

VIRUS-LIKE PARTICLES FOR THE TREATMENT OF SARS-COV2

RELATED APPLICATION DATA

The present application claims priority from United States Patent Application No. 63/508,537 filed 16 June 2023 entitled "Virus-Like Particles for the Treatment of SARS-CoV2", the entire contents of which is hereby incorporated by reference.

SEQUENCE LISTING

The present application is filed with a Sequence Listing in electronic form. The entire contents of the Sequence Listing are hereby incorporated by reference.

FIELD

The present disclosure relates to a virus-like particle (VLP) comprising one or more antigens for use as a vaccine. The present disclosure further relates to uses of the vaccine for the treatment of a SARS-CoV-2 infection or coronavirus disease 2019 (COVID-19).

BACKGROUND

Respiratory viral infections are a significant threat to human health and lives. Infections such as those caused by the influenza virus and severe acute respiratory syndrome coronavirus (SARS-CoV) have been known to cause global pandemics, killing millions of people worldwide. More recently, SARS-CoV-2 has been responsible for causing the worldwide pandemic of the severely infectious coronavirus disease 2019 (COVID-19).

SARS-CoV-2, belonging to the family *Coronaviridae* and the subfamily *orthocoronaviruses*, is an enveloped single-stranded positive-strand RNA virus which encodes non-structural proteins that play roles in viral replication and translation, and structural proteins including the spike protein (S protein), membrane protein (M protein), envelope protein (E protein) and nucleocapsid protein (N protein). The S protein is a transmembrane glycoprotein forming prominent homotrimers on the surface of the virus, consisting of two functional subunits S1 and S2 which have become the main targets for current genetic engineering vaccine development.

SARS-CoV-2 has a high propensity to mutate, with a significant number of mutant strains now identified globally. The predominant mutant strains include: the Alpha (B.1.1.7) mutant, the Beta (B.1.351) mutant, the Gamma (P1) mutant, the Epsilon (B.1.429) mutant, the Delta (B.1.617.2) mutant, the Kappa (B.1.617.1) mutant and the Omicron (B.1.1.529) mutant, which vary in their transmissibility, pathogenicity, and/or immune escape capabilities. Typically, these mutant strains are identified based on the number and location of mutations in the viral genome, specifically in the genome encoding the S protein of the mutant SARS-CoV-2.

The Omicron strain contains up to 36 amino acid mutation sites and this strain in particular has been shown to have an improved affinity for the ACE2 target receptor, thereby enhancing its toxicity and infectivity, and accelerating escape of the virus. There are currently few vaccines available that target specific strains of SARS-CoV-2 such as the Omicron strain. Currently available vaccines, such as those developed against the Wuhan (original) strain, which are not specifically targeted to evolving mutant strains, are known to provide for a reduced protective effect and/or immunogenicity.

There is therefore a need for the development of new vaccines that can specifically target SARS-CoV-2 strains, such as the omicron strain of SARS-CoV-2.

SUMMARY

The present disclosure is based on the inventors' identification of virus-like particles (VLPs) comprising one or more antigens from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) virus that are suitable for the treatment of a SARS-CoV-2 infection or coronavirus disease 2019 (COVID-19). The findings by the inventors provide basis for a VLP comprising one or more antigens from a SARS-CoV-2 that are suitable for the treatment of specific strains or variants of SARS-CoV-2, including variants of the omicron strain of SARS-CoV-2. Furthermore, the findings by the inventors provide basis for methods of treating or preventing or delaying progression of a disease or disorder such as a SARS-CoV-2 infection or COVID-19, as well as complications thereof including pneumonia, sepsis and acute respiratory distress syndrome (ARDS)) in a subject.

Accordingly, the present disclosure provides a recombinant virus-like particle (VLP) comprising one or more antigens from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), wherein the one or more antigens are selected from:

- (a) a spike (S) protein;
- (b) a membrane (M) protein;
- (c) an envelope (E) protein;
- (d) a nucleocapsid (N) protein,

wherein at least one antigen is from the omicron strain of SARS-CoV-2.

When discussing an "omicron strain" of SARS-COV-2 herein, the term will be understood to mean variants and sub-lineages arising from an omicron strain of SARS-COV-2. For example, an omicron strain of SARS-COV-2 includes BA.1 strains, BA.2 strains, XB strains, XBB strains, JN.1 strains, JN.2 strains, JN.3 strains, KP.1 strains, KP.2 strains.

In one example, each of the antigens are from the omicron strain of a SARS-Cov2. In one example, the VLP comprises an antigen from the S protein of the omicron strain of a SARS-CoV-2. In one example, the VLP comprises an S protein from an omicron variant of a SARS-CoV-2 selected from the group consisting of B.1.1.529, BA.1, BA.2, BA.4, BA.5, BA.2.12.1 and BA.2.75. In one example, the VLP comprises an S protein from an omicron variant of a SARS-

CoV-2 selected from the group consisting of B.1.1.529, a BA.1 strain, a BA.2 strain, a BA.4 strain, a BA.5 strain, BA.2.12.1, BA.2.75, a XB strain, a XBB strain, a JN.1 strain, a JN.2 strain, a JN.3 strain, a KP.1 strain and a KP.2 strain.

In one example, at least one antigen is an S protein from omicron variant BA.1 of a SARS-CoV-2 and the S protein comprises one or more or all of the mutations selected from the group consisting of A67V, T95I, Y145D, L212L, S371L, G446S, G496S, T547K, N856K, L981F, G142D, Q493R, G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K and del69-70.

In one example, at least one antigen is an S protein from omicron variant BA.2 of a SARS-CoV-2 and the S protein comprises one or more or all of the mutations selected from the group consisting of G142D, Q493R, del24-26, G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K, T19I, A27S, G142D, V213G, S371F, T376A, D405N and R408S.

In one example, at least one antigen is an S protein from omicron variant BA.4 or BA.5 of a SARS-CoV-2 and the S protein comprises one or more or all of the mutations selected from the group consisting of L452R, F486V, R493Q, del24-26, del69-70, G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K, T19I, A27S, G142D, V213G, S371F, T376A, D405N and R408S.

In one example, at least one antigen is an S protein from an omicron variant of a SARS-CoV-2 and the S protein comprises one or more or all of the mutations selected from the group consisting of T19I, Δ24-26, A27S, Δ144, G142D, G339H, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, N460K, S477N, T478K, F486P, Q498R, N501Y, Y505H, D614G, H655Y, N679K, N764K, D796Y, Q954H and N969K.

In one example, at least one antigen is an S protein from an omicron variant of a SARS-CoV-2 and the S protein comprises one or more or all of the mutations selected from the group consisting of T19I, Δ24-26, A27S, V83A, Δ144, G142D, H146Q, Q183E, V213E, G252V, G339H, R346T, L368I, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, N460K, S477N, T478K, E484A, F486P, F490S, Q498R, N501Y, Y505H, D614G, H655Y, N679K, N764K, D796Y, Q954H and N969K.

In one example, at least one antigen is an S protein from an omicron variant of a SARS-CoV-2 and the S protein comprises one or more or all of the mutations selected from the group consisting of 16insMPLF, T19I, R21T, Δ24-26, A27S, S50L, Δ69-70, V127F, Δ144, G142D, F157S, R158G, Δ211, L212I, V213G, H245N, A264D, I332V, G339H, K356T, S371F, S373P, S375F, T376A, R403K, D405N, R408S, K417N, N440K, V445P, G446S, N450D, L452W, N460K, S477N, T478K, N481K, Δ483, E484K, F486P, Q498R, N501Y, Y505H, E554K, A570V, D614G, P621S, H655Y, N679K, P681R, N764K, D796Y, Q954H, N969K and P1143L.

In one example, at least one antigen is an S protein from an omicron variant of a SARS-CoV-2 and the S protein comprises one or more or all of the mutations selected from the group consisting of T19I, Δ24-26, A27S, Δ144, G142D, G339H, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, L455S, N460K, S477N, T478K, F486P, Q498R, N501Y, Y505H, D614G, H655Y, N679K, N764K, D796Y, Q954H and N969K.

In one example, the at least one antigen is an S protein comprising one or more or all of the mutations R346T, F456L and T572I.

In one example, the VLP comprises an antigen from each of the S protein, M protein, and E protein of SARS-CoV-2. In another example, the VLP comprises an antigen from each of the S protein and M protein of a SARS-CoV-2. In another example, the VLP comprises an antigen from each of the S protein and E protein of a SARS-CoV-2. In another example, the VLP comprises an antigen from each of the E protein and M protein of a SARS-CoV-2.

In one example, the VLP comprises an antigen from each of the S protein, M protein, and E protein of the omicron strain of a SARS-CoV-2. In another example, the VLP comprises an antigen from each of the S protein and M protein of the omicron strain of a SARS-CoV-2. In another example, the VLP comprises an antigen from each of the S protein and E protein of the omicron strain of a SARS-CoV-2. In another example, the VLP comprises an antigen from each of the E protein and M protein of the omicron strain of a SARS-CoV-2. In an example, the omicron variant may be BA.1 or BA.2.

In an example, at least one of the antigens may be from the delta, beta, alpha, gamma or strain of a SARS-CoV-2 or from the SARS-CoV-2 strain 2019-nCoV/USA-WA1/2020. In another example, two of the antigens are from the delta, beta, alpha or gamma strain of a SARS-CoV-2 or from the SARS-CoV-2 strain 2019-nCoV/USA-WA1/2020. In another example, three of the antigens are from the delta, beta, alpha or gamma strain of a SARS-CoV-2 or from the SARS-CoV-2 strain 2019-nCoV/USA-WA1/2020. In another example, none of the antigens are from the delta, beta, alpha or gamma strain of a SARS-CoV-2 or from the SARS-CoV-2 strain 2019-nCoV/USA-WA1/2020. Thus, in an example, each of the S, M and E antigens are from the omicron strain of SARS-Cov2.

