additionally, in some examples, such compositions (or methods) are characterized in that they (e.g., when administered to a relevant population, which may in some examples be or comprise an adult population) induce a higher CD4+ T cell response than that observed with an alternative vaccine format (e.g., a peptide vaccine). In some examples involving modified nucleotides, such modified nucleotides may be present, for example, in a 3' UTR sequence, an antigen-encoding sequence, and/or a 5'UTR sequence. In some examples, modified nucleotides are or include one or more modified uracil residues and/or one or more modified cytosine residues.

Among other things, the present disclosure documents that provided (e.g., compositions comprising a lipid nanoparticle encapsulated mRNA encoding at least a portion (e.g., that is or comprises an epitope) of a SARS-CoV-2-encoded polypeptide (e.g., of a SARS-CoV-2-encoded S protein)) and/or methods are characterized by (e.g., when administered to a relevant population, which may in some examples be or comprise an adult population) sustained expression of an encoded polypeptide (e.g., of a SARS-CoV-2-encoded protein [such as an S protein] or portion thereof, which portion, in some examples, may be or comprise an epitope thereof). For example, in some examples, such compositions and/or methods are characterized in that, when administered to a human, they achieve detectable polypeptide expression in a biological sample (e.g., serum) from such human and, in some examples, such expression persists for a period of time that is at least at least 36 hours or longer, including, e.g., at least 48 hours, at least 60 hours, at least 72 hours, at least 96 hours, at least 120 hours, at least 148 hours, or longer.

Those skilled in the art, reading the present disclosure, will appreciate that it describes various mRNA constructs encoding at least a portion (e.g., that is or comprises an epitope) of a SARS-CoV-2-encoded polypeptide (e.g., of a SARS-CoV-2-encoded S protein)). Such person of ordinary skill, reading the present disclosure, will particularly appreciate that it describes various mRNA constructs encoding at least a portion of a SARS-CoV-2 S protein, for example at least an RBD portion of a SARS-CoV-2 S protein. Still further, such a person of ordinary skill, reading the present disclosure, will appreciate that it describes particular characteristics and/or advantages of mRNA constructs encoding at least a portion (e.g., that is or comprises an epitope) of a SARS-CoV-2-encoded polypeptide (e.g., of a SARS-CoV-2-encoded S protein). Among other things, the present disclosure particularly documents surprising and useful characteristics and/or advantages of certain mRNA constructs encoding a SARS-CoV-2 RBD portion and, in some examples, not encoding a full length SARS-CoV-2 S protein. Without wishing to be bound by any particular theory, the present disclosure suggests that provided

mRNA constructs that encode less than a full-length SARS-CoV-2 S protein, and particularly those that encode at least an RBD portion of such SARS-CoV-2 S protein may be particularly useful and/or effective for use as or in an immunogenic composition (e.g., a vaccine), and/or for achieving immunological effects as described herein (e.g., generation of SARS-CoV-2 neutralizing antibodies, and/or T cell responses (e.g., CD4+ and/or CD8+ T cell responses)).

In some examples, the present disclosure provides an RNA (e.g., mRNA) comprising an open reading frame encoding a polypeptide that comprises a receptor-binding portion of a SARS-CoV-2 S protein, which RNA is suitable for intracellular expression of the polypeptide. In some examples, such an encoded polypeptide does not comprise the complete S protein. In some examples, the encoded polypeptide comprises the receptor binding domain (RBD), for example, as shown in SEQ ID NO: 5. In some examples, the encoded polypeptide comprises the peptide according to SEQ ID NO: 29 or 31. In some examples, such an RNA (e.g., mRNA) may be complexed by a (poly)cationic polymer, polyplex(es), protein(s) or peptide(s). In some examples, such an RNA may be formulated in a lipid nanoparticle (e.g., ones described herein). In some examples, such an RNA (e.g., mRNA) may be particularly useful and/or effective for use as or in an immunogenic composition (e.g., a vaccine), and/or for achieving immunological effects as described herein (e.g., generation of SARS-CoV-2 neutralizing antibodies, and/or T cell responses (e.g., CD4+ and/or CD8+ T cell responses)). In some examples, such an RNA (e.g., mRNA) may be useful for vaccinating humans (including, e.g., humans known to have been exposed and/or infected by SARS-CoV-2, and/or humans not known to have been exposed to SARS-CoV-2).

Those skilled in the art, reading the present disclosure, will further appreciate that it describes various mRNA constructs comprising a nucleic acid sequence that encodes a full-length SARS-CoV-2 Spike protein (e.g., including examples in which such encoded SARS-CoV-2 Spike protein may comprise at least one or more amino acid substitutions, e.g., proline substitutions as described herein, and/or examples in which the mRNA sequence is codon-optimized

e.g., for mammalian, e.g., human, subjects). In some examples, such a full-length SARS-CoV-2 Spike protein may have an amino acid sequence that is or comprises that set forth in SEQ ID NO: 7. Still further, such a person of ordinary skill, reading the present disclosure, will appreciate, among other things, that it describes particular characteristics and/or advantages of certain mRNA constructs comprising a nucleic acid sequence that encodes a full-length SARS-CoV-2 Spike protein. Without wishing to be bound by any particular theory, the present disclosure suggests that provided mRNA constructs that encode a full-length SARS-CoV-2 S protein may be particularly useful and/or effective for use as or in an immunogenic composition (e.g., a vaccine) in particular subject population (e.g., particular age populations). For example, in some examples, such an mRNA composition may be particularly useful in younger (e.g., less than 25 years old, 20 years old, 18 years old, 15 years, 10 years old, or lower) subjects; alternatively or additionally, in some examples, such an mRNA composition may be particularly useful in elderly subjects (e.g., over 55 years old, 60 years old, 65 years old, 70 years old, 75 years old, 80 years old, 85 years old, or higher). In particular examples, an immunogenic composition comprising such an mRNA construct provided herein exhibits a minimal to modest increase (e.g., no more than 30% increase, no more than 20% increase, or no more than 10% increase, or lower) in dose level and/or dose number-dependent systemic reactogenicity (e.g., fever, fatigue, headache, chills, diarrhea, muscle pain, and/or joint pain, etc.) and/or local tolerability (e.g., pain, redness, and/or swelling, etc.), at least in some subjects (e.g., in some subject age groups); in some examples, such reactogenicity and/or local tolerability is observed particularly, in in younger age group (e.g., less than 25 years old, 20 years old, 18 years years old or lower) subjects, and/or in older (e.g., elderly) age group (e.g., 65-85 years old). In some examples, provided mRNA constructs that encode a full-length SARS-CoV-2 S protein may be particularly useful and/or effective for use as or in an immunogenic composition (e.g., a vaccine) for inducing SARS-CoV-2 neutralizing antibody response level in a population of subjects that are at high risk for severe diseases

associated with SARS-CoV-2 infection (e.g., an elderly population, for example, 65-85 year-old group). In some examples, a person of ordinary skill, reading the present disclosure, will appreciate, among other things, that provided mRNA constructs that encode a full-length SARS-CoV-2 S protein, which exhibit a favorable reactogenicity profile (e.g., as described herein) in younger and elderly age populations, may be particularly useful and/or effective for use as or in an immunogenic composition (e.g., a vaccine) for achieving immunological effects as described herein (e.g., generation of SARS-CoV-2 neutralizing antibodies, and/or T cell responses (e.g., CD4+ and/or CD8+ T cell responses)). In some examples, the present disclosure also suggests that provided mRNA constructs that encode a full-lenth SARS-CoV-2 S protein may be particularly effective to protect against SARS-CoV-2 infection, as characterized by earlier clearance of SARS-CoV-2 viral RNA in non-human mammalian subjects (e.g., rhesus macaques) that were immunized with immunogenic compositions comprising such mRNA constructs and subsequently challenged by SARS-CoV-2 strain. In some examples, such earlier clearance of SARS-CoV-2 viral RNA may be observed in the nose of non-human mammalian subjects (e.g., rhesus macaques) that were immunized with immunogenic compositions comprising such mRNA constructs and subsequently challenged by SARS-CoV-2 strain.

In some examples, the present disclosure provides an RNA (e.g., mRNA) comprising an open reading frame encoding a full-length SARS-CoV-2 S protein (e.g., a full-length SARS-CoV-2 S protein with one or more amino acid substitutions), which RNA is suitable for intracellular expression of the polypeptide. In some examples, the encoded polypeptide comprises the amino acid sequence of SEQ ID NO:_7. In some examples, such an RNA (e.g., mRNA) may be complexed by a (poly)cationic polymer, polyplex(es), protein(s) or peptide(s). In some examples, such an RNA may be formulated in a lipid nanoparticle (e.g., ones described herein).

In some examples, an immunogenic composition provided herein may comprise a plurality of (e.g., at least two or more, including, e.g., at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, etc.) immunoreactive epitopes of a SARS-CoV-2 polypeptide or variants thereof. In some such examples, such a plurality of immunoreactive epitopes may be encoded by a plurality of RNAs (e.g., mRNAs). In some such examples, such a plurality of immunoreactive epitopes may be encoded by a single RNA (e.g., mRNA). In some examples, nucleic acid sequences encoding a plurality of immunoreactive epitopes may be separated from each other in a single RNA (e.g., mRNA) by a linker (e.g., a peptide linker in some examples). Without wishing to be bound by any particular theory, in some examples, provided polyepitope immunogenic compositions (including, e.g., those that encode a full-length SARS-CoV-2 spike protein) may be particularly useful, when considering the genetic diversity of SARS-CoV-2 variants, to provide protection against numerous viral variants and/or may offer a greater opportunity for development of a diverse and/or otherwise robust (e.g., persistent, e.g., detectable about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60 or more days after administration of one or more doses) neutralizing antibody and/or T cell response, and in particular a particularly robust T_H1-type T cell (e.g., CD4+ and/or CD8+ T cell) response.

In some examples, the present disclosure documents that provided compositions and/or methods are characterized by (e.g., when administered to a relevant population, which may in some examples be or comprise an adult population) in that they achieve one or more particular therapeutic outcomes (e.g., effective immune responses as described herein and/or detectable expression of encoded SARS-CoV-2 S protein or an immunogenic fragment thereof) with a single administration; in some such examples, an outcome may be assessed, for example, as compared to that observed in absence of mRNA vaccines described herein. In some examples, a particular outcome may be achieved at a lower dose than required for one or more alternative strategies.

In some examples, the present disclosure provides an immunogenic composition comprising an isolated messenger ribonucleic acid (mRNA) polynucleotide, wherein the isolated mRNA polynucleotide comprises an open reading frame encoding a polypeptide that comprises a receptor-binding portion of a SARs-CoV-2 S protein, and wherein the isolated mRNA polynucleotide is formulated in at least one lipid nanoparticle. For example, in some examples, such a lipid nanoparticle may comprise a molar ratio of 20-60% ionizable cationic lipid, 5-25% non-cationic lipid (e.g., neutral lipid), 25-55% sterol or steroid, and 0.5-15% polymer-conjugated lipid (e.g., PEG-modified lipid). In some examples, a sterol or steroid included in a lipid nanoparticle may be or comprise cholesterol. In some examples, a neutral lipid may be or comprise 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC). In some examples, a polymer-conjugated lipid may be or comprise PEG2000 DMG. In some examples, such an immunogenic composition may comprise a total lipid content of about 1 mg to 10 mg, or 3 mg to 8 mg, or 4 mg to 6 mg. In some examples, such an immunogenic composition may comprise a total lipid content of about 5 mg/mL -15 mg/mL or 7.5 mg/mL- 12.5 mg/mL or 9-11 mg/mL. In some examples, such an isolated mRNA polynucleotide is provided in an effective amount to induce an immune response in a subject administered at least one dose of the immunogenic composition. In some examples, a polypeptide encoded by a provided isolated mRNA polynucleotide does not comprise the complete S protein. In some examples, such an isolated mRNA polynucleotide provided in an immunogenic composition is not self-replicating RNA.

In some examples, an immune response may comprise generation of a binding antibody titer against SARS-CoV-2 protein (including, e.g., a stabilized prefusion spike trimer in some examples) or a fragment thereof. In some examples, an immune response may comprise generation of a binding antibody titer against the receptor binding domain (RBD) of the SARS-CoV-2 spike protein. In some examples, a provided immunogenic composition has been established to achieve a detectable binding antibody titer after administration of a

first dose, with seroconversion in at least 70% (including, e.g., at least 80%, at least 90%, at least 95% and up to 100%) of a population of subjects receiving such a provided immunogenic composition, for example, by about 2 weeks.

In some examples, an immune response may comprise generation of a neutralizing antibody titer against SARS-CoV-2 protein (including, e.g., a stabilized prefusion spike trimer in some examples) or a fragment thereof. In some examples, an immune response may comprise generation of a neutralizing antibody titer against the receptor binding domain (RBD) of the SARS-CoV-2 spike protein. In some examples, a provided immunogenic composition has been established to achieve a neutralizing antibody titer in an appropriate system (e.g., in a human infected with SARS-CoV-2 and/or a population thereof, and/or in a model system therefor). For example, in some examples, such neutralizing antibody titer may have been demonstrated in one or more of a population of humans, a non-human primate model (e.g., rhesus macaques), and/or a mouse model.

In some examples, a neutralizing antibody titer is a titer that is (e.g., that has been established to be) sufficient to reduce viral infection of B cells relative to that observed for an appropriate control (e.g., an unvaccinated control subject, or a subject vaccinated with a live attenuated viral vaccine, an inactivated viral vaccine, or a protein subunit viral vaccine, or a combination thereof). In some such examples, such reduction is of at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more.

In some examples, a neutralizing antibody titer is a titer that is (e.g., that has been established to be) sufficient to reduce the rate of asymptomatic viral infection relative to that observed for an appropriate control (e.g., an unvaccinated control subject, or a subject vaccinated with a live attenuated viral vaccine, an inactivated viral vaccine, or a protein subunit viral vaccine, or a combination thereof). In some such examples, such reduction is of at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more. In some examples, such reduction can be characterized by assessment of SARS-

CoV-2 N protein serology. Significant protection against asymptomatic infection was also confirmed by real life observations (see also: Dagan N. et al., N Engl J Med. 2021, doi: 10.1056/NEJMoa2101765. Epub ahead of print. PMID: 33626250)

In some examples, a neutralizing antibody titer is a titer that is (e.g., that has been established to be) sufficient to reduce or block fusion of virus with epithelial cells and/or B cells of a vaccinated subject relative to that observed for an appropriate control (e.g., an unvaccinated control subject, or a subject vaccinated with a live attenuated viral vaccine, an inactivated viral vaccine, or a protein subunit viral vaccine, or a combination thereof). In some such examples, such reduction is of at least 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more.

In some examples, induction of a neutralizing antibody titer may be characterized by an elevation in the number of B cells, which in some examples may include plasma cells, class-switched IgG1- and IgG2-positive B cells, and/or germinal center B cells. In some examples, a provided immunogenic composition has been established to achieve such an elevation in the number of B cells in an appropriate system (e.g., in a human infected with SARS-CoV-2 and/or a population thereof, and/or in a model system therefor). For example, in some examples, such an elevation in the number of B cells may have been demonstrated in one or more of a population of humans, a non-human primate model (e.g., rhesus macaques), and/or a mouse model. In some examples, such an elevation in the number of B cells may have been demonstrated in draining lymph nodes and/or spleen of a mouse model after (e.g., at least 7 days, at least 8 days, at least 9 days, at least 10 days, at least 11 days, at least 12 days, at least 13 days, at least 14 days, after) immunization of such a mouse model with a provided immunogenic composition.

In some examples, induction of a neutralizing antibody titer may be characterized by a reduction in the number of circulating B cells in blood. In some examples, a provided immunogenic composition has been established to achieve such a reduction in the number of

circulating B cells in blood of an appropriate system (e.g., in a human infected with SARS-CoV-2 and/or a population thereof, and/or in a model system therefor). For example, in some examples, such a reduction in the number of circulating B cells in blood may have been demonstrated in one or more of a population of humans, a non-human primate model (e.g., rhesus macaques), and/or a mouse model. In some examples, such a reduction in the number of circulating B cells in blood may have been demonstrated in a mouse model after (e.g., at least 4 days, at least 5 days, at least 6 days, at least 7 days, at least 8 days, at least 9 days, at least 10 days, after) immunization of such a mouse model with a provided immunogenic composition. Without wishing to be bound by theory, a reduction in circulating B cells in blood may be due to B cell homing to lymphoid compartments.

In some examples, an immune response induced by a provided immunogenic composition may comprise an elevation in the number of T cells. In some examples, such an elevation in the number of T cells may include an elevation in the number of T follicular helper (T_{FH}) cells, which in some examples may comprise one or more subsets with ICOS upregulation. One of skilled in the art wil understand that proliferation of T_{FH} in germinal centres is integral for generation of an adaptive B-cell response, and also that in humans, T_{FH} occurring in the circulation after vaccination is typically correlated with a high frequency of antigen-specific antibodies. In some examples, a provided immunogenic composition has been established to achieve such an elevation in the number of T cells (e.g., T_{FH} cells) in an appropriate system (e.g., in a human infected with SARS-CoV-2 and/or a population thereof, and/or in a model system therefor). For example, in some examples, such an elevation in the number of T cells (e.g., T_{FH} cells) may have been demonstrated in one or more of a population of humans, a non-human primate model (e.g., rhesus macaques), and/or a mouse model. In some examples, such an elevation in the number of T cells (e.g., e.g., T_{FH} cells) may have been demonstrated in draining lymph nodes, spleen, and/or blood of a mouse model after (e.g., at least 4 days, at least 5 days, at least 6 days, at least 7 days, at least 8 days,

at least 9 days, at least 10 days, at least 11 days, at least 12 days, at least 13 days, at least 14 days, after) immunization of such a mouse model with a provided immunogenic composition. In some examples, a protective response against SARS-CoV-2 induced by a provided immunogenic composition has been established in an appropriate model system for SARS-CoV-2. For example, in some examples, such a protective response may have been demonstrated in an animal model, e.g., a non-human primate model (e.g., rhesus macaques) and/or a mouse model. In some examples, a non-human primate (e.g., rhesus macaque) or a polulation thereof that has/have received at least one immunization with a provided immunogenic composition is/are challenged with SARS-CoV-2, e.g., through intranasal and/or intratracheal route. In some examples, such a challenge may be performed several weeks (e.g., 5-10 weeks) after at least one immunization (including, e.g., at least two immunizations) with a provided immunogenic composition. In some examples, such a challenge may be performed when a detectable level of a SARS-CoV-2 neutralizing titer (e.g., antibody response to SARS-CoV-2 spike protein and/or a fragment thereof, including, e.g., but not limited to a stabilized prefusion spike trimer, S-2P, and/or antibody response to receptor-binding portion of SARS-CoV-2) is achieved in non-human primate(s) (e.g., rhesus macaque(s)) that has received at least one immunization (including, e.g., at least two immunizations) with a provided immunogenic composition. In some examples, a protective response is characterized by absence of or reduction in detectable viral RNA in bronchoalveolar lavage (BAL) and/or nasal swabs of challenged non-human primate(s) (e.g., rhesus macaque(s)). In some examples, immunogenic compositions described herein may have been characterized in that a larger percent of challenged animals, for example, non-human primates in a population (e.g., rhesus macaques), that have received at least one immunization (including, e.g., at least two immunizations) with a provided immunogenic composition display absence of detectable RNA in their BAL and/or nasal swab, as compared to a population of non-immunized animals, for example, non-human primates (e.g., rhesus

macaques). In some examples, immunogenic compositions described herein may have been characterized in that challenged animals, for example, non-human in a population (e.g., rhesus macaques), that have received at least one immunization (including, e.g., at least two immunizations) with a provided immunogenic composition may show clearance of viral RNA in nasal swab no later than 10 days, including, e.g., no later than 8 days, no later than 6 days, no later than 4 days, etc., as compared to a population of non-immunized animals, for example, non-human primates (e.g., rhesus macaques).

In some examples, immunogenic compositions described herein when administered tosubjects in need thereof do not substantially increase the risk of vaccine-associated enhanced respiratory disease. In some examples, such vaccine-associated enhanced respiratory disease may be associated with antibody-dependent enhancement of replication and/or with vaccine antigens that induced antibodies with poor neutralizing activity and Th2-biased responses. In some examples, immunogenic compositions described herein when administered to subjects in need thereof do not substantially increase the risk of antibody-dependent enhancement of replication.

In some examples, a single dose of an mRNA composition (e.g., formulated in lipid nanoparticles) can induce a therapeutic antibody response in less than 10 days of vaccination. In some examples, such a therapeutic antibody response may be characterized in that when such an mRNA vaccine can induce production of about 10-100 ug/mL IgG measured at 10 days after vaccination at a dose of 0.1 to 10 ug or 0.2- 5 ug in an animal model. In some examples, such a therapeutic antibody response may be characterized in that such an mRNA vaccine induces about 100-1000 ug/mL IgG measured at 20 days of vaccination at a dose of 0.1 to 10 ug or 0.2- 5 ug in an animal model. In some examples, a single dose may induce a pseudovirus-neutralization titer, as measured in an animal model, of 10-200 pVN50 titer 15 days after vaccination. In some examples, a single dose may induce a pseudovirus-

neutralization titer, as measured in an animal model, of 50-500 pVN50 titer 15 days after vaccination.

In some examples, a single dose of an mRNA composition can expand antigen-specific CD8 and/or CD4 T cell response by at least at 50% or more (including, e.g., at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or more), as compared to that observed in absence of such an mRNA construct encoding a SARS-COV2 immunogenic protein or fragment thereof (e.g., spike protein and/or receptor binding domain). In some examples, a single dose of an mRNA composition can expand antigen-specific CD8 and/or CD4 T cell response by at least at 1.5-fold or more (including, e.g., at least 2-fold, at least 3-fold, at least 5-fold, at least 10-fold, at least 50-fold, at least 100-fold, at least 500-fold, at least 1000-fold, or more), as compared to that observed in absence of such an mRNA construct encoding a SARS-COV2 immunogenic protein or fragment thereof (e.g., spike protein and/or receptor binding domain).

In some examples, a regimen (e.g., a single dose of an mRNA composition) can expand T cells that exhibit a Th1 phenotype (e.g., as characterized by expression of IFN-gamma, IL-2, IL-4, and/or IL-5) by at least at 50% or more (including, e.g., at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or more), as compared to that observed in absence of such an mRNA construct encoding a SARS-COV2 immunogenic protein or fragment thereof (e.g., spike protein and/or receptor binding domain). In some examples, a regimen (e.g., a single dose of an mRNA composition) can expand T cells that exhibit a Th1 phenotype (e.g., as characterized by expression of IFN-gamma, IL-2, IL-4, and/or IL-5), for example by at least at 1.5-fold or more (including, e.g., at least 2-fold, at least 3-fold, at least 5-fold, at least 10-fold, at least 50-fold, at least 100-fold, at least 500-fold, at least 1000-fold, or more), as compared to that observed in absence of such an mRNA construct encoding a SARS-COV2 immunogenic protein or fragment thereof (e.g., spike protein and/or receptor binding domain). In some examples, a T-cell phenotype may be or comprise a Th1-dominant cytokine profile (e.g.,

as characterized by INF-gamma positive and/or IL-2 positive), and/or no by or biologically insignificant IL-4 secretion.

In some examples, a regimen as described herein (e.g., one or more doses of an mRNA composition) induces and/or achieves production of RBD-specific CD4+ T cells. Among other things, the present disclosure documents that mRNA compositions encoding an RBD-containing portion of a SARS-CoV-2 spike protein (e.g., and not encoding a full-length SARS-CoV-2 spike protein) may be particularly useful and/or effective in such induction and/or production of RBD-specific CD4+ T cells. In some examples, RBD-specific CD4+ T-cells induced by an mRNA composition described herein (e.g., by an mRNA composition that encodings an RBD-containing-portion of a SARS-CoV-2 spike protein and, in some examples not encoding a full-length SARS-CoV-2 spike protein) demonstrate a Th1-dominant cytokine profile (e.g., as characterized by INF-gamma positive and/or IL-2 positive), and/or by no or biologically insignificant IL-4 secretion.

In some examples, characterization of CD4+ and/or CD8+ T cell responses (e.g., described herein) in subjects receiving mRNA compositions (e.g., as described herein) may be performed using ex vivo assays using PBMCs collected from the subjects, e.g., assays as described in the Examples.

In some examples, immunogenicity of mRNA compositions described herein may be assessed by one of or more of the following serological immunongenicity assays: detection of IgG, IgM, and/or IgA to SARS-CoV-2 S protein present in blood samples of a subject receiving a provided mRNA composition, and/or neutralization assays using SARS-CoV-2 pseudovirus and/or a wild-type SARS-CoV-2 virus.

In some examples, an mRNA composition (e.g., as described herein) provide a relatively low adverse effect (e.g., Grade 1-Grade 2 pain, redness and/or swelling) within 7 days after vaccinations at a dose of 10 ug – 100 ug or 1 ug-50 ug. In some examples, mRNA compositions (e.g., as described herein) provide a relatively low observation of systemic

events (e.g., Grade 1-Grade 2 fever, fatigue, headache, chills, vomiting, diarrhea, muscle pain, joint pain, medication, and combinations thereof) within 7 days after vaccinations at a dose of 10 ug – 100 ug.

In some examples, mRNA compositions are characterized in that when administered to subjects at 10-100 ug dose or 1 ug-50 ug, IgG directed to a SARS-CoV2 immunogenic protein or fragment thereof (e.g., spike protein and/or receptor binding domain) may be produced at a level of 100-100,000 U/mL or 500-50,000 U/mL 21 days after vaccination.

In some examples, an mRNA encodes a natively-folded trimeric receptor binding protein of SARS-CoV-2. In some examples, an mRNA encodes a variant of such receptor binding protein such that the encoded variant binds to ACE2 at a Kd of 10 pM or lower, including, e.g., at a Kd of 9 pM, 8 pM, 7 pM, 6 pM, 5 pM, 4 pM, or lower. In some examples, an mRNA encodes a variant of such receptor binding protein such that the encoded variant binds to ACE2 at a Kd of 5 pM. In some examples, an mRNA encodes a trimeric receptor binding portion of SARS-CoV-2 that comprises an ACE2 receptor binding site. In some examples, an mRNA comprises a coding sequence for a receptor-binding portion of SARS-CoV-2 and a trimerization domain (e.g., a natural trimerization domain (foldon) of T4 fibritin) such that the coding sequence directs expression of a trimeric protein that has an ACE2 receptor binding site and binds ACE2. In some examples, an mRNA encodes a trimeric receptor binding portion of SARS-CoV-2 or a variant thereof such that its Kd is smaller than that for a monomeric receptor-binding domain (RBD) of SARS-CoV-2. For example, in some examples, an mRNA encodes a trimeric receptor binding portion of SARS-CoV-2 or a variant thereof such that its Kd is at least 10-fold (including, e.g., at least 50-fold, at least 100-fold, at least 500-fold, at least 1000-fold, etc.) smaller than that for a RBD of SARS-CoV-2.

In some examples, a trimer receptor binding portion of SARS-CoV-2 encoded by an mRNA (e.g., as described herein) may be determined to have a size of about 3-4 angstroms when it is complexed with ACE2 and B^0AT1 neutral amino acid acid transporter in a closed

conformation, as characterized by electron cryomicroscopy (cryoEM). In some examples, geometric mean SARS-CoV-2 neutralizing titer that characterizes and/or is achieved by an mRNA composition or method as described herein can reach at least 1.5-fold, including, at least 2-fold, at least 2.5-fold, at least 3-fold, or higher, that of a COVID-19 convalescent human panel (e.g., a panel of sera from COVID-19 convalescing humans obtained 20-40 days after the onset of symptoms and at least 14 days after the start of asymptomatic convalescence.

In some examples, mRNA compositions as provided herein may be characterized in that subjects who have been treated with such compositions (e.g., with at least one dose, at least two doses, etc) may show reduced and/or more transient presence of viral RNA in relevant site(s) (e.g., nose and/or lungs, etc, and/or any other tissue susceptible to infection) as compared with an appropriate control (e.g., an established expected level for a comparable subject or population not having been so treated and having been exposed to virus under reasonably comparable exposure conditions)

In some examples, the RBD antigen expressed by an mRNA construct (e.g., as described herein) can be modified by addition of a T4-fibritin-derived "foldon" trimerization domain, for example, to increase its immunogenicity.

In some examples, mRNA compositions and/or methods described herein are characterized in that certain local reactions (e.g., pain, redness, and/or swelling, etc.) and/or systemic events (e.g., fever, fatigue, headache, etc.) may appear and/or peak at Day 2 after vaccination. In some examples, mRNA compositions described herein are characterized in that certain local reactions (e.g., pain, redness, and/or swelling, etc.) and/or systemic events (e.g., fever, fatigue, headache, etc.) may resolve by Day 7 after vaccination.

In some examples, mRNA compositions and/or methods described herein are characterized in that no Grade 1 or greater change in routine clinical laboratory values or laboratory abnormalities are observed in subjects receiving mRNA compositions (e.g., as

described herein). Examples of such clinical laboratory assays may include lymphocyte count, hematological changes, etc.

In some examples, mRNA compositions and/or methods described herein are characterized in that by 21 days after a first dose (e.g., 10-100 ug inclusive or 1 ug-50 ug inclusive), geometric mean concentrations (GMCs) of IgG directed to a SARS-CoV-2 S polypeptide or an immunogenic fragment thereof (e.g., RBD) may reach 200-3000 units/mL or 500-3000 units/mL or 500-2000 units/mL, compared to 602 units/mL for a panel of COVID-19 convalescent human sera. In some examples, mRNA compositions described herein are characterized in that by 7 days after a second dose (e.g., 10-30 ug inclusive; or 1 ug-50 ug inclusive), geometric mean concentrations (GMCs) of IgG directed to a SARS-CoV-2 spike polypeptide or an immunogenic fragment thereof (e.g., RBD) may increase by at least 8-fold or higher, including, e.g., at least 9-fold, at least 10-fold, at least 15-fold, at least 20-fold, at least 25-fold, at least 30-fold, at least 35-fold, at least 40-fold, or higher. In some examples, mRNA compositions described herein are characterized in that by 7 days after a second dose (e.g., 10-30 ug inclusive; or 1 ug-50 ug inclusive), geometric mean concentrations (GMCs) of IgG directed to a SARS-CoV-2 S polypeptide or an immunogenic fragment thereof (e.g., RBD) may increase to 1500 units/mL to 40,000 units/mL or 4000 units/mL to 40,000 units/mL. In some examples, antibody concentrations described herein can persist to at least 20 days or longer, including, e.g., at least 25 days, at least 30 days, at least 35 days, at least 40 days, at least 45 days, at least 50 days, after a first dose, or at least 10 days or longer, including, e.g., at least 15 days, at least 20 days, at least 25 days, or longer, after a second dose. In some examples, antibody concentrations can persist to 35 days after a first dose, or at least 14 days after a second dose.