In one example, the VLP comprises an antigen from each of the S protein, M protein, and E protein of the omicron strain of a SARS-CoV-2, wherein:

(a) the omicron variant is BA.1 and the S protein comprises one or more or all of the mutations selected from the group consisting of A67V, T95I, Y145D, L212L, S371L, G446S, G496S, T547K, N856K, L981F, G142D, Q493R, G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K and del69-70; or

(b) the omicron variant is BA.2 and the S protein comprises one or more or all of the mutations selected from the group consisting of G142D, Q493R, del24-26, G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y,

N679K, P681H, N764K, D796Y, Q954H, N969K, T19I, A27S, G142D, V213G, S371F, T376A, D405N and R408S;

(c) the omicron variant is BA.4 or BA.5 and the S protein comprises one or more or all of the mutations selected from the group consisting of L452R, F486V, R493Q, del24-26, del69-70, G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K, T19I, A27S, G142D, V213G, S371F, T376A, D405N and R408S;

(d) the S protein from an omicron variant of a SARS-CoV-2 comprises one or more or all of the mutations selected from the group consisting of T19I, Δ 24-26, A27S, Δ 144, G142D, G339H, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, N460K, S477N, T478K, F486P, Q498R, N501Y, Y505H, D614G, H655Y, N679K, N764K, D796Y, Q954H and N969K;

(e) the S protein from an omicron variant of a SARS-CoV-2 comprises one or more or all of the mutations selected from the group consisting of T19I, Δ 24-26, A27S, V83A, Δ 144, G142D, H146Q, Q183E, V213E, G252V, G339H, R346T, L368I, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, N460K, S477N, T478K, E484A, F486P, F490S, Q498R, N501Y, Y505H, D614G, H655Y, N679K, N764K, D796Y, Q954H and N969K;

(f) the S protein from an omicron variant of a SARS-CoV-2 comprises one or more or all of the mutations selected from the group consisting of 16insMPLF, T19I, R21T, Δ 24-26, A27S, S50L, Δ 69-70, V127F, Δ 144, G142D, F157S, R158G, Δ 211, L212I, V213G, H245N, A264D, I332V, G339H, K356T, S371F, S373P, S375F, T376A, R403K, D405N, R408S, K417N, N440K, V445P, G446S, N450D, L452W, N460K, S477N, T478K, N481K, Δ 483, E484K, F486P, Q498R, N501Y, Y505H, E554K, A570V, D614G, P621S, H655Y, N679K, P681R, N764K, D796Y, Q954H, N969K and P1143L;

(g) the S protein from an omicron variant of a SARS-CoV-2 comprises one or more or all of the mutations selected from the group consisting of T19I, Δ 24-26, A27S, Δ 144, G142D, G339H, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, L455S, N460K, S477N, T478K, F486P, Q498R, N501Y, Y505H, D614G, H655Y, N679K, N764K, D796Y, Q954H and N969K; or

(h) the S protein comprises one or more or all of the mutations R346T, F456L and T572I.

In one example, where the VLP comprises more than one antigen, the antigens are provided as a fusion polypeptide. For example, the VLP comprises each of the S protein, M protein and E protein, provided as a fusion polypeptide.

In one example, each of the antigens are formulated in separate VLPs or the same VLP. For example, each of the S protein, E protein and M protein may be formulated in the same VLP. In another example, each of the S protein, E protein and M protein may be formulated in separate VLPs. In yet another example, the S and E protein may be formulated in the same VLP and the

M protein may be formulated in a separate VLP. In another example, the S and M protein may be formulated in the same VLP and the E protein may be formulated in a separate VLP.

In one example, at least one of the antigens is from the delta, beta, alpha or gamma strain of SARS-CoV-2 or from the SARS-CoV-2 strain 2019-nCoV/USA-WA1/2020. For example, the S protein antigen is from the delta strain of SARS-CoV-2 and the E and M protein antigens are from the omicron strain of SARS-CoV-2. For example, the M protein antigen is from the delta strain of SARS-CoV-2 and the E and S protein antigens are from the omicron strain of SARS-CoV-2. In another example, two of the antigens are from the delta, beta, alpha or gamma strain of SARS-CoV-2 or from the SARS-CoV-2 strain 2019-nCoV/USA-WA1/2020. For example, the S protein and the M protein antigens are from the delta strain of SARS-CoV-2 and the E protein antigen is from the omicron strain of SARS-CoV-2. For example, the M protein and E protein antigens are from the delta strain of SARS-CoV-2 and the S protein antigen is from the omicron strain of SARS-CoV-2.

In an example, the VLP further comprises an antigen from the N protein of SARS-CoV-2.

In an example, one or more antigens is an immunogenic fragments of any of the proteins described herein.

In one example, the VLP comprises an antigen from each of the S protein, M protein, E protein and N protein of the omicron strain of SARS-CoV-2. In another example, the VLP comprises an antigen from each of the S protein, M protein and N protein of the omicron strain of SARS-CoV-2. In another example, the VLP comprises an antigen from each of the S protein, E protein and N protein of the omicron strain of SARS-CoV-2. In another example, the VLP comprises an antigen from each of the E protein, M protein and N protein of the omicron strain of SARS-CoV-2. In an example, the omicron variant may be BA.1 or BA.2.

In an example, at least one of the antigens may be from the delta, beta, alpha or gamma strain of SARS-CoV-2 or from the SARS-CoV-2 strain 2019-nCoV/USA-WA1/2020. For example, the S protein antigen is from the delta strain of SARS-CoV-2 and the E, N and M protein antigens are from the omicron strain of SARS-CoV-2. For example, the M protein antigen is from the 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2 and the E, N and S protein antigens are from the omicron strain of SARS-CoV-2. In another example, two of the antigens are from the delta, beta, alpha or gamma strain of SARS-CoV-2 or from the SARS-CoV-2 strain 2019-nCoV/USA-WA1/2020. For example, the S and M protein antigens are from the delta strain of SARS-CoV-2 and the E and N protein antigens are from the omicron strain of SARS-CoV-2. For example, the M and E protein antigens are from the 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2 and the N and S protein antigens are from the omicron strain of SARS-CoV-2. In another example, three of the antigens are from the delta, beta, alpha or gamma strain of SARS-CoV-2 or from the SARS-CoV-2 strain 2019-nCoV/USA-WA1/2020. For example, the S protein, M protein and E protein antigens are from the delta strain of SARS-CoV-2 and the N protein is from

the omicron strain of SARS-CoV-2. For example, the N protein, M protein and E protein antigens are from the 2019-nCoV/USA-WA1/2020 strain, delta strain and beta strain respectively, of SARS-CoV-2 and the S protein is from the omicron strain of SARS-CoV-2. In another example, none of the antigens are from the delta, beta, alpha or gamma strain of SARS-CoV-2 or from the SARS-CoV-2 strain 2019-nCoV/USA-WA1/2020. In another example, none of the antigens are from the delta, beta, alpha or gamma strain of SARS-CoV-2 or from the SARS-CoV-2 strain 2019-nCoV/USA-WA1/2020. Thus, in an example, all of the antigens are from the omicron strain of SARS-CoV-2

In one example, the VLP comprises an antigen from each of the S protein, M protein, E protein and N protein of the omicron strain of SARS-CoV-2, wherein:

(a) the omicron variant is BA.1 and the S protein comprises one or more or all of the mutations selected from the group consisting of A67V, T95I, Y145D, L212L, S371L, G446S, G496S, T547K, N856K, L981F, G142D, Q493R, G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K and del69-70; or

(b) the omicron variant is BA.2 and the S protein comprises one or more or all of the mutations selected from the group consisting of G142D, Q493R, del24-26, G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K, T19I, A27S, G142D, V213G, S371F, T376A, D405N and R408S; or

(c) the omicron variant is BA.4 or BA.5 and the S protein comprises one or more or all of the mutations selected from the group consisting of L452R, F486V, R493Q, del24-26, del69-70, G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K, T19I, A27S, G142D, V213G, S371F, T376A, D405N and R408S;

(d) the S protein from an omicron variant of a SARS-CoV-2 comprises one or more or all of the mutations selected from the group consisting of T19I, Δ24-26, A27S, Δ144, G142D, G339H, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, N460K, S477N, T478K, F486P, Q498R, N501Y, Y505H, D614G, H655Y, N679K, N764K, D796Y, Q954H and N969K;

(e) the S protein from an omicron variant of a SARS-CoV-2 comprises one or more or all of the mutations selected from the group consisting of T19I, Δ24-26, A27S, V83A, Δ144, G142D, H146Q, Q183E, V213E, G252V, G339H, R346T, L368I, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, N460K, S477N, T478K, E484A, F486P, F490S, Q498R, N501Y, Y505H, D614G, H655Y, N679K, N764K, D796Y, Q954H and N969K;

(f) the S protein from an omicron variant of a SARS-CoV-2 comprises one or more or all of the mutations selected from the group consisting of 16insMPLF, T19I, R21T, Δ24-26, A27S, S50L, Δ69-70, V127F, Δ144, G142D, F157S, R158G, Δ211, L212I, V213G, H245N, A264D,

I332V, G339H, K356T, S371F, S373P, S375F, T376A, R403K, D405N, R408S, K417N, N440K, V445P, G446S, N450D, L452W, N460K, S477N, T478K, N481K, Δ483, E484K, F486P, Q498R, N501Y, Y505H, E554K, A570V, D614G, P621S, H655Y, N679K, P681R, N764K, D796Y, Q954H, N969K and P1143L;

(g) the S protein from an omicron variant of a SARS-CoV-2 comprises one or more or all of the mutations selected from the group consisting of T19I, Δ24-26, A27S, Δ144, G142D, G339H, S371F, S373P, S375F, T376A, D405N, R408S, K417N, N440K, V445P, G446S, L455S, N460K, S477N, T478K, F486P, Q498R, N501Y, Y505H, D614G, H655Y, N679K, N764K, D796Y, Q954H and N969K; or

(h) the S protein comprises one or more or all of the mutations R346T, F456L and T572I.

In an example, each of the S protein, E protein, M protein and N protein are provided as a fusion polypeptide. In another example, each of the antigens are formulated in separate VLPs or the same VLP. For example, each of the S protein, E protein, M protein and N protein may be formulated in the same VLP. In another example, each of the S protein, E protein, M protein and N protein may be formulated in separate VLPs. For example, the S protein and E protein may be formulated in the same VLP and the M protein and N protein may be formulated in a separate VLP. In another example, the S protein, M protein and E protein may be formulated in the same VLP and the N protein may be formulated in a separate VLP.

In an example, the VLP has a diameter of between about 70nm and 160nm, between about 70nm and 150nm, between about 70nm and 140nm, between about 70nm and 130nm, between about 70nm and 120nm, between about 70nm and 110nm, between about 70nm and 100nm, or between about 70nm and 90nm. In another example, the VLP has a diameter of about 80nm.

In one example, the VLP is formulated in a lipid nanoparticle (LNP). For example, the VLP is encapsulated in a LNP. In another example, the VLP is bound to the LNP. In another example, the VLP is absorbed on the LNP.

In one example, the LNP further comprises a PEG-lipid, a structural lipid and/or a neutral lipid. For example, the LNP further comprises a PEG-lipid. In another example, the LNP further comprises a structural lipid. In another example, the LNP further comprises a neutral lipid.

In one example, the LNP comprises an ionisable lipid. For example, the ionisable lipid is a cationic lipid. In another example, the ionisable lipid is a zwitterionic lipid.

In one example, the LNP does not comprise an ionisable lipid.

In one example, each VLP is formulated together in a LNP. For example, a composition comprising each of the S protein, E protein and M protein antigens are formulated together in the LNP. In another example, a composition comprising each of the each of the S protein, E protein, M protein and N protein antigens are formulated together in the LNP.