In some examples, mRNA compositions described herein are characterized in that when measured at 7 days after a second dose (e.g., 1-50 ug inclusive), GMC of IgG directed to a SARS-CoV-2 S polypeptide or an immunogenic fragment thereof (e.g., RBD) is at least 30%

higher (including, e.g., at least 40% higher, at least 50% higher, at least 60%, higher, at least 70% higher, at least 80% higher, at least 90% higher, at least 95 % higher, as compared to antibody concentrations observed in a panel of COVID-19 convalescent human serum. In many examples, geometric mean concentration (GMC) of IgG described herein is GMCs of RBD-binding IgG.

In some examples, mRNA compositions described herein are characterized in that when measured at 7 days after a second dose (e.g., 10-50 ug inclusive), GMC of IgG directed to a SARS-CoV-2 S polypeptide or an immunogenic fragment thereof (e.g., RBD) is at least 1.1-fold higher (including, e.g., at least 1.5-fold, at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold higher, at least 7-fold higher, at least 8-fold higher, at least 9-fold higher, at least 10-fold higher, at least 15-fold higher, at least 20-fold higher, at least 25-fold higher, at least 30-fold higher), as compared to antibody concentrations observed in a panel of COVID-19 convalescent human serum, In many examples, geometric mean concentration (GMC) of IgG described herein is GMCs of RBD-binding IgG.

In some examples, mRNA compositions described herein are characterized in that when measured at 21 days after a second dose, GMC of IgG directed to a SARS-CoV-2 S polypeptide or an immunogenic fragment thereof (e.g., RBD) is at least 5-fold higher (including, e.g., at least 6-fold higher, at least 7-fold higher, at least 8-fold higher, at least 9-fold higher, at least 10-fold higher, at least 15-fold higher, at least 20-fold higher, at least 25-fold higher, at least 30-fold higher), as compared to antibody concentrations observed in a panel of COVID-19 convalescent human serum, In many examples, geometric mean concentration (GMC) of IgG described herein is GMCs of RBD-binding IgG.

In some examples, mRNA compositions and/or methods described herein are characterized in that an increase (e.g., at least 30%, at least 40%, at least 50%, or more) in SARS-CoV-2 neutralizing geometric mean titers (GMTs) is observed 21 days after a first dose. In some examples, mRNA compositions described herein are characterized in that a

substantially greater serum neutralizing GMTs are achieved 7 days after subjects receive a second dose (e.g., 10 μg-30 μg inclusive), reaching 150-300, compared to 94 for a COVID-19 convalescent serum panel.

In some examples, mRNA compositions and/or methods described herein are characterized in that 7 days after administration of the second dose, the protective efficacy is at least 60%, e.g., at least 70%, at least 80%, at least 90, or at least 95%. In one example, mRNA compositions and/or methods described herein are characterized in that 7 days after administration of the second dose, the protective efficacy is at least 70%. In one example, mRNA compositions and/or methods described herein are characterized in that 7 days after administration of the second dose, the protective efficacy is at least 80%. In one example, mRNA compositions and/or methods described herein are characterized in that 7 days after administration of the second dose, the protective efficacy is at least 90%. In one example, mRNA compositions and/or methods described herein are characterized in that 7 days after administration of the second dose, the protective efficacy is at least 95%.

In some examples, an RNA composition provided herein is characterized in that it induces an immune response against SARS-CoV-2 after at least 7 days after a dose (e.g., after a second dose). In some examples, an RNA composition provided herein is characterized in that it induces an immune response against SARS-CoV-2 in less than 14 days after a dose (e.g., after a second dose). In some examples, an RNA composition provided herein is characterized in that it induces an immune response against SARS-CoV-2 after at least 7 days after a vaccination regimen. In some examples, a vaccination regimen comprises a first dose and a second dose. In some examples, a first dose and a second dose are administered by at least 21 days apart. In some such examples, an immune response against SARS-CoV-2 is induced at least after 28 days after a first dose.

In some examples, mRNA compositions and/or methods described herein are characterized in that geometric mean concentration (GMCs) of antibodies directed to a SARS-

CoV-2 spike polypeptide or an immunogenic fragment thereof (e.g., RBD), as measured in serum from subjects receiving mRNA compositions of the present disclosure (e.g., at a dose of 10-30 ug inclusive), is substantially higher than in a convalescent serum panel (e.g., as described herein). In some examples where a subject may receive a second dose (e.g., 21 days after 1 first dose), geometric mean concentration (GMCs) of antibodies directed to a SARS-CoV-2 spike polypeptide or an immunogenic fragment thereof (e.g., RBD), as measured in serum from the subject, may be 8.0-fold to 50-fold higher than a convalescent serum panel GMC. In some examples where a subject may receive a second dose (e.g., 21 days after 1 first dose), geometric mean concentration (GMCs) of antibodies directed to a SARS-CoV-2 spike polypeptide or an immunogenic fragment thereof (e.g., RBD), as measured in serum from the subject, may be at least 8.0-fold or higher, including, e.g., at least 10-fold, at least 20-fold, at least 30-fold, at least 40-fold, at least 50-fold, at least 60-fold or higher, as compared to a convalescent serum panel GMC.

In some examples, mRNA compositions and/or methods described herein are characterized in that the SARS-CoV-2 neutralizing geometric mean titer, as measured at 28 days after a first dose or 7 days after a second dose, may be at least 1.5-fold or higher (including, e.g., at least 2-fold, at least 2.5-fold, at least 3-fold, at least 3.5-fold or higher), as compared to a neutralizing GMT of a convalescent serum panel.

In some examples, a regimen administered to a subject may be or comprise a single dose. In some examples, a regimen administered to a subject may comprise a plurality of doses (e.g., at least two doses, at least three doses, or more). In some examples, a regimen administered to a subject may comprise a first dose and a second dose, which are given at least 2 weeks apart, at least 3 weeks apart, at least 4 weeks apart, or more. In some examples, such doses may be at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least 12 months, or more apart. In

some examples, doses may be administered days apart, such as 1, 2, 3, 4, 5, 6, 7, 8, 9 ,10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60 or more days apart. In some examples, doses may be administered about 1 to about 3 weeks apart, or about 1 to about 4 weeks apart, or about 1 to about 5 weeks apart, or about 1 to about 6 weeks apart, or about 1 to more than 6 weeks apart. In some examples, doses may be separated by a period of about 7 to about 60 days, such as for example about 14 to about 48 days, etc. In some examples, a minimum number of days between doses may be about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 or more. In some examples, a maximum number of days between doses may be about 60, 59, 58, 57, 56, 55, 54, 53, 52, 51, 50, 49, 48, 47, 46, 45, 44, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, 30, 29, 28, 27, 26, 25, 24, 23, 22, 21, or fewer. In some examples, doses may be about 21 to about 28 days apart. In some examples, doses may be about 19 to about 42 days apart. In some examples, doses may be about 7 to about 28 days apart. In some examples, doses may be about 14 to about 24 days. In some examples, doses may be about 21 to about 42 days.

In some examples, particularly for compositions established to achieve elevated antibody and/or T-cell titres for a period of time longer than about 3 weeks – e.g., in some examples, a provided composition is established to achieve elevated antibody and/or T-cell titres (e.g., specific for a relevant portion of a SARS-CoV-2 spike protein) for a period of time longer than about 3 weeks; in some such examples, a dosing regimen may involve only a single dose, or may involve two or more doses, which may, in some examples, be separated from one another by a period of time that is longer than about 21 days or three weeks. For example, in some such examples, such period of time may be about 4 weeks, 5 weeks, 6 weeks 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks, 12 weeks, 13 weeks, 14 weeks, 15 wees, 16 weeks, 17 weeks, 18 weeks, 19 weeks, 20 weeks or more, or about 1

month, 2 months, 3 months, 4 months, 5 months, 6 months, 7 months, 8 months, 9 months, 10, months, 11 months, 12 months or more, or in some examples about a year or more.

In some examples, a first dose and a second dose (and/or other subsequent dose) may be administered by intramuscular injection. In some examples, a first dose and a second dose may be administered in the deltoid muscle. In some examples, a first dose and a second dose may be administered in the same arm. In some examples, an mRNA composition described herein is administered (e.g., by intramuscular injection) as a series of two doses (e.g., 0.3 mL each) 21 days part. In some examples, each dose is about 30 ug. In some examples, each dose may be higher than 30 ug, e.g., about 40 ug, about 50 ug, about 60 ug. In some examples, each dose may be lower than 30 ug, e.g., about 20 ug, about 10 ug, about 5 ug, etc. In some examples, each dose is about 3 ug or lower, e.g., about 1 ug. In some such examples, an mRNA composition described herein is administered to subjects of age 16 or older (including, e.g., 16-85 years). In some such examples, an mRNA composition described herein is administered to subjects of age 18-55. In some such examples, an mRNA composition escribed herein is administered to subjects of age 56-85. In some examples, an mRNA composition described herein is administered (e.g., by intramuscular injection) as a single dose.

In some examples, mRNA compositions and/or methods described herein are characterized in that RBD-specific IgG (e.g., polyclonal response) induced by such mRNA compositions and/or methods exhibit a higher binding affinity to RBD, as compared to a reference human monoclonal antibody with SARS-CoV-2 RBD-binding affinity (e.g., CR3022 as described in J. ter Meulen et al., PLOS Med. 3, e237 (2006).)

In some examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity across a panel (e.g., at least 10, at least 15, or more) of SARs-CoV-2 spike variants. In some examples, such SARs-CoV-2 spike variants include mutations in RBD (e.g., but not limited to Q321L,

V341I, A348T, N354D, S359N, V367F, K378R, R408I, Q409E, A435S, N439K, K458R, I472V, G476S, S477N, V483A, Y508H, H519P, etc., as compared to SEQ ID NO: 1), and/or mutations in spike protein (e.g., but not limited to D614G, etc., as compared to SEQ ID NO: 1). Those skilled in the art are aware of various spike variants, and/or resources that document them (e.g., the Table of mutating sites in Spike maintained by the COVID-19 Viral Genome Analysis Pipeline and found at https://cov.lanl.gov/components/sequence/COV/int_sites_tbls.comp) (last accessed 24 Aug 2020), and, reading the present specification, will appreciate that mRNA compositions and/or methods described herein can be characterized for there ability to induce sera in vaccinated subject that display neutralizing activity with respect to any or all of such variants and/or combinations thereof.

In particular examples, mRNA compositions encoding RBD of a SARS-CoV-2 spike protein are characterized in that sera of vaccinated subjects display neutralizing activity across a panel (e.g., at least 10, at least 15, or more) of SARs-CoV-2 spike variants including RBD variants (e.g., but not limited to Q321L, V341I, A348T, N354D, S359N, V367F, K378R, R408I, Q409E, A435S, N439K, K458R, I472V, G476S, S477N, V483A, Y508H, H519P, etc., as compared to SEQ ID NO: 1) and spike protein variants (e.g., but not limited to D614G, as compared to SEQ ID NO: 1).

In particular examples, mRNA compositions encoding a SARS-CoV-2 spike protein variant that includes two consecutive proline substitutions at amino acid positions 986 and 987, at the top of the central helix in the S2 subunit, are characterized in that sera of vaccinated subjects display neutralizing activity across a panel (e.g., at least 10, at least 15, or more) of SARs-CoV-2 spike variants including RBD variants (e.g., but not limited to Q321L, V341I, A348T, N354D, S359N, V367F, K378R, R408I, Q409E, A435S, N439K, K458R, I472V, G476S, S477N, V483A, Y508H, H519P, etc., as compared to SEQ ID NO: 1) and spike protein variants (e.g., but not limited to D614G, as compared to SEQ ID NO: 1). For example, in some examples, the mRNA composition encoding SEQ ID NO: 7 (S P2) elicits an immune response against any one of a SARs-CoV-2 spike variant including RBD variants (e.g., but not limited to Q321L, V341I,

A348T, N354D, S359N, V367F, K378R, R408I, Q409E, A435S, N439K, K458R, I472V, G476S, S477N, V483A, Y508H, H519P, etc., as compared to SEQ ID NO: 1) and spike protein variants (e.g., but not limited to D614G, as compared to SEQ ID NO: 1).

In some examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against one or more SARs-CoV-2 spike variants including a mutation at position 501 in spike protein as compared to SEQ ID NO: 1. In some examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against one or more SARs-CoV-2 spike variants including a N501Y mutation in spike protein as compared to SEQ ID NO: 1.

Said one or more SARs-CoV-2 spike variants including a mutation at position 501 in spike protein as compared to SEQ ID NO: 1 or said one or more SARs-CoV-2 spike variants including a N501Y mutation in spike protein as compared to SEQ ID NO: 1 may include one or more further mutations as compared to SEQ ID NO: 1 (e.g., but not limited to H69/V70 deletion, Y144 deletion, A570D, D614G, P681H, T716I, S982A, D1118H, D80A, D215G, E484K, A701V, L18F, R246I, K417N, L242/A243/L244 deletion etc., as compared to SEQ ID NO: 1).

In particular examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant "Variant of Concern 202012/01" (VOC-202012/01; also known as lineage B.1.1.7). The variant had previously been named the first Variant Under Investigation in December 2020 (VUI – 202012/01) by Public Health England, but was reclassified to a Variant of Concern (VOC-202012/01). VOC-202012/01 is a variant of SARS-CoV-2 which was first detected in October 2020 during the COVID-19 pandemic in the United Kingdom from a sample taken the previous month, and it quickly began to spread by mid-December. It is correlated with a significant increase in the rate of COVID-19 infection in United Kingdom; this increase is thought to be at least partly because of change N501Y inside the spike

glycoprotein's receptor-binding domain, which is needed for binding to ACE2 in human cells. The VOC-202012/01 variant is defined by 23 mutations: 13 non-synonymous mutations, 4 deletions, and 6 synonymous mutations (i.e., there are 17 mutations that change proteins and six that do not). The spike protein changes in VOC 202012/01 include deletion 69-70, deletion 144, N501Y, A570D, D614G, P681H, T716I, S982A, and D1118H. One of the most important changes in VOC-202012/01 seems to be N501Y, a change from asparagine (N) to tyrosine (Y) at amino-acid site 501. This mutation alone or in combination with the deletion at positions 69/70 in the N terminal domain (NTD) may enhance the transmissibility of the virus.

In particular examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including the following mutations: deletion 69-70, deletion 144, N501Y, A570D, D614G, P681H, T716I, S982A, and D1118H as compared to SEQ ID NO: 1.

In particular examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant "501.V2". This variant was first observed in samples from October 2020, and since then more than 300 cases with the 501.V2 variant have been confirmed by whole genome sequencing (WGS) in South Africa, where in December 2020 it was the dominant form of the virus. Preliminary results indicate that this variant may have an increased transmissibility. The 501.V2 variant is defined by multiple spike protein changes including: D80A, D215G, E484K, N501Y and A701V, and more recently collected viruses have additional changes: L18F, R246I, K417N, and deletion 242-244.

In particular examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including the following mutations: D80A, D215G, E484K, N501Y and A701V as compared to SEQ ID NO: 1, and optionally: L18F, R246I, K417N, and deletion 242-244 as

compared to SEQ ID NO: 1. Said SARs-CoV-2 spike variant may also include a D614G mutation as compared to SEQ ID NO: 1.

In some examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against one or more SARs-CoV-2 spike variants including a H69/V70 deletion in spike protein as compared to SEQ ID NO: 1.

In some examples, one or more SARs-CoV-2 spike variants including a H69/V70 deletion in spike protein as compared to SEQ ID NO: 1 may include one or more further mutations as compared to SEQ ID NO: 1 (e.g., but not limited to Y144 deletion, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H, D80A, D215G, E484K, A701V, L18F, R246I, K417N, L242/A243/L244 deletion, Y453F, I692V, S1147L, M1229I etc., as compared to SEQ ID NO: 1),

In particular examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant "Variant of Concern 202012/01" (VOC-202012/01; also known as lineage B.1.1.7).

In particular examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including the following mutations: deletion 69-70, deletion 144, N501Y, A570D, D614G, P681H, T716I, S982A, and D1118H as compared to SEQ ID NO: 1.

In particular examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant "Cluster 5", also referred to as ΔFVI-spike by the Danish State Serum Institute (SSI). It was discovered in North Jutland, Denmark, and is believed to have been spread from minks to humans via mink farms. In cluster 5, several different mutations in the spike protein of the virus have been confirmed. The specific mutations include 69–70deltaHV (a deletion of

the histidine and valine residues at the 69th and 70th position in the protein), Y453F (a change from tyrosine to phenylalanine at position 453), I692V (isoleucine to valine at position 692), M1229I (methionine to isoleucine at position 1229), and optionally S1147L (serine to leucine at position 1147).

In particular examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including the following mutations: deletion 69-70, Y453F, I692V, M1229I, and optionally S1147L, as compared to SEQ ID NO: 1.

In some examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against one or more SARs-CoV-2 spike variants including a mutation at position 614 in spike protein as compared to SEQ ID NO: 1. In some examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against one or more SARs-CoV-2 spike variants including a D614G mutation in spike protein as compared to SEQ ID NO: 1.

In some examples, one or more SARs-CoV-2 spike variants including a mutation at position 614 in spike protein as compared to SEQ ID NO: 1 or said one or more SARs-CoV-2 spike variants including a D614G mutation in spike protein as compared to SEQ ID NO: 1 may include one or more further mutations as compared to SEQ ID NO: 1 (e.g., but not limited to H69/V70 deletion, Y144 deletion, N501Y, A570D, P681H, T716I, S982A, D1118H, D80A, D215G, E484K, A701V, L18F, R246I, K417N, L242/A243/L244 deletion, Y453F, I692V, S1147L, M1229I etc., as compared to SEQ ID NO: 1).

In particular examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-

2 spike variant "Variant of Concern 202012/01" (VOC-202012/01; also known as lineage B.1.1.7).

In particular examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including the following mutations: deletion 69-70, deletion 144, N501Y, A570D, D614G, P681H, T716I, S982A, and D1118H as compared to SEQ ID NO: 1.

In particular examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including the following mutations: D80A, D215G, E484K, N501Y, A701V, and D614G as compared to SEQ ID NO: 1, and optionally: L18F, R246I, K417N, and deletion 242-244 as compared to SEQ ID NO: 1.

In some examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against one or more SARs-CoV-2 spike variants including a mutation at positions 501 and 614 in spike protein as compared to SEQ ID NO: 1. In some examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against one or more SARs-CoV-2 spike variants including a N501Y mutation and a D614G mutation in spike protein as compared to SEQ ID NO: 1.

In some examples, one or more SARs-CoV-2 spike variants including a mutation at positions 501 and 614 in spike protein as compared to SEQ ID NO: 1 or said one or more SARs-CoV-2 spike variants including a N501Y mutation and a D614G mutation in spike protein as compared to SEQ ID NO: 1 may include one or more further mutations as compared to SEQ ID NO: 1 (e.g., but not limited to H69/V70 deletion, Y144 deletion, A570D, P681H, T716I, S982A, D1118H, D80A, D215G, E484K, A701V, L18F, R246I, K417N, L242/A243/L244 deletion, Y453F, I692V, S1147L, M1229I etc., as compared to SEQ ID NO: 1).

In particular examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant "Variant of Concern 202012/01" (VOC-202012/01; also known as lineage B.1.1.7).

In particular examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including the following mutations: deletion 69-70, deletion 144, N501Y, A570D, D614G, P681H, T716I, S982A, and D1118H as compared to SEQ ID NO: 1.

In particular examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including the following mutations: D80A, D215G, E484K, N501Y, A701V, and D614G as compared to SEQ ID NO: 1, and optionally: L18F, R246I, K417N, and deletion 242-244 as compared to SEQ ID NO: 1.

In some examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against one or more SARs-CoV-2 spike variants including a mutation at position 484 in spike protein as compared to SEQ ID NO: 1. In some examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against one or more SARs-CoV-2 spike variants including a E484K mutation in spike protein as compared to SEQ ID NO: 1.

In some examples, one or more SARs-CoV-2 spike variants including a mutation at position 484 in spike protein as compared to SEQ ID NO: 1 or said one or more SARs-CoV-2 spike variants including a E484K mutation in spike protein as compared to SEQ ID NO: 1 may include one or more further mutations as compared to SEQ ID NO: 1 (e.g., but not limited to H69/V70 deletion, Y144 deletion, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H, D80A, D215G,

A701V, L18F, R246I, K417N, L242/A243/L244 deletion, Y453F, I692V, S1147L, M1229I, T20N, P26S, D138Y, R190S, K417T, H655Y, T1027I, V1176F etc., as compared to SEQ ID NO: 1).

In particular examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant "501.V2".

In particular examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including the following mutations: D80A, D215G, E484K, N501Y, and A701V, as compared to SEQ ID NO: 1, and optionally: L18F, R246I, K417N, and deletion 242-244 as compared to SEQ ID NO: 1. Said SARs-CoV-2 spike variant may also include a D614G mutation as compared to SEQ ID NO: 1.

Lineage B.1.1.248, known as the Brazil(ian) variant, is one of the variants of SARS-CoV-2 which has been named P.1 lineage and has 17 unique amino acid changes, 10 of which in its spike protein, including N501Y and E484K. B.1.1.248 originated from B.1.1.28. E484K is present in both B.1.1.28 and B.1.1.248. B.1.1.248 has a number of S-protein polymorphisms [L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, H655Y, T1027I, V1176F] and is similar in certain key RBD positions (K417, E484, N501) to variant described from South Africa.

In particular examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant "B.1.1.28".

In particular examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant "B.1.1.248".

In particular examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-

2 spike variant including the following mutations: L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, H655Y, T1027I, and V1176F as compared to SEQ ID NO: 1.

In some examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against one or more SARs-CoV-2 spike variants including a mutation at positions 501 and 484 in spike protein as compared to SEQ ID NO: 1. In some examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against one or more SARs-CoV-2 spike variants including a N501Y mutation and a E484K mutation in spike protein as compared to SEQ ID NO: 1.

In some examples, one or more SARs-CoV-2 spike variants including a mutation at positions 501 and 484 in spike protein as compared to SEQ ID NO: 1 or said one or more SARs-CoV-2 spike variants including a N501Y mutation and a E484K mutation in spike protein as compared to SEQ ID NO: 1 may include one or more further mutations as compared to SEQ ID NO: 1 (e.g., but not limited to H69/V70 deletion, Y144 deletion, A570D, D614G, P681H, T716I, S982A, D1118H, D80A, D215G, A701V, L18F, R246I, K417N, L242/A243/L244 deletion, Y453F, I692V, S1147L, M1229I, T20N, P26S, D138Y, R190S, K417T, H655Y, T1027I, V1176F etc., as compared to SEQ ID NO: 1).

In particular examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant "501.V2".

In particular examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including the following mutations: D80A, D215G, E484K, N501Y and A701V as compared to SEQ ID NO: 1, and optionally: L18F, R246I, K417N, and deletion 242-244 as compared to SEQ ID NO: 1. Said SARs-CoV-2 spike variant may also include a D614G mutation as compared to SEQ ID NO: 1.

In particular examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant "B.1.1.248".

In particular examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including the following mutations: L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, H655Y, T1027I, and V1176F as compared to SEQ ID NO: 1.

In some examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against one or more SARs-CoV-2 spike variants including a mutation at positions 501, 484 and 614 in spike protein as compared to SEQ ID NO: 1. In some examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against one or more SARs-CoV-2 spike variants including a N501Y mutation, a E484K mutation and a D614G mutation in spike protein as compared to SEQ ID NO: 1.

In some examples, one or more SARs-CoV-2 spike variants including a mutation at positions 501, 484 and 614 in spike protein as compared to SEQ ID NO: 1 or said one or more SARs-CoV-2 spike variants including a N501Y mutation, a E484K mutation and a D614G mutation in spike protein as compared to SEQ ID NO: 1 may include one or more further mutations as compared to SEQ ID NO: 1 (e.g., but not limited to H69/V70 deletion, Y144 deletion, A570D, P681H, T716I, S982A, D1118H, D80A, D215G, A701V, L18F, R246I, K417N, L242/A243/L244 deletion, Y453F, I692V, S1147L, M1229I, T20N, P26S, D138Y, R190S, K417T, H655Y, T1027I, V1176F etc., as compared to SEQ ID NO: 1).

In particular examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including the following mutations: D80A, D215G, E484K, N501Y, A701V, and

D614G as compared to SEQ ID NO: 1, and optionally: L18F, R246I, K417N, and deletion 242-244 as compared to SEQ ID NO: 1.

In some examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against one or more SARs-CoV-2 spike variants including a L242/A243/L244 deletion in spike protein as compared to SEQ ID NO: 1.

In some examples, one or more SARs-CoV-2 spike variants including a L242/A243/L244 deletion in spike protein as compared to SEQ ID NO: 1 may include one or more further mutations as compared to SEQ ID NO: 1 (e.g., but not limited to H69/V70 deletion, Y144 deletion, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H, D80A, D215G, E484K, A701V, L18F, R246I, K417N, Y453F, I692V, S1147L, M1229I, T20N, P26S, D138Y, R190S, K417T, H655Y, T1027I, V1176F etc., as compared to SEQ ID NO: 1).

In particular examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant "501.V2".

In particular examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including the following mutations: D80A, D215G, E484K, N501Y, A701V and deletion 242-244 as compared to SEQ ID NO: 1, and optionally: L18F, R246I, and K417N, as compared to SEQ ID NO: 1. Said SARs-CoV-2 spike variant may also include a D614G mutation as compared to SEQ ID NO: 1.

In some examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against one or more SARs-CoV-2 spike variants including a mutation at position 417 in spike protein as compared to SEQ ID NO: 1. In some examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing

activity against one or more SARs-CoV-2 spike variants including a K417N or K417T mutation in spike protein as compared to SEQ ID NO: 1.

In some examples, one or more SARs-CoV-2 spike variants including a mutation at position 417 in spike protein as compared to SEQ ID NO: 1 or said one or more SARs-CoV-2 spike variants including a K417N or K417T mutation in spike protein as compared to SEQ ID NO: 1 may include one or more further mutations as compared to SEQ ID NO: 1 (e.g., but not limited to H69/V70 deletion, Y144 deletion, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H, D80A, D215G, E484K, A701V, L18F, R246I, L242/A243/L244 deletion, Y453F, I692V, S1147L, M1229I, T20N, P26S, D138Y, R190S, H655Y, T1027I, V1176F etc., as compared to SEQ ID NO: 1).

In particular examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant "501.V2".

In particular examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including the following mutations: D80A, D215G, E484K, N501Y, A701V and K417N,, as compared to SEQ ID NO: 1, and optionally: L18F, R246I, and deletion 242-244 as compared to SEQ ID NO: 1. Said SARs-CoV-2 spike variant may also include a D614G mutation as compared to SEQ ID NO: 1.

In particular examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant "B.1.1.248".

In particular examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including the following mutations: L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, H655Y, T1027I, and V1176F as compared to SEQ ID NO: 1.

In some examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against one or more SARs-CoV-2 spike variants including a mutation at positions 417 and 484 and/or 501 in spike protein as compared to SEQ ID NO: 1. In some examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against one or more SARs-CoV-2 spike variants including a K417N or K417T mutation and a E484K and/or N501Y mutation in spike protein as compared to SEQ ID NO: 1.

In some examples, one or more SARs-CoV-2 spike variants including a mutation at positions 417 and 484 and/or 501 in spike protein as compared to SEQ ID NO: 1 or said one or more SARs-CoV-2 spike variants including a K417N or K417T mutation and a E484K and/or N501Y mutation in spike protein as compared to SEQ ID NO: 1 may include one or more further mutations as compared to SEQ ID NO: 1 (e.g., but not limited to H69/V70 deletion, Y144 deletion, A570D, D614G, P681H, T716I, S982A, D1118H, D80A, D215G, A701V, L18F, R246I, L242/A243/L244 deletion, Y453F, I692V, S1147L, M1229I, T20N, P26S, D138Y, R190S, H655Y, T1027I, V1176F etc., as compared to SEQ ID NO: 1).

In particular examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant "501.V2".

In particular examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including the following mutations: D80A, D215G, E484K, N501Y, A701V and K417N, as compared to SEQ ID NO: 1, and optionally: L18F, R246I, and deletion 242-244 as compared to SEQ ID NO: 1. Said SARs-CoV-2 spike variant may also include a D614G mutation as compared to SEQ ID NO: 1.

In particular examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant "B.1.1.248".

In particular examples, mRNA compositions and/or methods described herein are characterized in that sera of vaccinated subjects display neutralizing activity against SARs-CoV-2 spike variant including the following mutations: L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, H655Y, T1027I, and V1176F as compared to SEQ ID NO: 1.

The SARs-CoV-2 spike variants described herein may or may not include a D614G mutation as compared to SEQ ID NO: 1.

In some examples, mRNA compositions and/or methods described herein can provide protection against SARS-CoV-2 and/or decrease severity of SARS-CoV-2 infection in at least 50% of subjects receiving such mRNA compositions and/or methods.

In some examples, populations to be treated with mRNA compositions described herein include subjects of age 18-55. In some examples, populations to be treated with mRNA compositions described herein include subjects of age 56-85. In some examples, populations to be treated with mRNA compositions described herein include older subjects (e.g., over age 60, 65, 70, 75, 80, 85, etc, for example subjects of age 65-85). In some examples, populations to be treated with mRNA compositions described herein include subjects of age 18-85. In some examples, populations to be treated with mRNA compositions described herein include subjects of age 18 or younger. In some examples, populations to be treated with mRNA compositions described herein include subjects of age 12 or younger. In some examples, populations to be treated with mRNA compositions described herein include subjects of age 10 or younger. In some examples, populations to be treated with mRNA compositions described herein may include adolescent populations (e.g., individuals approximately 12 to approximately 17 years of age). In some examples, populations to be treated with mRNA compositions described herein include infants (e.g., less

than 1 year old). In some examples, populations to be treated with mRNA compositions described herein do not include infants (e.g., less than 1 year) whose mothers have received such mRNA compositions described herein during pregnancy. Without wishing to be bound by any particular theory, a rat study as shown in Example 31 has suggested that a SARS-CoV-2 neutralizing antibody response induced in female rats given such mRNA compositions during pregnancy can pass onto fetuses. In some examples, populations to be treated with mRNA compositions described herein include infants (e.g., less than 1 year) whose mothers did not receive such mRNA compositions described herein during pregnancy. In some examples, populations to be treated with mRNA compositions described herein may include pregnant women; in some examples, infants whose mothers were vaccinated during pregnancy (e.g., who received at least one dose, or alternatively only who received both doses), are not vaccinated during the first weeks, months, or even years (e.g., 1, 2, 3, 4, 5, 6, 7, 8 weeks or more, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 moths or more, or 1, 2, 3, 4, 5 years or more) post-birth. Alternatively or additionally, in some examples, infants whose whose mothers were vaccinated during pregnancy (e.g., who received at least one dose, or alternatively only who received both doses), receive reduced vaccination (e.g., lower doses and/or smaller numbers of administrations – e.g., boosters – and/or lower total exposure over a given period of time) after birth, for example during the first weeks, months, or even years (e.g., 1, 2, 3, 4, 5, 6, 7, 8 weeks or more, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 months or more, or 1, 2, 3, 4, 5 years or more) post-birthor may need reduced vaccination (e.g., lower doses and/or smaller numbers of administrations – e.g., boosters – over a given period of time), In some examples, compositions as provided herein are administered to populations that do not include pregnant women.