In one example, each VLP is formulated separately in the LNP. For example, where the composition comprises an S protein, E protein and M protein, each of the S protein, E protein and M protein are formulated in separate LNPs. In another example, where the composition

comprises an S protein, E protein, M protein and N protein, each of the S protein, E protein, M protein and N protein are formulated in separate LNPs. In another example, where the composition comprises an S protein, E protein, M protein and N protein, each of the S protein, E protein, M protein are formulated in the same LNP and the N protein is formulated in a separate LNP. In yet another example, where the composition comprises an S protein, E protein, M protein and N protein, each of the S protein and E protein are formulated in the same LNP and the M protein and N protein are formulated in a separate LNP. In another example, where the composition comprises an S protein, E protein, M protein and N protein, each of the E protein, M protein and N protein are formulated in the same LNP and the S protein is formulated in a separate LNP.

In another example, where the composition comprises an S protein, E protein and M protein, each of the S protein and E protein are formulated in the same LNP and the M protein is formulated in a separate LNP. In yet another example, where the composition comprises an S protein, E protein and M protein, each of the S protein and M protein are formulated in the same LNP and the E protein is formulated in a separate LNP. In another example, where the composition comprises an S protein, E protein and M protein, each of the E protein and M protein are formulated in the same LNP and the S protein is formulated in a separate LNP.

In one example, the present disclosure further provides an isolated, recombinant or synthetic nucleotide sequence encoding a VLP disclosed herein.

In another example, the present disclosure further provides an expression vector comprising a nucleotide sequence that encodes a VLP disclosed herein.

In one example, the present disclosure further provides a pharmaceutical composition comprising a VLP disclosed herein and a pharmaceutically acceptable carrier. In one example, the pharmaceutical composition is an immunogenic composition.

In another example, the present disclosure further provides a pharmaceutical composition comprising a VLP disclosed herein for use as a vaccine. In another example, present disclosure further provides a vaccine comprising the pharmaceutical or immunogenic composition.

In an example, the composition further comprises an adjuvant. In an example, the adjuvant is selected from the group consisting of Freund's adjuvant, incomplete Freund's adjuvants, aluminum phosphate, aluminum hydroxide, GMCSP, BCG, MDP compounds, such as thur-MDP and nor-MDP, CGP (MTP-PE), lipid A, monophosphoryl lipid A (MPL), RIBI, MPL, trehalose dimycolate (TDM), Novasomes®, QS21, Quil A (and derivatives and components thereof), calcium phosphate, calcium hydroxide, zinc hydroxide, MHC antigens, PolyI:C, MF59, glycolipid analogs, octadecyl esters of an amino acid, muramyl dipeptides, polyphosphazene, lipoproteins, ISCOM matrix, DC-Chol, ODA, cytokines, and other adjuvants and derivatives thereof. In an example, the adjuvant is MF59.

In an example, the effect of a composition comprising a VLP described herein, on treating, preventing or delaying progression of a SARS-CoV-2 infection or COVID-19, is enhanced in the

presence of an adjuvant such as MF59, when compared to the effect of a composition comprising a VLP described herein without an adjuvant.

In an example, the effect of a composition comprising a VLP described herein, on treating, preventing or delaying progression of pneumonia, sepsis or acute respiratory distress syndrome in a subject having COVID-19 is enhanced in the presence of an adjuvant such as MF59, when compared to the effect of a composition comprising a VLP described herein without an adjuvant.

In an example, the effect of a composition comprising a VLP described herein, on inducing an immune response in a subject is enhanced in the presence of an adjuvant such as MF59, when compared to the effect of a composition comprising a VLP described herein without an adjuvant.

In an example, the presence of an adjuvant in a composition comprising a VLP described herein enhances the induction of the immune response by the composition by at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, at least about 100%, at least about 200%, at least about 300%, at least about 400%, at least about 500% or more when compared to the effect of a composition comprising a VLP described herein without an adjuvant.

In an example, the adjuvant such as MF59 is administered at the same time as the administration of a composition of the disclosure. In another example, the adjuvant such as MF59 is administered sequentially to, preceding, or proceeding the administration of a composition of the disclosure.

In an example, the present disclosure provides a method of treating or preventing or delaying progression of a SARS-CoV-2 infection in a subject in need thereof, the method comprising administering a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein to the subject.

In an example, the present disclosure provides use of a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein in the manufacture of a medicament for treating or preventing or delaying progression of a SARS-CoV-2 infection in a subject.

In an example, the present disclosure provides a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein for use in the treatment or prevention or delaying progression of a SARS-CoV-2 infection in a subject.

In an example, the subject having a SARS-CoV-2 infection has at least one symptom of COVID-19. In an example, one such symptom of COVID-19 includes runny nose, cough, sore throat, fever, headache, muscle pain or fatigue. In another example, the at least one symptom is any one of the mild-COVID-19 symptoms described herein or known in the art. In another

example, the at least one symptom is any one of the moderate to severe COVID-19 symptoms described herein or known in the art.

Thus, in another example, the present disclosure provides a method of treating or preventing or delaying progression of COVID-19 in a subject in need thereof, the method comprising administering a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein to the subject.

In an example, the present disclosure provides use of a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein in the manufacture of a medicament for treating or preventing or delaying progression of COVID-19 in a subject.

In an example, the present disclosure provides a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein for use in the treatment or prevention or delaying progression of COVID-19 in a subject.

In an example, the subject in need is suspected of having, or has been diagnosed as having COVID-19. In an example, the subject in need is suspected of having, or has been diagnosed as having mild COVID-19. In an example, the subject in need is suspected of having, or has been diagnosed as having moderate COVID-19. In an example, the subject in need is suspected of having, or has been diagnosed as having severe COVID-19.

In an example, a method or use described herein further comprises a step of identifying a subject having or suspected of having mild COVID-19 based on a SARS-CoV-2 positive RT-PCR or molecular test result, and one or more of the following symptoms:

- fever;
- sore throat;
- headache;
- muscle pain (myalgia);
- gastrointestinal symptoms;
- cough;
- chest congestion;
- runny nose;
- wheezing;
- skin rash;
- eye irritation or discharge;
- chills;
- new or changing olfactory or taste disorders;
- red or bruised looking feet or toes;
- shaking chills or rigors;
- malaise (loss of appetite, generally unwell, fatigue, physical weakness).

In another example, a method or use described herein further comprises a step of identifying a subject having or suspected of having moderate COVID-19 based on a SARS-CoV-2 positive RT-PCR or molecular test result, and any one of the following new or worsening signs or symptoms:

- respiratory rate $2 \geq 20$ breaths/minute;
- abnormal saturation of oxygen but still $\geq 93\%$ on room air at sea level;
- clinical or radiologic evidence of pneumonia;
- radiologic evidence of DVT;
- shortness of breath or difficulty breathing;

or any two of the following new or worsening signs or symptoms:

- fever;
- heart rate $2 \geq 90$ beats/minute;
- shaking chills or rigors;
- new or changing olfactory or taste disorders;
- sore throat;
- malaise;
- headache;
- cough;
- muscle pain (myalgia);
- gastrointestinal symptoms;
- red or bruised looking feet or toes.

In another example, a method or use described herein further comprises a step of identifying a subject having or suspected of having severe COVID-19 based on a SARS-CoV-2 positive RT-PCR or molecular test result; and any one or more of the following:

- clinical signs at rest indicative of severe systemic illness (respiratory rate $2 \geq 30$ breaths/minute, heart rate $2 \geq 125$ beats/minute, $SpO_2 \leq 93\%$ on room air at sea level, or $PaO_2/FiO_2 \leq 300$ mmHg);
- respiratory failure (defined as needing high-flow oxygen, non-invasive ventilation, mechanical ventilation, or extracorporeal membrane oxygenation)
- evidence of shock (defined as systolic blood pressure ≤ 90 mmHg, diastolic blood pressure < 60 mmHg, or requiring vasopressors);
- significant acute renal, hepatic, or neurologic dysfunction;
- admission to the intensive care unit;
- death.

In an example, the present disclosure provides a method of inducing an immune response in a subject, the method comprising administering a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein to the subject in need thereof.

In an example, the present disclosure provides use of a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein in the manufacture of a medicament for inducing an immune response in a subject in need thereof.

In an example, the present disclosure provides a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein for use in inducing an immune response in a subject in need thereof.

In one example, the composition induces a humoral immune response in the subject. For example, the humoral immune response is an antibody-mediated immune response. For example, production of neutralizing antibodies. In another example, the composition induces a cell-mediated immune response. For example, the cell-mediated immune response includes activation of antigen-specific cytotoxic T cells. For example, the T cells are CD4 T cells and/or CD8 T cells. In one example, the T cells are CD4 T cells. In another example the T cells are CD8 T cells. In a further example, the T cells are CD4 and CD8 T cells.

In one example, administration of a VLP, a pharmaceutical composition, an immunogenic composition or a vaccine of the present disclosure induces a CD4 T cell mediated immune response.

In one example, administration of a VLP, a pharmaceutical composition, an immunogenic composition or a vaccine of the present disclosure induces a CD8 T cell mediated immune response.

In one example, administration of a VLP, the pharmaceutical composition, an immunogenic composition or a vaccine of the present disclosure induces a CD4 and a CD8 T cell mediated immune response.

In one example, the CD4 T cell mediated immune response is a Th0, a Th1 and/or a Th2 response. For example, the CD4 T cell mediated immune response is a Th0 response. In another example, the CD4 T cell mediated immune response is a Th1 response. In a further example, the CD4 T cell mediated immune response is a Th2 response. In one example, the CD4 T cell mediated immune response is a Th0 and Th1 response. In another example, the CD4 T cell mediated immune response is a Th0 and Th2 response. In a further example, the CD4 T cell mediated immune response is a Th1 and Th2 response. In another example, the CD4 T cell mediated immune response is a Th0, Th1 and Th2 response.

In one example, the Th0 response cytokines express interleukin 2 (IL2+) and/or tumor necrosis factor alpha (TNFα+); and/or are negative for interferon gamma (IFNγ-), IL5- and/or IL13-. For example, the cytokine is IL2+. In another example, the cytokine is TNFα+. In one example, the cytokine is IFNγ-. In another example, the cytokine is IL5-. In a further example, the cytokine is IL13-.

In one example, the Th1 response cytokines express interferon gamma (IFN γ); and/or are negative for IL5- and/or IL13-. For example, the cytokine is IFN γ +. In another example, the cytokine is IL5-. In a further example, the cytokine is IL13-.

In one example, the Th2 response cytokines express IL5+ and/or IL13+; and/or are negative for IFN γ . For example, the cytokine is IL5+. In a further example, the cytokine is IL13+. For example, the cytokine is IFN γ -.

In one example, the immune response is raised in response to the at least one antigen from the omicron strain of a SARS-CoV-2. For example, the immune response is raised in response to an S protein antigen from the omicron strain of a SARS-CoV-2 described herein. In one example, the immune response is raised in response to two, three or four antigens from the omicron strain of a SARS-CoV-2. For example, the immune response is raised in response to S protein, N protein, M protein and E protein antigens from the omicron strain of a SARS-CoV-2 described herein.

In another example, the immune response is sufficient to treat, prevent or delay progression of at least one symptom of a SARS-CoV-2 infection caused by the omicron strain of a SARS-CoV-2. In another example, the immune response is sufficient to treat, prevent or delay progression of at least one symptom of a SARS-CoV-2 infection caused by the delta, beta, alpha, gamma and/or 2019-nCoV/USA-WA1/2020 strain of the SARS-CoV-2.