In some particular examples, compositions as provided herein are administered to pregnant women according to a regimen that includes a first dose administered after about

24 weeks of gestation (e.g., after about 22, 23, 24, 25, 26, 27, 28 or more weeks of gestation); in some examples, compositions as provided herein are administered to pregnant women according to a regimen that includes a first dose administered before about 34 weeks of gestation (e.g., before about 30, 31, 32, 33, 34, 35, 36, 37, 38 weeks of gestation). In some examples, compositions as provided herein are administered to pregnant women according to a regimen that includes a first dose administered after about 24 weeks (e.g., after about 27 weeks of gestation, e.g., between about 24 weeks and 34 weeks, or between about 27 weeks and 34 weeks) of gestation and a second dose administered about 21 days later; in some examples both doses are administered prior to delivery. Without wishing to be bound by any particular theory, it is proposed that such a regimen (e.g., involving administration of a first dose after about 24 weeks, or 27 weeks of gestation and optionally before about 34 weeks of gestation), and optionally a second dose within about 21 days, ideally before delivery, may have certain advantages in terms of safety (e.g., reduced risk of premature delivery or of fetal morbidity or mortality) and/or efficacy (e.g., carryover vaccination imparted to the infant) relative to alternative dosing regimens (e.g., dosing at any time during pregnancy, refraining from dosing during pregnancy, and/or dosing later in pregnancy for example so that only one dose is administered during gestation. In some examples, as noted herein (see also Example 34), infants born of mothers vaccinated during pregnancy, e.g, according to a particular regimen as described herein, may not need further vaccination, or may need reduced vaccination (e.g., lower doses and/or smaller numbers of administrations – e.g., boosters –, and/or lower overall exposure over a given period of time), for a period of time (e.g., as noted herein) after birth.

In some examples, compositions as provided herein are administered to populations in which women are advised against becoming pregnant for a period of time after receipt of the vaccine (e.g., after receipt of a first dose of the vaccine, after receipt of a final dose of the vaccine, etc.); in some such examples, the period of time may be at least 1 week, at least

2 weeks, at least 3 weeks, at least 4 weeks, at least 5 weeks, at least 6 weeks, at least 7 weeks, at least 8 weeks, at least 9 weeks, at least 10 weeks or more, or may be at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, or more.

In some examples, populations to be treated with mRNA compositions described herein may include one or more populations with one or more particularly high risk conditions or history, e.g., as noted herein. For example, in some examples, populations to be treated with mRNA compositions described herein may include subjects whose profession and/or environmental exposure may dramatically increase their risk of getting SARS-CoV-2 infection (including, e.g., but not limited to mass transportation, prisoners, grocery store workers, residents in long-term care facilities, butchers or other meat processing workers, healthcare workers, and/or first responders, e.g., emergency responders). In particular examples, populations to be treated with mRNA compositions described herein may include healthcare workers and/or first responders, e.g., emergency responders. In some examples, populations to be treated with mRNA compositions described herein may include those with a history of smoking or vaping (e.g., within 6 months, 12 months or more, including a history of chronic smoking or vaping). In some examples, populations to be treated with mRNA compositions described herein may include certain ethnic groups that have been determined to be more susceptible to SARS-CoV-2 infection.

In some examples, populations to be treated with mRNA compositions described herein may include certain populations with a blood type that may have been determined to more susceptible to SARS-CoV-2 infection. In some examples, populations to be treated with mRNA compositions described herein may include immunocompromised subjects (e.g., those with HIV/AIDS; cancer and transplant patients who are taking certain immunosuppressive drugs; autoimmune diseases or other physiological conditions expected to warrant immunosuppressive therapy (e.g., within 3 months, within 6 months, or more); and those with

inherited diseases that affect the immune system (e.g., congenital agammaglobulinemia, congenital IgA deficiency)). In some examples, populations to be treated with mRNA compositions described herein may include those with an infectious disease. For example, in some examples, populations to be treated with mRNA compositions described herein may include those infected with human immunodeficiency virus (HIV) and/or a hepatitis virus (e.g., HBV, HCV). In some examples, populations to be treated with mRNA compositions described herein may include those with underlying medical conditions. Examples of such underlying medical conditions may include, but are not limited to hypertension, cardiovascular disease, diabetes, chronic respiratory disease, e.g., chronic pulmonary disease, asthma, etc., cancer, and other chronic diseases such as, e.g., lupus, rheumatoid arthritis, chonic liver diseases, chronic kidney diseases (e.g., Stage 3 or worse such as in some examples as characterized by a glomerular filtration rate (GFR) of less than 60 mL/min/1.73m^2). In some examples, populations to be treated with mRNA compositions described herein may include overweight or obese subjects, e.g., specifically including those with a body mass index (BMI) above about 30 kg/m^2. In some examples, populations to be treated with mRNA compositions described herein may include subjects who have prior diagnosis of COVID-19 or evidence of current or prior SARS-CoV-2 infection, e.g., based on serology or nasal swab. In some examples, populations to be treated include white and/or non-Hispanic/non-Latino.

In some examples, certain mRNA compositions described herein (e.g., BNT162b1) may be selected for administration to Asian populations (*e.g.*, Chinese populations), or in particular examples to older Asian populations (*e.g*, 60 years old or over, e.g., 60-85 or 65-85 years old).

In some examples, an mRNA composition as provided herein is administered to and/or assessed in subject(s) who have been determined not to show evidence of prior infection, and/or of present infection, before administration; in some examples, evidence of prior

infection and/or of present infection, may be or include evidence of intact virus, or any viral nucleic acid, protein, lipid etc. present in the subject (e.g., in a biological sample thereof, such as blood, cells, mucus, and/or tissue), and/or evidence of a subject's immune response to the same. In some examples, an mRNA composition as provided herein is administered to and/or assessed in subject(s) who have been determined to show evidence of prior infection, and/or of present infection, before administration; in some examples, evidence of prior infection and/or of present infection, may be or include evidence of intact virus, or any viral nucleic acid, protein, lipid etc. present in the subject (e.g., in a biological sample thereof, such as blood, cells, mucus, and/or tissue), and/or evidence of a subject's immune response to the same. In some examples, a subject is considered to have a prior infection based on having a positive N-binding antibody test result or positive nucleic acid amplification test (NAAT) result on the day of Dose 1.

In some examples, an RNA (e.g., mRNA) composition as provided herein is administered to a subject who has been informed of a risk of side effects that may include one or more of, for example: chills, fever, headache, injection site pain, muscle pain, tiredness; in some examples, an RNA (e.g., mRNA) composition is administered to a subject who has been invited to notify a healthcare provider if one or more such side effects occurs, is experienced as more than mild or moderate, persists for a period of more than a day or a few days, or if any serious or unexpected event is experienced that the subject reasonably considers may be associated with receipt of the composition. In some examples, an RNA (e.g., mRNA) composition as provided herein is administered to a subject who has been invited to notify a healthcare provider of particular medical conditions which may include, for example, one or more of allergies, bleeding disorder or taking a blood thinner medication, breastfeeding, fever, immunocompromised state or taking medication that affects the immune system, pregnancy or plan to become pregnant, etc. In some examples, an RNA (e.g., mRNA) composition as provided herein is administered to a subject who has been invited to notify a healthcare

provider of having received another COVID-19 vaccine. In some examples, an RNA (e.g., mRNA) composition as provided herein is administered to a subject not having one of the following medical conditions: experiencing febrile illness, receiving immunosuppressant therapy, receiving anticoagulant therapy, suffering from a bleeding disorder (e.g., one that would contraindicate intramuscular injection), or pregnancy and/or breatfeeding/lactation. In some examples, an RNA (e.g., mRNA) composition as provided herein is administered to a subject not having received another COVID-19 vaccine. In some examples, an RNA (e.g., mRNA) composition as provided herein is administered to a subject who has not had an allergic reaction to any component of the RNA (e.g., mRNA) composition. Examples of such allergic reaction may include, but are not limited to difficulty breathing, swelling of fact and/or throat, fast hearbeat, rash, dizziness and/or weakness. In some examples, an RNA (e.g., mRNA) composition as provided herein is administered to a subject who received a first dose and did not have an allergic reaction (e.g., as described herein) to the first dose. In some examples where allergic reaction occurs in subject(s) after receiving a dose of an RNA (e.g., mRNA) composition as provided herein, such subject(s) may be administered one or more interventions such as treatment to manage and/or reduce symptom(s) of such allergic reactions, for example, fever-reducing and/or anti-inflammatory agents.

In some examples, a subject who has received at least one dose of an RNA (e.g., mRNA) composition as provided herein is informed of avoiding being exposed to a coronavirus (e.g., SARS-CoV-2) unless and until several days (e.g., at least 7 days, at least 8 days, 9 days, at least 10 days, at least 11 days, at least 12 days, at least 13 days, at least 14 days, etc.) have passed since administration of a second dose. For example, a subject who has received at at least one dose of an RNA (e.g., mRNA) composition as provided herein is informed of taking precautionary measures against SARS-CoV-2 infection (e.g., remaining socially distant, wearing masks, frequent hand-washing, etc.) unless and until several days (e.g., at least 7 days, at least 8 days, 9 days, at least 10 days, at least 11 days, at least 12 days, at least 13 days, at

least 14 days, etc.) have passed since administration of a second dose. Accordingly, in some examples, methods of administering an RNA (e.g., mRNA) composition as provided herein comprise administering a second dose of such an RNA (e.g., mRNA) composition as provided herein to a subject who received a first dose and took precautionary measures to avoid being exposed to a coronavirus (e.g., SARS-CoV-2).

In some examples, mRNA compositions described herein may be delivered to a draining lymph node of a subject in need thereof, for example, for vaccine priming. In some examples, such delivery may be performed by intramuscular administration of a provided mRNA composition. In some examples, different particular mRNA compositions may be administered to different subject population(s); alternatively or additionally, in some examples, different dosing regimens may be administered to different subject populations. For example, in some examples, mRNA compositions administered to particular subject population(s) may be characterized by one or more particular effects (e.g., incidence and/or degree of effect) in those subject populations. In some examples, such effect(s) may be or comprise, for example titer and/or persistence of neutralizing antibodies and/or T cells (e.g., T_H1-type T cells such as $CD4^+$ and/or $CD8^+$ T cells), protection against challenge (e.g., via injection and/or nasal exposure, etc), incidence, severity, and/or persistence of side effects (e.g., reactogenicity), etc.

In some examples, one or more mRNA compositions described herein may be administered according to a regimen established to reduce COVID-19 incidence per 1000 person-years, e.g., based on a laboratory test such as nucleic acid amplification test (NAAT). In some examples, one or more mRNA compositions described herein may be administered according to a regimen established to reduce COVID-19 incidence per 1000 person-years based on a laboratory test such as nucleic acid amplification test (NAAT) in subjects receiving at least one dose of a provided mRNA composition with no serological or

virological evidence (e.g., up to 7 days after receipt of the last dose) of past SARS-CoV-2 infection. In some examples, one or more mRNA compositions described herein may be administered according to a regimen established to reduce confirmed severe COVID-19 incidence per 1000 person-years. In some examples, one or more mRNA compositions described herein may be administered according to a regimen established to reduce confirmed severe COVID-19 incidence per 1000 person-years in subjects receiving at least one dose of a provided mRNA composition with no serological or virological evidence of past SARS-CoV-2 infection.

In some examples, one or more mRNA compositions described herein may be administered according to a regimen established to produce neutralizing antibodies directed to a SARS-CoV-2 spike polypeptide and/or an immunogenic fragment thereof (e.g., RBD) as measured in serum from a subject that achieves or exceeds a reference level (e.g., a reference level determined based on human SARS-CoV-2 infection/COVID-19 convalescent sera) for a period of time and/or induction of cell-mediated immune response (e.g., a T cell response against SARS-CoV-2), including, e.g., in some examples induction of T cells that recognize at least one or more MHC-restricted (e.g., MHC class I-restricted) eptiopes within a SARS-CoV-2 spike polypeptide and/or an immunogenic fragment thereof (e.g., RBD) for a period of time. In some such examples, the period of time may be at least 2 months, 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, at least 10 months, at least 11 months, at least 12 months or longer. In some examples, one or more epitopes recognized by vaccine-induced T cells (e.g., CD8+ T cells) may be presented on a MHC class I allele that is present in at least 50% of subjects in a population, including, e.g., at least 60%, at least 70%, at least 80%, at least 90%, or more; in some such examples, the MHC class I allele may be HLA-B*0702, HLA-A*2402, HLA-B*3501, HLA-B*4401, or HLA-A*0201. In some examples, an epitope may comprise HLA-A*0201 YLQPRTFLL; HLA-A*0201 RLQSLQTYV; HLA-A*2402 QYIKWPWYI; HLA-A*2402

NYNYLYRLF; HLA-A*2402 KWPWYIWLGF; HLA-B*3501 QPTESIVRF; HLA-B*3501 IPFAMQMAY; or HLA-B*3501 LPFNDGVYF.

In some examples, efficacy is assessed as COVID-19 incidence per 1000 person-years in individuals without serological or virological ecidence of past SARS-CoV-2 infection before and during vaccination regimen; alternatively or additionally, in some examples, efficacy is assessed as COVID-19 incidence per 1000 person-years in subjects with and without evidence of past SARS-CoV-2 infection before and during vaccination regimen. In some such examples, such incidence is of COVID-19 cases confirmed within a specific time period after the final vaccination dose (e.g., a first dose in a single-dose regimen; a second dose in a two-dose regimen, etc); in some examples, such time period may be within (i.e., up to and including 7 days) a particular number of days (e.g., 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 days or more). In some examples, such time period may be within 7 days or within 14 days or within 21 days or within 28 days. In some examples, such time period may be within 7 days. In some examples, such time period may be within 14 days.