In an example, the present disclosure provides a method for reducing SARS-CoV-2 viral load in a subject with COVID-19 comprising administering a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein to a subject in need thereof.

In an example, the present disclosure provides use of a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein in the preparation of a medicament for reducing SARS-CoV-2 viral load in a subject with COVID-19.

In an example, the present disclosure provides a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein for use in reducing SARS-CoV-2 viral load in a subject with COVID-19.

In an example, COVID-19 has been confirmed by quantitative reverse-transcriptase polymerase chain reaction.

In an example, the present disclosure provides a method for treating, preventing or delaying progression of pneumonia in a subject with COVID-19 comprising administering a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein to the subject in need thereof.

In an example, the present disclosure provides use of a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a

vaccine disclosed herein in the preparation of a medicament for treating, preventing or delaying progression of pneumonia in a subject with COVID-19.

In an example, the present disclosure provides a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein for use in treating, preventing or delaying progression of pneumonia in a subject with COVID-19.

In an example, COVID-19 has been confirmed by quantitative reverse-transcriptase polymerase chain reaction.

In an example, the present disclosure provides a method for treating, preventing or delaying progression of acute respiratory distress syndrome in a subject with COVID-19 comprising administering a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein to the subject in need thereof.

In an example, the present disclosure provides use of a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein in the preparation of a medicament for treating, preventing or delaying progression of acute respiratory distress syndrome in a subject with COVID-19.

In an example, the present disclosure provides a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein for use in treating, preventing or delaying progression of acute respiratory distress syndrome in a subject with COVID-19.

In an example, COVID-19 has been confirmed by quantitative reverse-transcriptase polymerase chain reaction.

In an example, the present disclosure provides a method for treating, preventing or delaying progression of sepsis in a subject with COVID-19 comprising administering a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein to the subject in need thereof.

In an example, the present disclosure provides use of a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein in the preparation of a medicament treating, preventing or delaying progression of sepsis in a subject with COVID-19.

In an example, the present disclosure provides a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein for use in treating, preventing or delaying progression of sepsis in a subject with COVID-19.

In an example, COVID-19 has been confirmed by quantitative reverse-transcriptase polymerase chain reaction.

In an example, the present disclosure provides a method for preventing or decreasing mortality in a subject with COVID-19 comprising administering a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein to a subject in need thereof.

In an example, the present disclosure provides use of a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein in the preparation of a medicament for preventing or decreasing mortality in a subject with COVID-19.

In an example, the present disclosure provides a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein for use in preventing or decreasing mortality in a subject with COVID-19.

In an example, COVID-19 has been confirmed by quantitative reverse-transcriptase polymerase chain reaction.

In an example, COVID-19 has been caused by the omicron strain of the SARS-CoV-2. In another example, COVID-19 has been caused by the delta, beta, alpha or gamma strain 2019-nCoV/USA-WA1/2020 SARS-CoV-2 strain. In another example, COVID-19 has been caused by more than one strain of SARS-CoV-2, for example, the omicron and delta strains.

In an example, the subject is a human of 18 years of age or older. In another example, the subject is a human of any age, e.g., from about 1 month to 100 years old, e.g., from about 2 months to about 80 years old, from about 6 months of age to about 3 years old, from about 3 years to about 18 years old, from about 12 years to about 18 years old, from about 18 years to about 55 years old, from about 50 years to about 75 years old, from about 40 years to about 65 years old. In another example, the subject is a human from 2 years of age. In another example, subject is a human from 18 years of age, a human from 30 years of age, a human from 40 years of age, a human from 50 years of age, a human from 60 years of age, a human from 70 years of age, a human from 80 years of age or a human from about 90 years of age. In another example, the subject is less than 2 years of age, less than 18 months of age, less than 12 months of age, less than 6 months of age or less than 3 months of age.

In an example, a composition or vaccine described herein is administered in a one dose regimen. In another example, the composition is administered in a two, three or four dose regimen. In this example, the doses may be administered about 1, 2 or 3 months apart.

In an example of the present disclosure, there is provided a eukaryotic cell for expressing a VLP described herein. In an example, the cell comprises one or more polynucleotides encoding antigens from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), wherein the antigens are selected from:

- (a) a spike (S) protein;
- (b) a membrane (M) protein;
- (c) an envelope (E) protein;

(d) a nucleocapsid (N) protein

wherein at least one antigen is from the omicron strain of SARS-Cov2.

In an example, the eukaryotic cell comprises polynucleotides encoding an S protein, an M protein and an E protein, wherein each protein is from the the omicron strain of SARS-Cov2. In another example, the eukaryotic cell comprises polynucleotides encoding an S protein, an M protein, an E protein and an N protein, wherein each protein is from the the omicron strain of SARS-Cov2. In another example, the omicron strain is a BA.1 variant. In yet another example, the omicron strain is a BA.2 variant. In an example, the polynucleotides encode each of the S protein, an M protein, an E protein and/or an N protein as a fusion polypeptide.

In an example, the eukaryotic cell is a CHO cell, baby hamster kidney-21 (BHK-21) cell, human embryonic kidney 293 (HEK293) cell, CAP-T cell line derived from human amniocytes, Vero 9, or an east lanning line-0 (ELL-0) cell.

In an example of the present disclosure, there is provided a method for producing a VLP comprising:

(a) providing an expression vector comprising polynucleotides encoding one or more antigens from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), wherein the antigens are selected from:

- (i) a spike (S) protein;
- (ii) a membrane (M) protein;
- (iii) an envelope (E) protein;
- (iv) a nucleocapsid (N) protein

wherein at least one antigen is from the omicron strain of SARS-Cov2;

(b) providing a host cell, and

(c) transfecting said host cell with said vector to produce virus-like particles (VLPs) comprising the one or more antigens,

wherein the polynucleotides are expressed under conditions sufficient for the formation of VLPs.

In an example, the method further comprises purifying the VLPs.

In an example, the polynucleotides encoding antigens from each of the S protein, M protein and E protein are expressed from monocistronic polynucleotides, wherein the polynucleotides are operably linked to a promoter sufficient to drive expression of the antigens encoded by the polynucleotides. In another example, the polynucleotides encoding antigens from each of the S protein, M protein and E protein are expressed from a polycistronic polynucleotide, wherein the polynucleotides are operably linked to a promoter sufficient to drive expression of the antigens encoded by the polynucleotides.

In an example, the present disclosure also provides a kit comprising at least one composition or vaccine of the disclosure.

In one example, the kit comprises a composition or vaccine of the present disclosure, optionally in a delivery system and/or a pharmaceutically acceptable carrier or diluent, packaged

with instructions for use in treating or preventing or delaying progression of a SARS-CoV-2 infection and/or a COVID-19 infection in a subject in need thereof. In an example, composition or vaccine comprises a VLP comprising an antigen from each of the S protein, M protein, and E protein of the SARS-CoV-2 and optionally an adjuvant such as MF59. In another example, composition or vaccine comprises a VLP comprising an antigen from each of the S protein, M protein, E protein and N protein of the SARS-CoV-2 and optionally an adjuvant such as MF59. In another example, the kit further comprises a delivery system and/or a pharmaceutically acceptable carrier or diluent, packaged with instructions to administer the VLP to a subject who is suffering from or at risk of suffering from a SARS-CoV-2 infection or COVID-19.

Thus, in one example, the kit comprises:

(a) a VLP disclosed herein, a pharmaceutical composition disclosed herein, an immunogenic composition disclosed herein or a vaccine disclosed herein;

(b) instructions for use thereof; and optionally

(c) a pharmaceutically acceptable carrier, excipient or diluent.

In one example, the composition, the immunogenic composition or the pharmaceutical composition of the disclosure is supplied in a vial. In another example, the immunogenic composition or the pharmaceutical composition of the disclosure is supplied in a syringe.

Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present disclosure as it existed before the priority date of each of the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Overview of production of VLPs comprising various SARS-CoV-2 antigens in cell culture.

Figure 2. Effect of VLP1, which comprises N protein, S protein, M protein and E protein antigens from the omicron strain of SARS-CoV-2, and VLP2, which comprises S protein, M protein and E protein antigens from the omicron strain of SARS-CoV-2, on LV microneutralisation, PV microneutralisation and ACE-2 binding inhibition. The effect of VLP-1 and VLP-2 were also tested in the presence of adjuvant MF-59.

Figure 3. Effect of VLP1 which comprises N protein, S protein, M protein and E protein antigens from the omicron strain of SARS-CoV-2, and VLP2, which comprises S protein, M protein and E protein antigens from the omicron strain of SARS-CoV-2, on PV microneutralisation when administered in the presence of adjuvant MF-59.

DETAILED DESCRIPTION

General

Throughout this specification, unless specifically stated otherwise or the context requires otherwise, reference to a single step, composition of matter, group of steps or group of compositions of matter shall be taken to encompass one and a plurality (i.e. one or more) of those steps, compositions of matter, groups of steps or groups of compositions of matter.

Those skilled in the art will appreciate that the present disclosure is susceptible to variations and modifications other than those specifically described. It is to be understood that the disclosure includes all such variations and modifications. The disclosure also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations or any two or more of said steps or features.

The present disclosure is not to be limited in scope by the specific examples described herein, which are intended for the purpose of exemplification only. Functionally-equivalent products, compositions and methods are clearly within the scope of the present disclosure.

Any example of the present disclosure herein shall be taken to apply *mutatis mutandis* to any other example of the disclosure unless specifically stated otherwise. Stated another way, any specific example of the present disclosure can be combined with any other specific example of the disclosure (except where mutually exclusive).

Any example of the present disclosure disclosing a specific feature or group of features or method or method steps will be taken to provide explicit support for disclaiming the specific feature or group of features or method or method steps.

Unless specifically defined otherwise, all technical and scientific terms used herein shall be taken to have the same meaning as commonly understood by one of ordinary skill in the art (for example, in cell culture, molecular genetics, immunology, immunohistochemistry, protein chemistry, and biochemistry).

Unless otherwise indicated, the recombinant protein, cell culture, and immunological techniques utilized in the present disclosure are standard procedures, well known to those skilled in the art. Such techniques are described and explained throughout the literature in sources such as, J. Perbal, *A Practical Guide to Molecular Cloning*, John Wiley and Sons (1984), J. Sambrook *et al.* *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbour Laboratory Press (1989), T.A. Brown (editor), *Essential Molecular Biology: A Practical Approach*, Volumes 1 and 2, IRL Press (1991), D.M. Glover and B.D. Hames (editors), *DNA Cloning: A Practical Approach*, Volumes 1-4, IRL Press (1995 and 1996), and F.M. Ausubel *et al.* (editors), *Current Protocols in Molecular Biology*, Greene Pub. Associates and Wiley-Interscience (1988, including all updates until present), Ed Harlow and David Lane (editors) *Antibodies: A Laboratory Manual*, Cold Spring Harbour Laboratory, (1988), and J.E. Coligan *et al.* (editors) *Current Protocols in Immunology*, John Wiley & Sons (including all updates until present).