In some examples (e.g., in some examples of assessing efficacy), a subject is determined to have experienced COVID-19 infection if one or more of the following is established: detection of SARS-CoV-2 nucleic acid in a sample from the subject, detection of antibodies that specifically recognize SARS-CoV-2 (e.g., a SARS-Co-V-2 spike protein), one or more symptoms of COVID-19 infection, and combinations thereof. In some such examples, detection of SARS-CoV-2 nucleic acid may involve, for example, NAAT testing on a mid-turbinatae swap sample. In some such examples, detection of relevant antibodies may involve serological testing of a blood sample or portion thereof. In some such examples, symptoms of COVID-19 infection may be or include: fever, new or increased cough, new or increased shortness of breath, chills, new or increased muscle pain, new loss of taste or smell, sore throat, diarrhea, vomiting and combinations thereof. In some such

examples, symptoms of COVID-19 infection may be or include: fever, new or increased cough, new or increased shortness of breath, chills, new or increased muscle pain, new loss of taste or smell, sore throat, diarrhea, vomiting, fatigue, headache, nasal congestion or runny nose, nausea, and combinations thereof. In some such examples, a subject is determined to have experienced COVID-19 infection if such subject both has experienced one such symptom and also has received a positive test for SARS-CoV-2 nucleic acid or antibodies, or both. In some such examples, a subject is determined to have experienced COVID-19 infection if such subject both has experienced one such symptom and also has received a positive test for SARS-CoV-2 nucleic acid. In some such examples, a subject is determined to have experienced COVID-19 infection if such subject both has experienced one such symptom and also has received a positive test for SARS-CoV-2 antibodies.

In some examples (e.g., in some examples of assessing efficacy), a subject is determined to have experienced severe COVID-19 infection if such subject has experienced one or more of: clinical signs at rest indicative or severe systemic illness (e.g., one or more of respiratory rate at greater than or equal to 30 breaths per minute, heart rate at or above 125 beats per minute, SpO_2 less than or equal to 93% on room air at sea level or a PaO_2/FiO_2 below 300 m Hg), respiratory failure (e.g., one or more of needing high-flow oxygen, noninvasive ventilation, mechanical ventilation, ECMO), evidence of shock (systolic blood pressure below 90 mm Hg, diastolic blood pressure below 60mm Hg, requiring vasopressors), significant acute renal, hepatic, or neurologic dystfunction, admission ot an intensive care unit, death, and combinations thereof.

In some examples, one or more mRNA compositions described herein may be administered according to a regimen established to reduce the percentage of subjects reporting at least one of the following: (i) one or more local reactions (e.g., as described herein) for up to 7 days

following each dose; (ii) one or more systemic events for up to 7 days following each dose; (iii) adverse events (e.g., as described herein) from a first dose to 1 month after the last dose; and/or (iv) serious adverse events (e.g., as described herein) from a first dose to 6 months after the last dose.

In some examples, one or more subjects who have received an RNA (e.g., mRNA) composition as described herein may be monitored (e.g., for a period of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 days or more, including, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 weeks or more, including for example 1, 2, 3, 4, 5, 6, 7, 8, 9 ,10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 months or more, including for example 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 years or more) to assess, for example, presence of an immune response to component(s) of the administered composition, evidence of exposure to and/or immune response to SARS-CoV-2 or another coronavirus, evidence of any adverse event, etc. In some examples, monitoring may be via tele-visit. Alternatively or additionally, in some examples, monitoring may be in-person.

In some examples, a treatment effect conferred by one or more mRNA compositions described herein may be characterized by (i) a SARS-CoV-2 anti-S1 binding antibody level above a pre-determined threshold; (ii) a SARS-CoV-2 anti-RBD binding antibody level above a pre-determined threshold; and/or (iii) a SARS-CoV-2 serum neutralizing titer above a threshold level, e.g., at baseline, 1 month, 3 months, 6 months, 9 months, 12 months, 18 months, and/or 24 months after completion of vaccination. In some examples, anti-S1 binding antibody and/or anti-RBD binding antibody levels and/or serum neutralizing titers may be characterized by geometric mean concentration (GMC), geometric mean titer (GMT), or geometric mean fold-rise (GMFR).

In some examples, a treatment effect conferred by one or more mRNA compositions described herein may be characterized in that percentage of treated subjects showing a SARS-CoV-2 serum neutralizing titer above a pre-determined threshold, e.g., at baseline, 1 month, 3 months, 6 months, 9 months, 12 months, 18 months, and/or 24 months after completion of

vaccination, is higher than the percentage of non-treated subjects showing a SARS-CoV-2 serum neutralizing titer above such a pre-determined threshold (e.g., as described herein). In some examples, a serum neutralizing titer may be characterized by geometric mean concentration (GMC), geometric mean titer (GMT), or geometric mean fold-rise (GMFR).

In some examples, a treatment effect conferred by one or more mRNA compositions described herein may be characterized by detection of SARS-CoV-2 NVA-specific binding antibody.

In some examples, a treatment effect conferred by one or more mRNA compositions described herein may be characterized by SARS-CoV-2 detection by nucleic acid amplification test.

In some examples, a treatment effect conferred by one or more mRNA compositions described herein may be characterized by induction of cell-mediated immune response (e.g., a T cell response against SARS-CoV-2), including, e.g., in some examples induction of T cells that recognize at least one or more MHC-restricted (e.g., MHC class I-restricted) eptiopes within a SARS-CoV-2 spike polypeptide and/or an immunogenic fragment thereof (e.g., RBD). In some examples, one or more epitopes recognized by vaccine-induced T cells (e.g., CD8+ T cells) may be presented on a MHC class I allele that is present in at least 50% of subjects in a population, including, e.g., at least 60%, at least 70%, at least 80%, at least 90%, or more; in some such examples, the MHC class I allele may be HLA-B*0702, HLA-A*2402, HLA-B*3501, HLA-B*4401, or HLA-A*0201. In some examples, an epitope may comprise HLA-A*0201 YLQPRTFLL; HLA-A*0201 RLQSLQTYV; HLA-A*2402 QYIKWPWYI; HLA-A*2402 NYNYLYRLF; HLA-A*2402 KWPWYIWLGF; HLA-B*3501 QPTESIVRF; HLA-B*3501 IPFAMQMAY; or HLA-B*3501 LPFNDGVYF.

In some examples, primary vaccine efficacy (VE) of one or more mRNA compositions described herein may be established when there is sufficient evidence (posterior probability) that either primary VE1 or both primary VE1 and primary VE2 are >30% or higher (including,

e.g., greater than 40%, greater than 50%, greater than 60%, greater than 70%, greater than 80%, greater than 90%, greater than 95%, greater than 96%, greater than 97%, greater than 98%, or higher), wherein primary VE is defined as primary VE = 100 × (1 – IRR); and IRR is calculated as the ratio of COVID-19 illness rate in the vaccine group to the corresponding illness rate in the placebo group. Primary VE1 represents VE for prophylactic mRNA compositions described herein against confirmed COVID-19 in participants without evidence of infection before vaccination, and primary VE2 represents VE for prophylactic mRNA compositions described herein against confirmed COVID-19 in all participants after vaccination. In some examples, primary VE1 and VE2 can be evaluated sequentially to control the overall type I error of 2.5% (hierarchical testing). In some examples where one or more RNA (e.g., mRNA) compositions described herein are demonstrated to achieve primary VE endponts as discussed above, secondary VE endpoints (e.g., confirmed severe COVID-19 in participants without evidence of infection before vaccination and confirmed severe COVID-19 in all participants) can be evaluated sequentially, e.g., by the same method used for the primary VE endpoint evaluation (hierarchical testing) as discussed above. In some examples, evaluation of primary and/or secondary VE endpoints may be based on at least 20,000 or more subjects (e.g., at least 25,000 or more subjects) randomized in a 1:1 ratio to the vaccine or placebo group, e.g., based on the following assumptions: (i) 1.0% illness rate per year in the placebo group, and (ii) 20% of the participants being non-evaluable or having serological evidence of prior infection with SARS-CoV-2, potentially making them immune to further infection.

In some examples, one or more mRNA compositions described herein may be administered according to a regimen established to achieve maintenance and/or continued enhancement of an immune response. For example, in some examples, an administration regimen may include a first dose optionally followed by one or more subsequent doses; in some examples, need for, timing of, and/or magnitude of any such subsequent dose(s)

may be selected to maintain, enhance, and/or modify one or more immune responses or features thereof. In some examples, number, timing, and/or amount(s) of dose(s) have been established to be effective when administered to a relevant population. In some examples, number, timing and/or amount(s) of dose(s) may be adjusted for an individual subject; for example, in some examples, one or more features of an immune response in an individual subject may be assessed at least once (and optionally more than once, for example multiple times, typically spaced apart, often at pre-selected intervals) after receipt of a first dose. For example, presence of antibodies, B cells, and/or T cells (e.g., $CD4^+$ and/or $CD8^+$ T cells), and/or of cytokines secreted thereby and/or identity of and/or extent of responses to particular antigen(s) and/or epitope(s) may be assessed. In some examples, need for, timing of, and/or amount of a subsequent dose may be determined in light of such assessments.

As noted hereinabove, in some examples, one or more subjects who have received an RNA (e.g., mRNA) composition as described herein may be monitored (e.g., for a period of at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 days or more, including, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 weeks or more, including for example 1, 2, 3, 4, 5, 6, 7, 8, 9 ,10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 months or more, including for example 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 years or more) from receipt of any particular dose to assess, for example, presence of an immune response to component(s) of the administered composition, evidence of exposure to and/or immune response to SARS-CoV-2 or another coronavirus, evidence of any adverse event, etc, including to perform assessment of one or more of presence of antibodies, B cells, and/or T cells (e.g., $CD4^+$ and/or $CD8^+$ T cells), and/or of cytokines secreted thereby and/or identity of and/or extent of responses to particular antigen(s) and/or epitope(s) may be assessed. Administration of a composition as described herein may be in accordance with a regimen that includes one or more such monitoring steps.

For example, in some examples, need for, timing of, and/or amount of a second dose relative to a first dose (and/or of a subsequent dose relative to a prior dose) is assessed, determined, and/or selected such that administration of such second (or subsequent) dose achieves amplification or modification of an immune response (e.g., as described herein) observed after the first (or other prior) dose. In some examples, such amplification of an immune response (e.g., ones described herein) may be at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or higher, as compared to the level of an immune response observed after the first dose. In some examples, such amplification of an immune response may be at least 1.5 fold, at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, at least 10-fold, at least 20-fold, at least 30-fold, or higher, as compared to the level of an immune response observed after the first dose.

In some examples, need for, timing of, and/or amount of a second (or subsequent) dose relative to a first (or other prior) dose is assessed, determined, and/or selected such that administration of the later dose extends the durability of an immune response (e.g., as described herein) observed after the earlier dose; in some such examples, the durability may be extended by at least 1 week, at least 2 weeks, at least 3 weeks, at least 1 month, at least 2 months, at least 3 months, at least 4 months, at least 5 months, at least 6 months, at least 7 months, at least 8 months, at least 9 months, or longer. In some examples, an immune response observed after the first dose may be characterized by production of neutralizing antibodies directed to a SARS-CoV-2 spike polypeptide and/or an immunogenic fragment thereof (e.g., RBD) as measured in serum from a subject and/or induction of cell-mediated immune response (e.g., a T cell response against SARS-CoV-2), including, e.g., in some examples induction of T cells that recognize at least one or more MHC-restricted (e.g., MHC class I-restricted) eptiopes within a SARS-CoV-2 spike polypeptide and/or an immunogenic fragment thereof (e.g., RBD). In some examples, one or more epitopes

recognized by vaccine-induced T cells (e.g., CD8+ T cells) may be presented on a MHC class I allele that is present in at least 50% of subjects in a population, including, e.g., at least 60%, at least 70%, at least 80%, at least 90%, or more; in some such examples, the MHC class I allele may be HLA-B*0702, HLA-A*2402, HLA-B*3501, HLA-B*4401, or HLA-A*0201. In some examples, an epitope may comprise HLA-A*0201 YLQPRTFLL; HLA-A*0201 RLQSLQTYV; HLA-A*2402 QYIKWPWYI; HLA-A*2402 NYNYLYRLF; HLA-A*2402 KWPWYIWLGF; HLA-B*3501 QPTESIVRF; HLA-B*3501 IPFAMQMAY; or HLA-B*3501 LPFNDGVYF.

In some examples, need for, timing of, and/or amount of a second dose relative to a first dose (or other subsequent dose relative to a prior dose) is assessed, determined and/or selected such that administration of such second (or subsequent) dose maintains or exceeds a reference level of an immune response; in some such examples, the reference level is determined based on human SARS-CoV-2 infection/COVID-19 convalescent sera and/ro PBMC samples drawn from subjects (e.g., at least a period of time such as at least 14 days or longer, including, e.g., 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 25 days, 30 days, 35 days, 40 days, 45 days, 50 days, 55 days, 60 days, or longer, after PCR-confirmed diagnosis when the subjects were asymptomatic. In some examples, an immune response may be characterized by production of neutralizing antibodies directed to a SARS-CoV-2 spike polypeptide and/or an immunogenic fragment thereof (e.g., RBD) as measured in serum from a subject and/or induction of cell-mediated immune response (e.g., a T cell response against SARS-CoV-2), including, e.g., in some examples induction of T cells that recognize at least one or more MHC-restricted (e.g., MHC class I-restricted) eptiopes within a SARS-CoV-2 spike polypeptide and/or an immunogenic fragment thereof (e.g., RBD). In some examples, one or more epitopes recognized by vaccine-induced T cells (e.g., CD8+ T cells) may be presented on a MHC class I allele that is present in at least 50% of subjects in a population, including, e.g., at least 60%, at least 70%, at least 80%, at least 90%, or more; in some such examples, the MHC class I allele may be HLA-B*0702, HLA-A*2402, HLA-B*3501, HLA-B*4401, or HLA-

A*0201. In some examples, an epitope may comprise HLA-A*0201 YLQPRTFLL; HLA-A*0201 RLQSLQTYV; HLA-A*2402 QYIKWPWYI; HLA-A*2402 NYNYLYRLF; HLA-A*2402 KWPWYIWLGF; HLA-B*3501 QPTESIVRF; HLA-B*3501 IPFAMQMAY; or HLA-B*3501 LPFNDGVYF.

In some examples, determination of need for, timing of, and/or amount of a second (or subsequent) dose may include one or more steps of assessing, after (e.g., 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 days or longer after) a first (or other prior) dose, presence and/or expression levels of neutralizing antibodies directed to a SARS-CoV-2 spike polypeptide and/or an immunogenic fragment thereof (e.g., RBD) as measured in serum from a subject and/or induction of cell-mediated immune response (e.g., a T cell response against SARS-CoV-2), including, e.g., in some examples induction of T cells that recognize at least one or more MHC-restricted (e.g., MHC class I-restricted) eptiopes within a SARS-CoV-2 spike polypeptide and/or an immunogenic fragment thereof (e.g., RBD). In some examples, one or more epitopes recognized by vaccine-induced T cells (e.g., CD8+ T cells) may be presented on a MHC class I allele that is present in at least 50% of subjects in a population, including, e.g., at least 60%, at least 70%, at least 80%, at least 90%, or more; in some such examples, the MHC class I allele may be HLA-B*0702, HLA-A*2402, HLA-B*3501, HLA-B*4401, or HLA-A*0201. In some examples, an epitope may comprise HLA-A*0201 YLQPRTFLL; HLA-A*0201 RLQSLQTYV; HLA-A*2402 QYIKWPWYI; HLA-A*2402 NYNYLYRLF; HLA-A*2402 KWPWYIWLGF; HLA-B*3501 QPTESIVRF; HLA-B*3501 IPFAMQMAY; or HLA-B*3501 LPFNDGVYF.

In some examples, a kit as provided herein may comprise a real-time monitoring logging device, which, for example in some examples, is capable of providing shipment temperatures, shipment time and/or location.