The term “and/or”, e.g., “X and/or Y” shall be understood to mean either “X and Y” or “X or Y” and shall be taken to provide explicit support for both meanings or for either meaning.

Throughout this specification the word “comprise”, or variations such as “comprises” or “comprising”, will be understood to imply the inclusion of a stated element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

As used herein the term “derived from” shall be taken to indicate that a specified integer can be obtained from a particular source albeit not necessarily directly from that source. Similarly, the term “based on” shall be taken to indicate that a specified integer can be developed or used from a particular source albeit not necessarily directly from that source.

Selected Definitions

As used herein, the term “fragment” refers to a portion of a nucleotide sequence or polypeptide of a reference nucleotide sequence or polypeptide disclosed herein which maintains a defined activity of the full length nucleotide sequence or polypeptide. In one example, the defined activity is inducing an immune response in a subject administered with a composition of the present disclosure.

As used herein, the term “variant” refers to a nucleotide sequence or polypeptide (e.g. antigenic polypeptide) with difference(s) in one or more nucleotide sequence(s) or amino acid sequence(s) to a reference nucleotide sequence or polypeptide disclosed herein which maintains a defined activity of the nucleotide sequence or polypeptide. The difference(s) in one or more nucleotide sequence(s) or amino acid sequence(s) results from one or modification(s) made to the nucleotide sequence or polypeptide of the present disclosure. In one example, the modification is a chemical modification of one or more nucleotide(s) of the nucleotide sequence. For example, at least one naturally occurring nucleotide of the RNA is replaced with a chemically modified nucleotide (e.g. pseudouridine (ψ), and 1-methylpseudouridine ($m1\psi$)). In one example, the modification comprises increasing the G/C content of the nucleotide sequence. In one example, the modification comprises codon optimization of the nucleotide sequence. In one example, the defined activity is inducing an immune response in a subject administered with a composition of the present disclosure.

In one example, the variant has at least 70%, or 75%, or 80%, or 85%, or 90%, or 91%, or 92%, or 93%, or 94%, or 95%, or 96%, or 97%, or 98%, or 99% of sequence identity with a sequence disclosed herein. In one example, the variant has at least 70%, or 75%, or 80%, or 85%, or 90%, or 91%, or 92%, or 93%, or 94%, or 95%, or 96%, or 97%, or 98%, or 99% of sequence identity with a sequence disclosed herein. The nucleotide sequence or polypeptide variant disclosed herein can have one or more nucleotide(s) or amino acid(s) deleted or substituted by different nucleotide(s) or amino acid(s). In one example, the substitution is a conservative substitution. A skilled person will appreciate that a conservative substitution with reference to a

polypeptide involves replacement of an amino acid in the polypeptide with a different amino acid with similar biochemical properties (e.g. charge, hydrophobicity and size). In one example, the substitution is a non-conservative substitution.

As used herein, the term “encode”, “encodes” or “encoding” refers to a region of a RNA capable of undergoing translation into a polypeptide.

As used herein, the term “antigen” refers to a molecule or structure containing one or more epitopes that induce, elicit, augment or boost a cellular and/or humoral immune response. Antigens can include, for example, proteins and peptides from a pathogen such as a virus, bacteria, fungus, protozoan, plant or from a tumour. For example, an antigen is derived from a gene of interest. In another example, the antigenic proteins are from one or more of a spike (S) protein; a membrane (M) protein; an envelope (E) protein; and a nucleocapsid (N) protein of a SARS-CoV-2.

As used herein the term "adjuvant" refers to a compound that, when used in combination with a specific immunogen (e.g. a VLP) in a formulation, augments or otherwise alters or modifies the resultant immune response. Modification of the immune response includes intensification or broadening the specificity of either or both antibody and cellular immune responses. Modification of the immune response can also mean decreasing or suppressing certain antigen-specific immune responses.

The term “naked” as used herein refers to nucleic acids that are substantially free of other macromolecules, such as lipids, polymers and proteins. A “naked” nucleic acid is not formulated with other macromolecules to improve cellular uptake. Accordingly, a naked nucleic acid is not encapsulated in, absorbed on, or bound to a lipid nanoparticle (LNP), a liposome or a polymeric microparticle.

As used herein, the term “nucleotide sequence” or “nucleic acid sequence” will be understood to mean a series of contiguous nucleotides (or bases) covalently linked to a phosphodiester backbone. By convention, sequences are presented from the 5' end to the 3' end, unless otherwise specified.

As used herein, the term “operably linked to” means positioning a translation initiation sequence (e.g. a Kozak consensus sequence, an internal ribosome entry site (IRES), a subgenomic (SG) promoter) or a stability element (e.g., an 5'-UTR) relative to a nucleic acid such that expression of the nucleic acid is controlled or regulated by the sequence or element. For example, a translation initiation sequence can be operably linked to the 5' end of the one or more polynucleotide sequence(s) disclosed herein.

The term “polypeptide” or “polypeptide chain” will be understood to mean a series of contiguous amino acids linked by peptide bonds. For example, a protein shall be taken to include a single polypeptide chain i.e., a series of contiguous amino acids linked by peptide bonds or a series of polypeptide chains covalently or non-covalently linked to one another (i.e., a polypeptide complex). The series of polypeptide chains can be covalently linked using a suitable

chemical or a disulfide bond. Examples of non-covalent bonds include hydrogen bonds, ionic bonds, Van der Waals forces, and hydrophobic interactions.

The term “recombinant” shall be understood to mean the product of artificial genetic recombination.

As used herein, the term “lipid nanoparticle” or “LNP” shall be understood to refer to any lipid composition, including, but not limited to, liposomes or vesicles, where an aqueous volume is encapsulated by amphipathic lipid bilayers (e.g., single; unilamellar or multiple; multilamellar), micelle-like lipid nanoparticles having a non-aqueous core and solid lipid nanoparticles.

As used herein, the terms “disease”, “disorder” or “condition” refers to a disruption of or interference with normal function.

As used herein, a subject “at risk” of developing a SARS-CoV-2 infection may have or may not have detectable disease or symptoms of a SARS-CoV-2 infection, and may have or may not have displayed detectable disease or symptoms of SARS-CoV-2 infection prior to the treatment according to the present disclosure. “At risk” denotes that a subject has one or more risk factors, which are measurable parameters that correlate with development of the SARS-CoV-2 infection, as known in the art and/or described herein.

As used herein, the terms “treatment” or “treating” of a subject includes the application or administration of a compound or composition of the invention to a subject (or application or administration of a compound of the invention to a cell or tissue from a subject) with the purpose of delaying, slowing, stabilizing, curing, healing, alleviating, relieving, altering, remedying, less worsening, ameliorating, improving, or affecting the disease or condition, the symptom of the disease or condition, or the risk of (or susceptibility to) the disease or condition. The term “treating” refers to any indication of success in the treatment or amelioration of an injury, pathology or condition, including any objective or subjective parameter such as abatement; remission; lessening of the rate of worsening; lessening severity of the disease; stabilization, diminishing of symptoms or making the injury, pathology or condition more tolerable to the subject; slowing in the rate of degeneration or decline; or making the final point of degeneration less debilitating.

As used herein, “preventing” or “prevention” is intended to refer to at least the reduction of likelihood of the risk of (or susceptibility to) acquiring a disease or disorder (i.e., causing at least one of the clinical symptoms of the disease not to develop in a patient that may be exposed to or predisposed to the disease but does not yet experience or display symptoms of the disease). Biological and physiological parameters for identifying such patients are provided herein and are also well known by physicians.

As used herein, the phrase “delaying progression of” includes reducing or slowing down the progression of the disease or condition in an individual and/or at least one symptom of a disease or condition.

An “effective amount” refers to at least an amount effective, at dosages and for periods of time necessary, to achieve the desired result. For example, the desired result can be a therapeutic or prophylactic result. An effective amount can be provided in one or more administrations. In some examples of the present disclosure, the term “effective amount” is meant an amount necessary to effect treatment of a disease or condition as hereinbefore described. In some examples of the present disclosure, the term “effective amount” is meant an amount necessary to effect a change associated with a disease or condition as hereinbefore described. The effective amount can vary according to the disease or condition to be treated or factor to be altered and also according to the weight, age, racial background, sex, health and/or physical condition and other factors relevant to the mammal being treated. Typically, the effective amount will fall within a relatively broad range (e.g. a “dosage” range) that can be determined through routine trial and experimentation by a medical practitioner. Accordingly, this term is not to be construed to limit the disclosure to a specific quantity. The effective amount can be administered in a single dose or in a dose repeated once or several times over a treatment period.

A “therapeutically effective amount” is at least the minimum concentration required to effect a measurable improvement of a particular disease or condition. A therapeutically effective amount herein can vary according to factors such as the disease state, age, sex, and weight of the patient, and the ability of the VLP of the present disclosure to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the VLP are outweighed by the therapeutically beneficial effects.

As used herein, the term “prophylactically effective amount” shall be taken to mean a sufficient quantity of a VLP of the disclosure to prevent or inhibit or delay the onset of one or more detectable symptoms of a disease or disorder.

A “subject” can also be any animal that is susceptible to infection by SARS-CoV-2. A subject of this invention can be a mammal and in particular embodiments is a human, which can be an infant, a child, an adult or an elderly adult. A “subject at risk of infection by SARS-CoV-2” or a “subject at risk of SARS-CoV-2 infection” is any subject who may be or has been exposed to a SARS-CoV-2. The subject may be a primary contact of an individual diagnosed with a SARS-CoV-2 infection. “Subject” includes any human or non-human animal. Thus, in addition to being useful for human treatment, the compounds of the present invention may also be useful for veterinary treatment of mammals, including companion animals and farm animals, such as, but not limited to dogs, cats, horses, cows, sheep, and pigs.

As used herein, the term “virus-like particle”, “VLP”, “virus-like particles” or “VLPs” shall be taken to mean a multi-subunit protein- and lipid-based structure, made up elements required to produce a virus-like particle (VLP) which resembles the form and/or size of a virus particle but does not contain the genetic material of the virus. The VLP or VLPs display antigens which present conformational epitopes that elicit T cell and/or B cell immune responses but are unable to replicate and/or infect a host cell. For example, a VLP of the present disclosure

comprises one or more antigens from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) selected from a spike (S) protein; a membrane (M) protein; an envelope (E) protein; and a nucleocapsid (N) protein that are suitable for use as a vaccine.

SARS-CoV-2 Antigens

In an example, the present disclosure provides for VLPs comprising one or more antigens from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) selected from a spike (S) protein; a membrane (M) protein; an envelope (E) protein; and a nucleocapsid (N) protein, wherein at least one of the antigens is from the omicron strain of a SARS-CoV-2. In the context of the present disclosure, the antigen is therefore a pathogenic antigen. For example, the antigen of interest is an antigen polypeptide, an immunogenic fragment and/or variant thereof which can induce an immune response in the subject.

The SARS-CoV-2 genome encodes for at least four main structural proteins: the spike (S), membrane (M), envelope (E), nucleocapsid (N) proteins and other accessory proteins which aid the replicative processes and facilitate entry into cells. The M protein is the most abundant component of the virus envelope, which directs the assembly of coronaviruses through interactions with all other structural proteins. The E protein is a small membrane protein or viroporin that is thought to promote budding of virus particles by pinching off cellular membrane surfaces. The S protein is a class I fusion protein that mediates attachment of SARS-CoV-2 to the major cell surface receptor human Angiotensin Converting Enzyme 2 (ACE2). Due to its exposed conformation on the surface of the virus, the S protein is highly immunogenic and is the main focus of current vaccine development. The N protein packages the RNA genome to form the nucleocapsid and whilst not necessarily required for envelope formation; it appears to play an important role in the assembly and stability of the complete virion, and in enhancement of VLP yields.

The S protein comprises three domains: (i) a large ectodomain; (ii) a transmembrane domain (which passes through the viral envelope in a single pass); and (iii) a short intracellular tail. The ectodomain consists of three receptor-binding subunits (3 x S1) and a trimeric stalk made of three membrane-fusion subunits (3 x S2). Thus, the SARS-CoV-2 S protein is a homotrimer. During virus entry, S1 binds to a receptor on the host cell surface for viral attachment, and S2 fuses the host and viral membranes, allowing viral genomes to enter host cells. Receptor binding and membrane fusion are the initial and critical steps in the coronavirus infection cycle. There is significant divergence in the receptors targeted by different CoVs.

The structure of the SARS-CoV-2 S protein is described, for example, in Cai et al. (Science (2020) 369:1586-1592)), which is herein incorporated by reference in its entirety. Each S1 subunit of a SARS-CoV-2 S protein comprises an N-terminal domain (NTD), receptor binding domain (RBD), two C terminal domains (CTDs). Prior to fusion with the host cell membrane, the S1 subunits of the SARS-CoV-2 S protein protect the S2 subunits. On binding to ACE2, the

SARS-CoV-2 S protein refolds in a "jack-knife" manner, forming a long-central coiled coil and ultimately leading to membrane fusion and viral entry to a host cell.

Given the propensity of RNA viruses such as SARS-CoV-2 to mutate, the present inventors provide for VLPs that comprise S proteins that may include mutations found in different strains of SARS-CoV-2, such that the vaccine compositions find particular utility in the treatment of targeted strains of SARS-CoV-2 including the omicron strain of SARS-CoV-2.

In one example, the SARS-CoV-2 S protein forming a VLP of the disclosure may include mutations that are present in the omicron strain of SARS-CoV-2. In one example, where the variant is omicron variant BA.1, the S protein comprises one or more or all of the mutations selected from the group consisting of A67V, T95I, Y145D, L212L, S371L, G446S, G496S, T547K, N856K, L981F, G142D, Q493R, G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K and del69-70.

In another example, the variant is omicron variant BA.2 and the S protein comprises one or more or all of the mutations selected from the group consisting of G142D, Q493R, del24-26, G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K, T19I, A27S, G142D, V213G, S371F, T376A, D405N and R408S.

In another example, the variant is omicron variant BA.4 or BA.5 and the S protein comprises one or more or all of the mutations selected from the group consisting of L452R, F486V, R493Q, del24-26, del69-70, G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K, T19I, A27S, G142D, V213G, S371F, T376A, D405N and R408S.

The VLPs, compositions and vaccines of the present disclosure may also be useful for the treatment of other variant SARS-CoV-2. In particular, VLPs, compositions and vaccines of the present disclosure find utility in the treatment of B.1.1.7 SARS-CoV-2 strain (also known as 201/501Y.V1, which was first detected in the UK, now known as the Alpha variant); the B.1.351 SARS-CoV-2 strain (also known as 20H/501.V2, which was first detected in South Africa, now known as the Beta variant), the P1 SARS-CoV-2 strain (also known as 20J/501 Y.V3, which was first detected in Japan and Brazil, now known as the Gamma variant), the B1.427 and B1.429 SARS-CoV-2 strains (first detected in California, now known as the Epsilon variant), and/or the B.1.617.2 SARS-CoV-2 strain (which was first detected in India, now known as the Delta variant). The mRNA, conventional mRNA, self-replicating RNA, compositions and vaccines of the present disclosure may also be useful for the treatment of the Wuhan (original) strain of SARS-CoV-2.

According to the CDC (SARS-CoV-2 Variant Classifications and Definitions ([cdc.gov](https://www.cdc.gov/coronavirus/2019-ncov/variant-classifications))), the Alpha variant has been found to comprise the following mutations to the S protein: 69deletion, 70deletion, 144deletion, (E484K*), (S494P*), N501Y, A570D, D614G, P681H, T716I, S982A,

D1118H, and (K1191N*) with the key mutations being deletion of residues 69/70 and 144Y, as well as N501Y, A570D, D614G and P681H substitutions. The Beta variant has been found to comprise the following mutations: D80A, D215G, 241deletion, 242deletion, 243deletion, K417N, E484K, N501Y, D614G, and A701V with the key mutations being K417N, E484K, N501Y and D614G substitutions. The Gamma variant has been found to comprise the following mutations: L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T10271 with the key mutations being E484K, K417N/T, N501Y and D614G. The Delta variant has been found to comprise the following mutations: T19R, (G142D*), 156deletion, 157deletion, R158G, L452R, T478K, D614G, P681R, and D950N with the key mutations being L452R, E484Q and T478K. The Epsilon variant has been found to comprise the following mutations: S131, W152C, 30 L452R, D614G with the key mutation being L452R. Thus, the present disclosure encompasses VLPs comprising antigens from a S protein which include or or more or all of the above mutations.

In one example, the VLP comprises an antigen from each of the S protein, M protein, and E protein of SARS-CoV-2. In another example, the VLP comprises an antigen from each of the S protein and M protein of a SARS-CoV-2. In another example, the VLP comprises an antigen from each of the S protein and E protein of a SARS-CoV-2. In another example, the VLP comprises an antigen from each of the E protein and M protein of a SARS-CoV-2.

In one example, the VLP comprises an antigen from each of the S protein, M protein, and E protein of the omicron strain of a SARS-CoV-2. In another example, the VLP comprises an antigen from each of the S protein and M protein of the omicron strain of a SARS-CoV-2. In another example, the VLP comprises an antigen from each of the S protein and E protein of the omicron strain of a SARS-CoV-2. In another example, the VLP comprises an antigen from each of the E protein and M protein of the omicron strain of a SARS-CoV-2. In an example, the omicron variant may be BA.1 or BA.2.

In an example, at least one of the antigens may be from the delta, beta, alpha, gamma or strain of a SARS-CoV-2 or from the SARS-CoV-2 strain 2019-nCoV/USA-WA1/2020. In another example, two of the antigens are from the delta, beta, alpha or gamma strain of a SARS-CoV-2 or from the SARS-CoV-2 strain 2019-nCoV/USA-WA1/2020. In another example, three of the antigens are from the delta, beta, alpha or gamma strain of a SARS-CoV-2 or from the SARS-CoV-2 strain 2019-nCoV/USA-WA1/2020. In another example, none of the antigens are from the delta, beta, alpha or gamma strain of a SARS-CoV-2 or from the SARS-CoV-2 strain 2019-nCoV/USA-WA1/2020. Thus, in an example, each of the S, M and E antigens are from the omicron strain of SARS-Cov2.

In one example, the VLP comprises an antigen from each of the S protein, M protein, and E protein of the omicron strain of a SARS-CoV-2, wherein:

(a) the omicron variant is BA.1 and the S protein comprises one or more or all of the mutations selected from the group consisting of A67V, T95I, Y145D, L212L, S371L, G446S,

G496S, T547K, N856K, L981F, G142D, Q493R, G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K and del69-70; or

(b) the omicron variant is BA.2 and the S protein comprises one or more or all of the mutations selected from the group consisting of G142D, Q493R, del24-26, G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K, T19I, A27S, G142D, V213G, S371F, T376A, D405N and R408S; or

(c) the omicron variant is BA.4 or BA.5 and the S protein comprises one or more or all of the mutations selected from the group consisting of L452R, F486V, R493Q, del24-26, del69-70, G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K, T19I, A27S, G142D, V213G, S371F, T376A, D405N and R408S.

In one example, at least one of the antigens is from the delta, beta, alpha or gamma strain of SARS-CoV-2 or from the SARS-CoV-2 strain 2019-nCoV/USA-WA1/2020. For example, the S protein antigen is from the delta strain of SARS-CoV-2 and the E and M protein antigens are from the omicron strain of SARS-CoV-2. For example, the M protein antigen is from the delta strain of SARS-CoV-2 and the E and S protein antigens are from the omicron strain of SARS-CoV-2. In another example, two of the antigens are from the delta, beta, alpha or gamma strain of SARS-CoV-2 or from the SARS-CoV-2 strain 2019-nCoV/USA-WA1/2020. For example, the S protein and the M protein antigens are from the delta strain of SARS-CoV-2 and the E protein antigen is from the omicron strain of SARS-CoV-2. For example, the M protein and E protein antigens are from the delta strain of SARS-CoV-2 and the S protein antigen is from the omicron strain of SARS-CoV-2.

In an example, the VLP further comprises an antigen from the N protein of SARS-CoV-2.

In one example, the VLP comprises an antigen from each of the S protein, M protein, E protein and N protein of the omicron strain of SARS-CoV-2. In another example, the VLP comprises an antigen from each of the S protein, M protein and N protein of the omicron strain of SARS-CoV-2. In another example, the VLP comprises an antigen from each of the S protein, E protein and N protein of the omicron strain of SARS-CoV-2. In another example, the VLP comprises an antigen from each of the E protein, M protein and N protein of the omicron strain of SARS-CoV-2. In an example, the omicron variant may be BA.1 or BA.2.