In some examples, an RNA (e.g., mRNA) composition as described herein may be shipped, stored, and/or utilized, in a container (such as a vial or syringe), e.g., a glass container (such

as a glass vial or syringe), which, in some examples, may be a single-dose container or a multi-dose container (e.g., may be arranged and constructed to hold, and/or in some examples may hold, a single dose, or multiple doses of a product for administration). In some examples, a multi-dose container (such as a multi-dose vial or syringe) may be arranged and constructed to hold, and/or may hold 2, 3, 4, 5, 6, 7, 8, 9, 10 or more doses; in some particular examples, it may be designed to hold and/or may hold 5 doses. In some examples, a single-dose or multi-dose container (such as a single-dose or multi-dose vial or syringe) may be arranged and constructed to hold and/or may hold a volume or amount greater than the indicated number of doses, e.g., in order to permit some loss in transfer and/or administration. In some examples, an RNA (e.g., mRNA) composition as described herein may be shipped, stored, and/or utilized, in a preservative-free glass container (e.g., a preservative-free glass vial or syringe, e.g., a single-dose or multi-dose preservative-free glass vial or syringe). In some examples, an RNA (e.g., mRNA) composition as described herein may be shipped, stored, and/or utilized, in a preservative-free glass container (e.g., a preservative-free glass vial or syringe, e.g., a single-dose or multi-dose preservative-free glass vial or syringe) that contains 0.45 ml of frozen liquid (e.g., including 5 doses). In some examples, an RNA (e.g., mRNA) composition as described herein and/or a container (e.g., a vial or syringe) in which it is disposed, is shipped, stored, and/or utilized may be maintained at a temperature below room temperature, at or below 4 °C, at or below 0 °C, at or below -20 °C, at or below -60 °C, at or below -70 °C, at or below -80 °C , at or below -90 °C, etc. In some examples, an RNA (e.g., mRNA) composition as described herein and/or a container (e.g., a viral or syringe) in which it is disposed, is shipped, stored, and/or utilized may be maintained at a temperature between -80°C and -60°C and in some examples protected from light. In some examples, an RNA (e.g., mRNA) composition as described herein and/or a container (e.g., a viral or syringe) in which it is disposed, is shipped, stored, and/or utilized may be maintained at a temperature below about 25°C, and in some examples protected

from light. In some examples, an RNA (e.g., mRNA) composition as described herein and/or a container (e.g., a viral or syringe) in which it is disposed, is shipped, stored, and/or utilized may be maintained at a temperature below about 5°C (e.g., below about 4°C), and in some examples protected from light. In some examples, an RNA (e.g., mRNA) composition as described herein and/or a container (e.g., a viral or syringe) in which it is disposed, is shipped, stored, and/or utilized may be maintained at a temperature below about -20°C, and in some examples protected from light. In some examples, an RNA (e.g., mRNA) composition as described herein and/or a container (e.g., a viral or syringe) in which it is disposed, is shipped, stored, and/or utilized may be maintained at a temperature above about -60°C (e.g., in some examples at or above about -20°C, and in some examples at or above about 4-5°C, in either case optionally below about 25°C), and in some examples protected from light, or otherwise without affirmative steps (e.g., cooling measures) taken to achieve a storage temperature materially below about -20°C.

In some examples, an RNA (e.g., mRNA) composition as described herein and/or a container (e.g., a vial or syringe) in which it is disposed is shipped, stored, and/or utilized together with and/or in the context of a thermally protective material or container and/or of a temperature adjusting material. For example, in some examples, an RNA (e.g., mRNA) composition as described herein and/or a container (e.g., a vial or syringe) in which it is disposed is shipped, stored, and/or utilized together with ice and/or dry ice and/or with an insulating material. In some particular examples, a container (e.g., a vial or syringe) in which an RNA (e.g., mRNA) composition is disposed is positioned in a tray or other retaining device and is further contacted with (or otherwise in the presence of) temperature adjusting (e.g., ice and/or dry ice) material and/or insulating material. In some examples, multiple containers (e.g., multiple vials or syringes such as single use or multi-use vials or syringes as described herein) in which a provided RNA (e.g., mRNA) composition is disposed are co-localized (e.g., in a common tray, rack, box, etc.) and packaged with (or otherwise in the

presence of) temperature adjusting (e.g., ice and/or dry ice) material and/or insulating material. To give but one example, in some examples, multiple containers (e.g., multiple vials or syringes such as single use or multi-use vials or syringes as described herein) in which an RNA (e.g., mRNA) composition is disposed are positioned in a common tray or rack, and multiple such trays or racks are stacked in a carton that is surrounded by a temperature adjusting material (e.g., dry ice) in a thermal (e.g., insulated) shipper. In some examples, temperature adjusting material is replenished periodically (e.g., within 24 hours of arrival at a site, and/or every 2 hours, 4 hours, 6 hours, 8 hours, 10 hours, 12 hours, 14 hours, 16 hours, 18 hours, 20 hours, 22 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 7 days, 8 days, 9 days, 10 days, etc.). Preferably, re-entry into a thermal shipper should be infrequent, and desirably should not occur more than twice a day. In some examples, a thermal shipper is re-closed within 5, 4, 3, 2, or 1 minute, or less, of having been opened. In some examples, a provided RNA (e.g., mRNA) composition that has been stored within a thermal shipper for a period of time, optionally within a particular temperature range remains useful. For example, in some examples, if a thermal shipper as described herein containing a provided RNA (e.g., mRNA) composition is or has been maintained (e.g., stored) at a temperature within a range of about 15 °C to about 25 °C, the RNA (e.g., mRNA) composition may be used for up to 10 days; that is, in some examples, a provided RNA (e.g., mRNA) composition that has been maintained within a thermal shipper, which thermal shipper is at a temperature within a range of about 15 °C to about 25 °C, for a period of not more than 10 days is administered to a subject. Alternatively or additionally, in some examples, if a provided RNA (e.g., mRNA) composition is or has been maintained (e.g., stored) within a thermal shipper, which thermal shipper has been maintained (e.g., stored) at a temperature within a range of about 15 °C to about 25 °C, it may be used for up to 10 days; that is, in some examples, a provided RNA (e.g., mRNA) composition that has been maintained within a thermal shipper, which thermal shipper has been maintained at a

temperature within a range of about 15 °C to about 25 °C for a period of not more than 10 days is administered to a subject.

In some examples, a provided RNA (e.g., mRNA) composition is shipped and/or stored in a frozen state. In some examples, a provided RNA (e.g., mRNA composition is shipped and/or stored as a frozen suspension, which in some examples does not contain preservative. In some examples, a frozen RNA (e.g., mRNA) composition is thawed. In some examples, a thawed RNA (e.g., mRNA) composition (e.g., a suspension) may contain white to off-white opaque amorphous particles. In some examples, a thawed RNA (e.g., mRNA) composition may be used for up to a small number (e.g., 1, 2, 3, 4, 5, or 6) of days after thawing if maintained (e.g., stored) at a temperature at or below room temperature (e.g., below about 30 °C, 25 °C, 20 °C, 15 °C, 10 °C, 8 °C, 4 °C, etc). In some examples, a thawed RNA (e.g., mRNA) composition may be used after being stored (e.g., for such small number of days) at a temperature between about 2 °C and about 8 °C; alternatively or additionally, a thawed RNA (e.g., mRNA) composition may be used within a small number (e.g., 1, 2, 3, 4, 5, 6) of hours after thawing at room temperature. Thus, in some examples, a provided RNA (e.g., mRNA) composition that has been thawed and maintained at a temperature at or below room temperature, and in some examples between about 2 °C and about 8 °C, for not more than 6, 5, 4, 3, 2, or 1 days is administered to a subject. Alternatively or additionally, in some examples, a provided RNA (e.g., mRNA) composition that has been thawed and maintained at room temperature for not more than 6, 5, 4, 3, 2, or 1 hours is administered to a subject. In some examples, a provided RNA (e.g., mRNA) composition is shipped and/or stored in a concentrated state. In some examples, such a concentrated composition is diluted prior to administration. In some examples, a diluted composition is administered within a period of about 10, 9, 8, 7, 6, 5, 4, 3, 2, or 1 hour(s) post-dilution; in some examples, such administration is within 6 hours post-dilution. Thus, in some examples, diluted preparation of a provided RNA (e.g., mRNA) composition is

administered to a subject within 6 hours post-dilution (e.g., as described herein after having been maintained at an appropriate temperature, e.g., at a temperature below room temperature, at or below 4 °C, at or below 0 °C, at or below -20 °C, at or below -60 °C, at or below -70 °C, at or below - 80 °C, etc, and typically at or above about 2 °C, for example between about 2 °C and about 8 °C or between about 2 °C and about 25 °C). In some examples, unusued composition is discarded within several hours (e.g., about 10, about 9, about 8, about 7, about 6, about 5 or fewer hours) after dilution; in some examples, unused composition is discarded within 6 hours of dilution.

In some examples, an RNA (e.g., mRNA) composition that is stored, shipped or utilized (e.g., a frozen composition, a liquid concentrated composition, a diluted liquid composition, etc.) may have been maintained at a temperature materially above -60°C for a period of time of at least 1, 2, 3, 4, 5, 6, 7 days or more, or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 weeks or more, or at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 months or more; in some such examples, such composition may have been maintained at a temperature at or above about -20°C for such period of time, and/or at a temperature up to or about 4-5°C for such period of time, and/or may have been maintained at a temperature above about 4-5°C, and optionally about 25°C for a period of time up that is less than two (2) months and/or optionally up to about one (1) month. In some examples, such composition may not have been stored, shipped or utilized (or otherwise exposed to) a temperature materially above about 4-5°C, and in particular not at or near a temperature of about 25°C for a period of time as long as about 2 weeks, or in some examples 1 week. In some examples, such composition may not have been stored, shipped or utilized (or otherwise exposed to) a temperature materially above about -20°C, and in particular not at or near a temperature of about 4-5°C for a period of time as long as about 12 months, 11 months, 10 months, 9 months, 8 months, 7 months, 6 months, 5 months, 4 months, 3 months, 2 months, or, in some examples, for a period of

time as long as about 8 weeks or 6 weeks or materially more than about 2 months or, in some examples, 3 months or, in some examples 4 months.

In some examples, an RNA (e.g., mRNA) composition that is stored, shipped or utilized (e.g., a frozen composition, a liquid concentrated composition, a diluted liquid composition, etc.) may be protected from light. In some examples, one or more steps may be taken to reduce or minimize exposure to light for such compositions (e.g., which may be disposed within a container such as a vial or a syringe). In some examples, exposure to direct sunlight and/or to ultraviolent light is avoided. In some examples, a diluted solution may be handled and/or utilized under normal room light conditions (e.g., without particular steps taken to minimize or reduce exposure to room light). It should be understood that strict adherence to aseptic techniques is desirable during handling (e.g., diluting and/or administration) of an RNA (e.g., mRNA) composition as described herein. In some examples, an RNA (e.g., mRNA) composition as described herein is not administered (e.g., is not injected) intravenously. In some examples, an RNA (e.g., mRNA) composition as described herein is not administered (e.g., is not injected) intradermally. In some examples, an RNA (e.g., mRNA) composition as described herein is not administered (e.g., is not injected) subcutaneously. In some examples, an RNA (e.g., mRNA) composition as described herein is not administered (e.g., is not injected) any of intravenously, intradermally, or subcutaneously. In some examples, an RNA (e.g., mRNA) composition as described herein is not administered to a subject with a known hypersensitivity to any ingredient thereof. In some examples, a subject to whom an RNA (e.g., mRNA) composition has been administered is monitored for one or more signs of anaphylaxis. In some examples, a subject to whom an RNA (e.g., mRNA) composition is administered had previously received at least one dose of a different vaccine for SARS-CoV-2; in some examples, a subject to whom an RNA (e.g., mRNA) composition is administered had not previously received a different vaccine for SARS-CoV-2. In some examples, a subject’s temperature is taken

promptly prior to administration of an RNA (e.g., mRNA) composition (e.g., shortly before or after thawing, dilution, and/or administration of such composition); in some examples, if such subject is determined to be febrile, administration is delayed or canceled. In some examples, an RNA (e.g., mRNA) composition as described herein is not administered to a subject who is receiving anticoagulant therapy or is suffering from or susceptible to a bleeding disorder or condition that would contraindicate intramuscular injection. In some examples, an RNA (e.g., mRNA) composition as described herein is administered by a healthcare professional who has communicated with the subject receiving the composition information relating to side effects and risks. In some examples, an RNA (e.g., mRNA) composition as described herein is administered by a healthcare professional who has agreed to submit an adverse event report for any serious adverse events, which may include for example one or more of death, development of a disability or congenital anomaly/birth defect (e.g., in a child of the subject), in-patient hospitalization (including prolongation of an existing hospitalization), a life-threatening event, a medical or surgical intervention to prevent death, a persistent or significant or substantial disruption of the ability to conduct normal life functions; or another important medical event that may jeopardize the individual and may require medical or surgical intervention (treatment) to prevent one of the other outcomes.

In some examples, provided RNA compositions are administered to a population of individuals under 18 years of age, or under 17 years of age, or under 16 years of age, or under 15 years of age, or under 14 years of age, or under 13 years of age, for example according to a regimen established to have a rate of incidence for one or more of the local reaction events indicated below that does not exceed the rate of incidence indicated below:

- pain at the injection site (75% after a first dose and/or a second dose, and/or a lower incidence after a second dose, e.g., 65% after a second dose);
- redness at the injection site (less than 5% after a first dose and/or a second dose); and/or

- swelling at the injection site (less than 5% after a first dose and/or a second dose).

In some examples, provided RNA compositions are administered to a population of individuals under 18 years of age, or under 17 years of age, or under 16 years of age, or under 15 years of age, or under 14 years of age, or under 13 years of age, for example according to a regimen established to have a rate of incidence for one or more of the systemic reaction events indicated below that does not exceed the rate of incidence indicated below:

- fatigue (55% after a first dose and/or a second dose);
- headache (50% after a first dose and/or a second dose);
- muscle pain (40% after a first dose and/or a second dose);
- chills (40% after a first dose and/or a second dose);
- joint pain (20% after a first dose and/or a second dose);
- fever (25% after a first dose and/or a second dose);
- vomiting (10% after a first dose and/or a second dose); and/or
- diarrhea (10% after a first dose and/or a second dose).

In some examples, medication that alleviates one or more symptoms of one or more local reaction and/or systemic reaction events (e.g., described herein) are administered to individuals under 18 years of age, or under 17 years of age, or under 16 years of age, or under 15 years of age, or under 14 years of age, or under 13 years of age who have been administered with provided RNA compositions and have experienced one or more of the local and/or systemic reaction events (e.g., described herein). In some examples, antipyretic and/or pain medication can be administered to such individuals.

In a further aspect, the present disclosure provides a container system comprising: a) a primary container; b) a payload container that is configured to receive at least one tray; and c) a dry ice container; wherein the at least one tray has dimensions A x B x H, where A is 228 to 233 mm, B is 228 to 233 mm, and H is 38 to 46 mm. In some examples, the present disclosure provides a kit and/or container system comprising: a) a primary container; b) a payload container; c) at least one tray for placement within the payload container, wherein the at least one tray contains a temperature-sensitive material; and d) a dry ice container; wherein the at least one tray has dimensions A x B x H, where A is about 228 to about 233

mm, B is about 228 to about 233 mm, and H is about 38 to

about 46 mm. For example, the A dimension can be about 228 mm, 229 mm, 230 mm, 231 mm, 232 mm, or about 233 mm; the B dimension can be about 228 mm, 229 mm, 230 mm, 231 mm, 232 mm, or about 233 mm; and the H dimension can be about 38 mm, 39 mm, 40 mm, 41 mm, 42 mm, 43 mm, 44 mm, 45 mm, or about 46 mm. Further, for example, the payload container in such a kit can have dimensions such as 229 ± 10 mm x 229 ± 10 mm x 229 ± 10 mm.