In an example, at least one of the antigens may be from the delta, beta, alpha or gamma strain of SARS-CoV-2 or from the SARS-CoV-2 strain 2019-nCoV/USA-WA1/2020. For example, the S protein antigen is from the delta strain of SARS-CoV-2 and the E, N and M protein antigens are from the omicron strain of SARS-CoV-2. For example, the M protein antigen is from the 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2 and the E, N and S protein antigens are

from the omicron strain of SARS-CoV-2. In another example, two of the antigens are from the delta, beta, alpha or gamma strain of SARS-CoV-2 or from the SARS-CoV-2 strain 2019-nCoV/USA-WA1/2020. For example, the S and M protein antigens are from the delta strain of SARS-CoV-2 and the E and N protein antigens are from the omicron strain of SARS-CoV-2. For example, the M and E protein antigens are from the 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2 and the N and S protein antigens are from the omicron strain of SARS-CoV-2. In another example, three of the antigens are from the delta, beta, alpha or gamma strain of SARS-CoV-2 or from the SARS-CoV-2 strain 2019-nCoV/USA-WA1/2020. For example, the S protein, M protein and E protein antigens are from the delta strain of SARS-CoV-2 and the N protein is from the omicron strain of SARS-CoV-2. For example, the N protein, M protein and E protein antigens are from the 2019-nCoV/USA-WA1/2020 strain, delta strain and beta strain respectively, of SARS-CoV-2 and the S protein is from the omicron strain of SARS-CoV-2. In another example, none of the antigens are from the delta, beta, alpha or gamma strain of SARS-CoV-2 or from the SARS-CoV-2 strain 2019-nCoV/USA-WA1/2020. In another example, none of the antigens are from the delta, beta, alpha or gamma strain of SARS-CoV-2 or from the SARS-CoV-2 strain 2019-nCoV/USA-WA1/2020. Thus, in an example, all of the antigens are from the omicron strain of SARS-CoV-2

In one example, the VLP comprises an antigen from each of the S protein, M protein, E protein and N protein of the omicron strain of SARS-CoV-2, wherein:

(a) the omicron variant is BA.1 and the S protein comprises one or more or all of the mutations selected from the group consisting of A67V, T95I, Y145D, L212L, S371L, G446S, G496S, T547K, N856K, L981F, G142D, Q493R, G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K and del69-70; or

(b) the omicron variant is BA.2 and the S protein comprises one or more or all of the mutations selected from the group consisting of G142D, Q493R, del24-26, G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K, T19I, A27S, G142D, V213G, S371F, T376A, D405N and R408S; or

(c) the omicron variant is BA.4 or BA.5 and the S protein comprises one or more or all of the mutations selected from the group consisting of L452R, F486V, R493Q, del24-26, del69-70, G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K, T19I, A27S, G142D, V213G, S371F, T376A, D405N and R408S.

Polynucleotides

The present disclosure also provides polynucleotides that when expressed under sufficient conditions, encode a VLP disclosed herein and are suitable for forming a composition or vaccine

for treating a SARS-CoV-2 infection or COVID-19. The term polynucleotide encompasses both DNA and RNA sequences. Herein, the terms "nucleic acid", "nucleic acid molecule" and "polynucleotide" are used interchangeably. Thus, the antigens derived from SARS-CoV-2 (e.g. SARS-CoV-2 S, M, E and/or N protein) may be encoded or expressed by DNA or RNA comprised within one or more expression cassettes or vectors.

By way of non-limiting example, where the VLP comprises more than one antigen from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), said more than one antigen may be expressed by a monocistronic polynucleotide, or each of said SARS-CoV-2 antigens may be expressed by polycistronic polynucleotides. For example, the S, M, E and/or N protein antigens may be expressed by polycistronic polynucleotides or a monocistronic polynucleotide.

The one or more polynucleotide (e.g. a DNA or RNA) encoding the one or more SARS-CoV-2 spike protein or immunogenic fragments thereof may be optimised for expression in a cell. The term "optimised" as used herein relates to optimisation for expression of the one or more SARS-CoV-2 spike protein or immunogenic fragment thereof, and includes both codon optimisation and/or other modifications to the polynucleotide (both in terms of the nucleic acid sequence and other modifications) which increase the level and/or duration of expression of the one or more SARS-CoV-2 spike protein from the polynucleotide within the cell.

The one or more polynucleotide (e.g. a DNA or RNA) according to the disclosure may be comprised in an expression vector to facilitate expression of the one or more SARS-CoV-2 antigens. Typically, in such an expression construct said one or more polynucleotide is operably linked to a suitable promoter(s). The one or more polynucleotide may be linked to a suitable terminator sequence(s). The one or more polynucleotide may be linked to both a promoter(s) and terminator(s). Suitable promoter and terminator sequences are well known in the art.

The one or more polynucleotide (e.g. DNA or RNA) encoding at least one SARS-CoV-2 antigen may additionally comprise a leader sequence(s), for example to assist in the secretion of the at least one SARS-CoV-2 antigen. Any suitable leader sequence may be used, including conventional leader sequences known in the art. Suitable leader sequences include human tissue plasminogen activator leader sequence (tPA), which is routinely used in viral and DNA based vaccines and for protein vaccines to aid secretion from mammalian cells.

One or more viral vectors, expression vectors or DNA vectors (or DNA plasmids) may comprise one or more polynucleotides encoding at least one antigen derived from SARS-CoV-2 as described herein. Preferably, said one or more viral vector or DNA vector (or DNA plasmid) encodes at least one antigen as described herein. Multiple SARS-CoV-2 antigens may be expressed by a single viral vector or DNA vector (or DNA plasmid) by multiple viral vectors or DNA vectors (or DNA plasmids) or a combination thereof. By way of non-limiting example, said one or more SARS-CoV-2 antigens may be expressed by a single viral vector or DNA vector (or DNA plasmid) or each of said SARS-CoV-2 antigens may be expressed by a separate viral vector or DNA vector (or DNA plasmid).

The one or more vector(s) may be a DNA vector, such as a DNA plasmid. The one or more vector(s) may be an RNA vector, such as a mRNA vector or a self-amplifying RNA vector. The one or more DNA and/or RNA vector(s) of the invention is typically capable of expression in eukaryotic cells, particularly any host cell type described herein.

Typically the DNA and/or RNA vector(s) are capable of expression in a human, *e. coli* or yeast cell. The one or more vector may be a phage vector, such as an AAV/phage hybrid vector as described in Hajitou et al., Cell 2006; 125(2) pp. 385-398; herein incorporated by reference.

The nucleic acid molecules and vectors of the invention may be made using any suitable process known in the art. Thus, the nucleic acid molecules may be made using chemical synthesis techniques. Alternatively, the nucleic acid molecules and vectors of the invention may be made using molecular biology techniques.

Virus-like particles

In a composition or vaccine of the disclosure, the one or more antigens from a SARS-CoV-2 is comprised in a virus-like particle (VLP).

Virus-like particles (VLPs) are particles which resemble viruses but do not contain viral nucleic acid and are therefore non-infectious. They commonly contain one or more virus capsid or envelope proteins which are capable of self-assembly to form the VLP. VLPs have been produced from components of a wide variety of virus families (Noad and Roy (2003), Trends in Microbiology, 11:438-444; Grgacic et al., (2006), Methods, 40:60-65). Some VLPs have been approved as therapeutic vaccines, for example Engerix-B (for hepatitis B), Cervarix and Gardasil (for human papilloma viruses).

Multiple SARS-CoV-2 antigens, for example may be comprised in a single VLP or a number of VLPs. By way of non-limiting example, one or more SARS-CoV-2 antigens (for example an S protein, an M protein and an E protein) may be comprised in a single VLP, or each of said SARS-CoV-2 antigens (for example each of an S protein, an M protein and an E protein) may be comprised in separate VLPs.

Accordingly, the one or more antigens derived from SARS-CoV-2 may be comprised in one or more VLPs. The one or more VLPs comprising the at least one antigen of the disclosure may comprise an S protein from SARS-CoV-2 or an immunogenic fragment thereof, that has a common antigenic cross-reactivity with said S protein. The one or more VLP comprising the at least one antigen of the disclosure may comprise an M protein from SARS-CoV-2 or an immunogenic fragment thereof, that has a common antigenic cross-reactivity with said M protein. The one or more VLPs comprising the at least one antigen of the disclosure may comprise an E protein from SARS-CoV-2 or an immunogenic fragment thereof, that has a common antigenic cross-reactivity with said E protein. The one or more VLP comprising the at least one antigen of the disclosure may comprise an N protein from SARS-CoV-2 or an immunogenic fragment

thereof, that has a common antigenic cross-reactivity with said N protein. Alternatively, VLP comprising the at least one antigen of the disclosure may comprise any combination of the above.

The skilled person will understand that VLPs can be synthesized through the individual expression of viral structural proteins, which can then self-assemble into the virus-like structure. Combinations of structural capsid proteins from different viruses can be used to create recombinant VLPs. In addition, antigens or immunogenic fragments thereof can be fused to the surface of VLPs. By way of non-limiting example, antigens or immunogenic fragments thereof of the invention may be coupled to a VLP using the SpyCatcher-SpyTag system (as described by Brune, Biswas, Howarth).

Fusion proteins

Said one or more VLPs may comprise at least one fusion protein of the at least one antigen described herein. For example, the VLP may comprise a fusion protein comprising an S protein, an M protein and an E protein; or comprising an S protein, an M protein, an E protein and an N protein. Alternatively, the one or more antigens from SARS-CoV-2 may comprise more than one more fusion protein. For example, the VLP may comprise a fusion protein comprising an S protein and an M protein and another fusion protein comprising an E protein and an N protein described herein.

In one example, a fusion protein of the invention comprises a non-SARS-CoV-2 domain or element, typically a non-SARS-CoV-2 protein, polypeptide or peptide domain or element. Said one or more fusion protein may comprise the at least one SARS-CoV-2 antigen and one or more of: Hepatitis B surface antigen (HBsAg); human papillomavirus (HPV) 18 L1 protein; HPV 16 L1 protein; and/or Hepatitis E P239, preferably Hepatitis B surface antigen.

In one example, said one or more fusion protein may take the form of a VLP. Without being bound by theory, this is because HPSAg, HPV 18 L1 protein, HPB 16 L1 protein and Hepatitis E P239 protein are known to spontaneously form VLPs when expressed recombinantly, and this structure is retained when HPSAg, HPV 18 L1 protein, HPB 16 L1 protein and/or Hepatitis E P239 protein are present in fusion protein form combined with a SARS-CoV-2 antigen.

A fusion protein of the disclosure may comprise a linker (also referred to interchangeably herein as a linker peptide, a spacer or a spacer peptide). A linker may be used to join two or more functional domains of a fusion protein of the invention. Typically, where a linker is present, it is used to join an SARS-CoV-2 antigen (e.g., a S protein) of the fusion protein to another SARS-CoV-2 antigen (e.g., a N protein) of the fusion protein. Use of linkers in fusion proteins is routine in the art, and any conventional linker protein may be used in fusion proteins of the invention, provided that the resulting fusion protein retains the desired functional properties of the SARS-CoV-2 antigens.

A linker may be a short peptide of up to about 30 amino acids, such as about 5-30 amino acids, about 5-25 amino acids, about 5-20 amino acids, about 10-20 amino acids, about 5-15 amino acids or about 10-15 amino acids in length. In some embodiments, the linker is about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19 or about 20 amino acids in length.

In an example, a rigid linker may be used in fusion proteins of the invention. Rigid linkers are conventionally used when it is necessary to keep a fixed distance between the different domains/portions of a fusion protein and to maintain their independent functions. Rigid linkers may also be used when the spatial separation of the fusion protein domains is critical to preserve the stability or bioactivity of the fusion proteins. An empirical rigid linker with the sequence of A(EAAAK)_nA (SEQ ID NO: 1), wherein $n = 2-5$, displayed α -helical conformation, which is stabilized by Glu-Lys+salt bridges. A non-limiting example of a rigid linker is EAAAKEAAAKEAAAK (SEQ ID NO: 2), also referred to as EAAAKh (SEQ ID NO: 3). Rigid linkers may be used for expression of fusion proteins of the invention in mammalian cells, such as HEK 293 cells.