Further, for example, the primary container (or thermal shipper) can have internal dimensions of about 200mm to about 300mm X about 200mm to about 300mm X about 200mm to about 300mm; and external dimensions of about 300 mm to about 500 mm X about 300mm to about 500mm X about 350mm to about 700mm. For example, the primary container can have internal dimensions of A x B x C, wherein A and B are each independently about 200mm, 220mm, 230mm, 240mm, 245 mm, 255mm, 260mm, 265mm, 270mm, 280mm, 290mm, or about 300mm; and wherein C is independently about 200mm, 220mm, 230mm, 235 mm, 237mm, 238 mm, 239 mm, 240mm, 241mm, 242mm, 243 mm, 244mm, 245 mm, 255mm, 260mm, 265mm, 270mm, 280mm, 290mm, or about 300mm. Further, for example, the primary container can have external dimensions of A x B x C, wherein A and B are each independently about 300mm, 320mm, 340mm, 360mm, 380mm, 390mm, 395mm, 400mm, 405mm, 410mm, 420mm, 440mm, 460mm, 480mm or about 500mm; and wherein C is independently about 350mm, 370mm, 390mm, 410mm, 430mm, 450mm, 470mm, 490mm, 510mm, 520mm, 530mm, 540mm, 550mm, 555mm, 560mm, 565mm, 570mm, 575mm, 580mm, 600mm, 620mm, 640mm, 660mm, 680mm, or about 700 mm.

Further, for example, the kits and/or container systems disclosed herein are capable of maintaining the temperature of the material within the tray, and/or the interior of the payload container, at −10°C or lower, −20°C or lower, −30°C or lower, −40°C or lower, −50°C or lower, −60°C or lower, −70°C or lower, −80°C or lower, or −90°C or lower for at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or at least 10 days. In a further example, the kits and/or container systems can

further comprise a temperature monitoring system. For example, the temperature monitoring system can comprise a temperature sensor and a display, wherein the temperature monitoring system displays or warns when the temperature of the material, or the temperature of a specific region within the container system attains a temperature greater than a specific threshold temperature. For example, such threshold temperature can be about −10°C, −20°C, −30°C, −40°C, −50°C, −60°C, −70°C, −80°C, or about −90°C.

Further, for example, the kits and/or container systems disclosed herein can have the payload container placed at the bottom of the primary container, and further wherein the dry ice container is placed on top (or on bottom) of the payload container.

Further, for example, the kits and/or container systems disclosed herein can have the at least one tray placed inside the payload container. For example, there can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more trays within the payload container.

Further, for example, the temperature-sensitive material can be contained within at least one glass vial, wherein the at least one glass vial is placed within the tray. The temperature-sensitive material can also be contained within a specimen tube, a bag, or a syringe. Such vials, syringes, tubes, and/or bags can be single-dose, or multi-dose.

Further, for example, the trays described herein can each contain any number of vials, such as 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 125, 150, 175, 185, 195, 200 or more.

In yet another aspect, further disclosed herein is a method of transporting a temperature-sensitive material comprising the steps of: a) placing the material in a kit or container system according as disclosed herein; and b) transporting the kit or container system to an intended destination. In some examples, the temperature inside the payload container and/or its location is continuously monitored throughout the duration of the transportation. In some examples, the transportation is carried out on land, air, and/or water. In some examples, the transportation is carried out via land vehicle (such as delivery truck or van), airplane (or other modes of air transportation such as drone or helicopter), and/or boat. In further examples,

the temperature inside the payload container is maintained at −10°C or lower, −20°C or lower, −30°C or lower, −40°C or lower, −50°C or lower, −60°C or lower, −70°C or lower, −80°C or lower, or −90°C or lower throughout the duration of the transportation. In further examples, there are at least 10, 15, 20, 30, 40, 50, 60, 70, 80, 90, 100, 125, 150, 175, 185, 195, 200 or more vials in each tray through the transportation. In further examples, there are at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or at least 10 trays within the payload container throughout the transportation. In further examples, the location of the kit or container system is periodically or continuously monitored through use of a global positioning system (GPS).

In another aspect, the present dislosure relates to a payload container having dimensions of about 229 mm x 229 mm x 229 mm, and is configured to receive at least five trays within the payload container, and wherein each tray is configured to hold at least 100 vials of temperature-sensitive material. In yet another aspect, the present disclosure provides a payload container having dimensions of about 229 mm x 229 mm x 229 mm, wherein at least 5 trays are placed within the payload container, and wherein each tray contains at least 100 vials of temperature-sensitive material. Further disclosed herein is a payload container having dimensions A x B x C, wherein each of the A, B, and C dimensions can independently be about 225 mm, 226 mm, 227 mm, 228 mm, 229 mm, 230 mm, 231 mm or about 232 mm. Further, for example, at least 1, 2, 3, 4, 5, 6, 7, 8, 9, or at least 10 trays are placed within the payload container, wherein each tray contains at least 50, 75, 100, 125, 150, 160, 170, 180, 185, 190, 195, or at least 200 vials of temperature-sensitive material.

In another aspect, the present dislosure relates to a tray configured for carrying temperature-sensitive material, wherein the tray has dimensions A x B x H, where A is 228 to 233 mm, B is 228 to 233 mm, and H is 38 to 46 mm. In another aspect, the present dislosure relates to a tray configured for carrying temperature-sensitive material, wherein the tray has dimensions A x B x H, where A is 228 to 233 mm, B is 228 to 233 mm, and H is 38 to 46 mm, and wherein the tray contains at least 150 vials. Further disclosed herein is a tray for carrying temperature-sensitive material, wherein the tray has dimensions A x B x H, wherein: A is about 227 mm, 228 mm, 229 mm, 230 mm, 231 mm, 232 mm, or about 233 mm; B is about 227 mm, 228 mm, 229 mm, 230 mm, 231 mm, 232 mm, or about 233 mm; and H is about 38 mm, 39

mm, 40 mm, 41 mm, 42 mm, 43 mm, 44 mm, 45 mm, or about 46 mm. Further, for example, a tray contains at least 50, 75, 100, 125, 150, 160, 170, 180, 185, 190, 195, or at least 200 vials of temperature-sensitive material. In some examples, the tray is made of polypropylene (e.g. Akylux®, Biplex®, or equivalent thereof).

In a further example, the kit and/or container system of the present disclosure can be used to store a temperature-sensitive material for up to 10 days if stored at 15°C to 25°C without opening. For example, the temperature-sensitive material can be stored for 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 days under such conditions. In a further example, after the primary container

is opened, it can be replenished with dry ice within 24 hours. For example, replenishment can occur within 1 hour, within 2 hours, within 4 hours, within 8 hours, within 12 hours, within 16 hours, within 20 hours, or within 24 hours of being opened following transportation. Further for example, the amount of dry ice used to replenish the kit or container system can be up to 1 kg, 5kg, 10kg, 15 kg, 20 kg, 21kg, 22 kg, 22 kg, 23 kg, 24 kg, 25 kg or up to 30 kg. Dry ice that can be used includes various sizes, such as 1mm pellets up to 20 mm pellets. The kit or container system can be re-iced, for example, every 1 day, every 2 days, every 3 days, every 4 days, every 5 days, every 6 days, every 7 days, every 8 days, every 9 days, or every 10 days. In a further example, the kit or container system is opened not more than once per day, or not more than twice per day. In a further example, the kit or container system can be closed within 1 minute (or less), within 2 minutes (or less), within 3 minutes (or less), within 4 minutes (or less), or within 5 minutes (or less) after opening. In some examples, the temperature-sensitive material can be stored at about 2°C to about 8°C up to 2 days or at room temperature for no more than 1 hours, or no more than 2 hours after thawing.

Claims:

1. A kit comprising: a) a primary container; b) a payload container; c) at least one tray placed within the payload container; and d) a dry ice container; wherein the at least one tray has dimensions A x B x H, where A is 228 to 233 mm, B is 228 to 233 mm, and H is 38 to 46 mm.

2. The kit according to claim 1, wherein the payload container has the dimensions of about 229 mm x 229 mm x 229 mm.

3. The kit according to any of claims 1-2, wherein the at least one tray contains a temperature-sensitive material.

4. The kit according to claim 3, wherein the kit is capable of maintaining the temperature of the material within the tray at −50°C or lower for at least 10 days.

5. The kit according to any of claims 3-4, wherein the kit is capable of maintaining the temperature of the material within the tray at −70°C or lower for at least 10 days.

6. The kit according to any of claims 3-5, wherein the kit is capable of maintaining the temperature of the material within the tray at −80°C for at least 10 days.

7. The kit according to any of claims 1-6, further comprising a temperature monitoring system.

8. The kit according to any of claims 1-7, further comprising a light sensor.

9. The kit according to any of claims 3-8, wherein the temperature monitoring system comprises a temperature sensor and a display, wherein the temperature monitoring system is capable of displaying or warning when the temperature inside the payload container is greater than about −80° C.

10. The kit according to any of claims 1-9, wherein the primary container comprises a top portion and a bottom portion, and the primary container is configured

to receive the payload container in the bottom portion and to receive the dry ice container in the top portion.

11. The kit according to any of claims 1-10, wherein the payload container is configured to receive the at least one tray.

12. The kit according to any of claims 1-11, wherein the payload container is capable of containing one or more trays within the payload container simultaneously.

13. The kit according to any of claims 1-12, wherein the payload container is capable of containing 1, 2, 3, 4, or 5 trays within the payload container simultaneously.

14. The kit according to any of claim 3-13, wherein the temperature-sensitive material is contained within at least one glass vial, and wherein the at least one glass vial is placed within the tray.

15. The kit according to claim 14, where the at least one glass vial is a multi-dose vial.

16. The kit according to any of claims 1-15, wherein each tray is configured to contain at least 25 vials, or wherein at least one tray contains at least 25 vials.

17. The kit according to any of claims 1-16, wherein each tray is configured to contain at least 50 vials, or wherein at least one tray contains at least 50 vials.

18. The kit according to any of claims 1-17, wherein each tray is configured to contain at least 75 vials, or wherein at least one tray containst at least 75 vials.

19. The kit according to any of claims 1-18, wherein each tray is configured to contain at least 125 vials, or wherein at least one tray contains at least 125 vials.

20. The kit according to any of claims 1-19, wherein each tray is configured to contain at least 150 vials, or wherein at least one tray contains at least 150 vials.

21. The kit according to any of claims 1-20, wherein each tray is configured to contain at least 195 vials, or wherein at least one tray contains at least 195 vials.

22. A container system comprising: a) a primary container; b) a payload container that is configured to receive at least one tray; and c) a dry ice container; wherein the at least one tray has dimensions A x B x H, where A is 228 to 233 mm, B is 228 to 233 mm, and H is 38 to 46 mm.

23. The container system according to claim 19, wherein the payload container has the dimensions A x B x H, where A is 228 to 233 mm, B is 228 to 233 mm, and H is 228 to 233 mm.

24. The container system according to claim 20, wherein the payload container has the dimensions of about 229 mm x 229 mm x 229 mm.

25. The container system according to any of claims 22-24, wherein the at least one tray contains a temperature-sensitive material.

26. The container system according to any of claims 22-25, further comprising a temperature monitoring system.

27. The container system according to any of claims 22-25, further comprising a light sensor.

28. The container system according to any of claims 25-27, wherein the system is capable of maintaining the temperature of the material within the tray at −50°C or lower for at least 10 days.

29. The container system according to any of claims 25-28, wherein the system is capable of maintaining the temperature of the material within the tray at −70°C or lower for at least 10 days.

30. The container system according to any of claims 25-29, wherein the system is capable of maintaining the temperature of the material within the tray at −80°C or lower for at least 10 days.

31. The container system according to any of claims 22-30, wherein the primary container comprises a top portion and a bottom portion, and the primary container is

configured to receive the payload container in the bottom portion and to receive the dry ice container in the top portion.

32. The container system according to any of claims 22-31, wherein the payload container is configured to receive the at least one tray.

33. The container system according to any of claims 22-32, wherein the payload container is capable of containing one or more trays within the payload container simultaneously.

34. The container system according to any of claims 22-33, wherein the payload container is capable of containing 1, 2, 3, 4, or 5 trays within the payload container simultaneously.

35. The container system according to any of claims 25-34, wherein the temperature-sensitive material is contained within at least one glass vial, and wherein the at least one glass vial is placed within the tray.

36. The container system according to claim 35, wherein the at least one glass vial is a multi-dose vial.

37. The container system according to any of claims 22-36, wherein each tray is configured to contain at least 25 vials, or wherein at least one tray contains at least 25 vials.

38. The container system according to any of claims 22-37, wherein each tray is configured to contain at least 100 vials, or wherein at least one tray contains at least 100 vials.

39. The container system according to any of claims 22-38, wherein each tray is configured to contain at least 195 vials, or wherein at least one tray contains at least 195 vials.

40. A method of transporting a temperature-sensitive material comprising the steps of: a) placing the material in a kit according to any of claims 1-21, or in a

container system according to any of claims 23-39; and b) transporting the kit or container system.

41. The method according to claim 40, wherein the temperature inside the payload container is continuously monitored throughout the duration of the transporting .

42. The method according to any of claims 40-41, where the transporting is carried out on land, air, and/or water.

43. The method according to any of claims claim 40-42, where the transporting is carried out via land vehicle, airplane, and/or boat.

44. The method according to any of claims 40-43, wherein the temperature inside the payload container is maintained at −70°C or lower throughout the duration of the transporting.

45. The method according to any of claims 40-44, wherein the temperature inside the payload container is maintained at −80°C or lower throughout the duration of the transporting.

46. The method according to any of claims 40-45, wherein there are at least 150 vials in each tray.

47. The method according to any of claims 40-46, wherein there are 195 vials in each tray.

48. The method according to any of claims 40-47, wherein there are at least 5 trays within the payload container.

49. The method according to any of claims 40-48, wherein the location of the kit or container system is at least periodically monitored through use of a global positioning system (GPS).

50. A payload container having dimensions of about 229 mm x 229 mm x 229 mm, and is configured to receive at least five trays within the payload container, and

wherein each tray is configured to hold at least 100 vials of temperature-sensitive material.

51. A payload container having dimensions of about 229 mm x 229 mm x 229 mm, wherein at least 5 trays are placed within the payload container, and wherein each tray contains at least 100 vials of temperature-sensitive material.

52. The payload container according to any of claims 50-51, wherein each tray contains at least 150 vials of temperature-sensitive material.

53. The payload container according to any of claims 50-52, wherein each tray contains 195 vials of temperature-sensitive material.

54. A tray configured for carrying temperature-sensitive material, wherein the tray has dimensions A x B x H, where A is 228 to 233 mm, B is 228 to 233 mm, and H is 38 to 46 mm.

55. The tray according to claim 54, configured to hold at least 150 vials within the tray.

56. The tray according to any of claims 54-55, configured to hold 195 vials within the tray.

57. A tray configured for carrying temperature-sensitive material, wherein the tray has dimensions A x B x H, where A is 228 to 233 mm, B is 228 to 233 mm, and H is 38 to 46 mm, and wherein the tray contains at least 150 vials.

58. The tray according to claim 57, wherein the tray contains 195 vials.