In some embodiments, flexible linkers may be used in fusion proteins of the invention. Flexible linkers are conventionally used when the joined domains require a certain degree of movement or interaction. Flexible linkers usually comprise or consist of small amino acid residues, such as glycine, threonine, arginine, serine, asparagine, glutamine, alanine, aspartic acid, proline, glutamic acid, lysine, leucine and/or valine, particularly glycine, serine, alanine, leucine and/or valine. Flexible linkers comprising or consisting of glycine, serine and/or alanine are preferred, with glycine and serine being particularly preferred. Accordingly, the most commonly used flexible linkers have sequences consisting primarily of stretches of Gly and Ser residues ("GS" linker), which comprise a sequence of (Gly-Gly-Gly-Gly-Ser)_n (SEQ ID NO: 4). Non-limiting examples of GS linkers include GS₅; GS₁₀; GS₁₅; GS₂₀; and GS₂₅. Flexible linkers may be used for expression of fusion proteins of the invention in bacterial cells, such as *e. coli* cells.

Methods of Production

Suitable methods for the production of a VLP of the present disclosure will be apparent to the skilled person and/or described herein.

Typically, the plasmid DNA is produced by inserting the polynucleotide sequence encoding at least one antigen into a DNA vector. Suitable DNA vectors for use will be apparent to the skilled person, and the polynucleotide sequences of the present disclosure can be purchased from any commercial supplier. Insertion of the nucleotide sequence(s) into the DNA vector may be performed using standard methods in the art.

In one example, a protein antigen described herein is produced using a plasmid DNA. The skilled person will understand that plasmid DNA is relatively stable. Briefly, competent

bacterial cells (e.g., *Escherichia coli*) cells are transformed with a DNA plasmid encoding the a protein antigen described herein. Individual bacterial colonies are isolated and the resultant plasmid DNA amplified in *E. coli* cultures.

In one example, the plasmid DNA is isolated following fermentation. For example, the plasmid DNA is isolated using a commercially available kit (e.g., Maxiprep DNA kit), or other routine methods known to the skilled person. Following isolation, plasmid DNA is linearized by restriction digest (i.e., using a restricting enzyme). Restriction enzymes are removed using methods known in the art, including for example phenol/chloroform extraction and ethanol precipitation.

Compositions

The present disclosure provides an immunogenic composition comprising a VLP of the present disclosure. In an example, the immunogenic composition is a vaccine. The present disclosure also provides a pharmaceutical composition comprising an immunogenic composition of the present disclosure and a pharmaceutically acceptable carrier.

In an example, the VLPs described herein may be administered in a composition comprising an adjuvant for enhancing immunogenicity. In an example, the adjuvant is selected from the group consisting of Freund's adjuvant, incomplete Freund's adjuvants, aluminum phosphate, aluminum hydroxide, GMCSP, BCG, MDP compounds, such as thur-MDP and nor-MDP, CGP (MTP-PE), lipid A, monophosphoryl lipid A (MPL), RIBI, MPL, trehalose dimycolate (TDM), Novasomes®, QS21, Quil A (and derivatives and components thereof), calcium phosphate, calcium hydroxide, zinc hydroxide, MHC antigens, PolyI:C, MF59, glycolipid analogs, octadecyl esters of an amino acid, muramyl dipeptides, polyphosphazene, lipoproteins, ISCOM matrix, DC-Chol, ODA, cytokines, and other adjuvants and derivatives thereof. In an example, the adjuvant is MF59. In an example, MF59 is administered at the same time as the administration of a VLP, composition or vaccine of the disclosure. In another example, MF59 is administered sequentially to, preceding, or proceeding the administration of a VLP, composition or vaccine of the disclosure.

It will be apparent to the skilled person and/or described herein, that the VLP of the present disclosure may be present as a VLP or in combination with lipids, polymers or other delivery system that facilitates entry into the cells.

Delivery systems

In one example, the pharmaceutical composition of the present disclosure further comprises a lipid nanoparticle (LNP) and/or a polymeric microparticle. For example, the VLP is encapsulated in, bound to or adsorbed on a LNP and/or a polymeric microparticle.

Lipid Nanoparticles

In one example, the pharmaceutical composition of the present disclosure further comprises a LNP.

It will be apparent that the term “lipid nanoparticle” or “LNP” shall be understood to refer to any lipid composition, including, but not limited to, liposomes or vesicles, where an aqueous volume is encapsulated by amphipathic lipid bilayers (e.g., single; unilamellar or multiple; multilamellar), micelle-like lipid nanoparticles having a non-aqueous core and solid lipid nanoparticles. Methods of preparing a LNP are known to the skilled person and/or described herein. In one example, LNP are prepared using a staggered herribone mixer. For example, as described in US patent application 20120276209. In another example, liposomes are prepared using a microfluidic device. For example, as described in WO2018220553.

Lipid nanoparticles suitable for use in the present disclosure will be apparent to the skilled person and/or are described herein. For example, the LNP comprises an ionisable lipid.

As used herein, the term “ionisable lipid” or “ionisable lipids” shall refer to a lipid having at least one protonatable or deprotonatable group. For example, the lipid is positively charged at a pH at or below physiological pH (e.g. pH 7.4), and neutral at a second pH (e.g. at or above physiological pH). For example, the lipid is a cationic lipid.

Suitable ionisable lipids can have an anionic, cationic or zwitterionic hydrophilic head group. Exemplary phospholipids (anionic or zwitterionic) for use in the present disclosure include, for example, phosphatidylethanolamines, phosphatidylcholines, phosphatidylserines, and phosphatidylglycerols. In one example, the lipid is a cationic lipid. Exemplary cationic lipids include, but are not limited to, dioleoyl trimethylammonium propane (DOTAP), 1,2-distearoyloxy-N,N-dimethyl-3-aminopropane (DSDMA), 1,2-dioleoyloxy-N,N-dimethyl-3-aminopropane (DODMA), 1,2-dilinoleoyloxy-N,N-dimethyl-3-aminopropane (DLinDMA), 2,5-bis((9z,12z)-octadeca-9,12,dien-1-yloxy)benzyl-4-(dimethylamino)butanoate (LKY750). In one example, the phospholipid is 2,5-bis((9z,12z)-octadeca-9,12,dien-1-yloxy)benzyl-4-(dimethylamino)butanoate (LKY750). Exemplary zwitterionic lipids include, but are not limited to, acyl zwitterionic lipids and ether zwitterionic lipids, such as dipalmitoylphosphatidylcholine (DPPC), dioleoylphosphatidylcholine (DOPC) and dodecylphosphocholine. The lipids can be saturated or unsaturated.

Lipid moieties suitable for use in the LNP will be apparent to the skilled person and include, for example, a fatty acid, an isoprenoid and combinations thereof. In one example, the lipid moiety is selected from the group consisting of an isoprenoid, a triglyceride, a phospholipid, a cholesteryl ester and combinations thereof.

In one example, the lipid nanoparticle additionally comprises a PEG-lipid, a sterol structural lipid and/or a neutral lipid. In one example, the lipid nanoparticle does not comprise a cationic lipid.

PEG-lipids

In one example, the present disclosure provides a LNP comprising a PEGylated lipid.

It will be apparent to the skilled person that reference to a PEGylated lipid is a lipid that has been modified with polyethylene glycol. Exemplary PEGylated lipids include, but are not limited to, PEG-modified phosphatidylethanolamines, PEG-modified phosphatidic acids, PEG-modified ceramides, PEG-modified dialkylamines, PEG-modified diacylglycerols, and PEG-modified dialkylglycerols. For example, a PEG lipid includes PEG-c-DOMG, PEG-DMG, PEG-DLPE, PEG-DMPE, PEG-DPPC, a PEG-DSPE lipid and combinations thereof.

Neutral lipids

In one example, the present disclosure provides a LNP comprising a neutral lipid.

Suitable neutral or zwitterionic lipids for use in the present disclosure will be apparent to the skilled person and include, for example, 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), 1,2-dilinoleoyl-sn-glycero-3-phosphocholine (DLPC), 1,2-dimyristoyl-sn-glycero-phosphocholine (DMPC), 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), 1,2-diundecanoyl-sn-glycero-phosphocholine (DUPC), 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC), 1,2-di-O-octadecenyl-sn-glycero-3-phosphocholine (18:0 Diether PC), 1-oleoyl-2-cholesterylhemisuccinoyl-sn-glycero-3-phosphocholine (OChemPC), 1-hexadecyl-sn-glycero-3-phosphocholine (C16 Lyso PC), 1,2-dilinolenoyl-sn-glycero-3-phosphocholine, 1,2-diarachidonoyl-sn-glycero-3-phosphocholine, 1,2-didocosaheptaenoyl-sn-glycero-3-phosphocholine, 1,2-diphytanoyl-sn-glycero-3-phosphoethanolamine (ME 16.0 PE), 1,2-distearoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinoleoyl-sn-glycero-3-phosphoethanolamine, 1,2-dilinolenoyl-sn-glycero-3-phosphoethanolamine, 1,2-diarachidonoyl-sn-glycero-3-phosphoethanolamine, 1,2-didocosaheptaenoyl-sn-glycero-3-phosphoethanolamine, 1,2-dioleoyl-sn-glycero-3-phospho-rac-(1-glycerol) sodium salt (DOPG), and sphingomyelin. The lipids can be saturated or unsaturated.

Structural lipids

In one example, the present disclosure provides a LNP comprising a structural lipid.

Exemplary structural lipids include, but are not limited to, cholesterol fecosterol, sitosterol, campesterol, stigmasterol, brassicasterol, ergosterol, tomatidine, tomatine, ursolic acid and alpha-tocopherol.

In one example, the structural lipid is a sterol. For example, the structural lipid is cholesterol. In another example, the structural lipid is campesterol.

Polymeric microparticles

In one example, the pharmaceutical composition of the present disclosure further comprises a polymeric microparticle.

The skilled person will be aware that various polymers can form microparticles to encapsulate or adsorb the protein antigens or VLPs of the present disclosure. It will be apparent that use of a substantially non-toxic polymer means that particles are safe, and the use of a biodegradable polymer means that the particles can be metabolised after delivery to avoid long-term persistence. Useful polymers are also sterilisable, to assist in the preparation of pharmaceutical grade formulations.

Exemplary non-toxic and biodegradable polymers include, but are not limited to, poly(α -hydroxy acids), polyhydroxy butyric acids, polylactones (including polycaprolactones), polydioxanones, polyvalerolactone, polyorthoesters, polyanhydrides, polycyanoacrylates, tyrosine-derived polycarbonates, polyvinyl- pyrrolidinones or polyester-amides, and combinations thereof.

Pharmaceutically acceptable carrier

Suitably, in compositions or methods for administration of the VLP, vaccine or composition of the disclosure to a subject, the VLP, vaccine or composition is combined with a pharmaceutically acceptable carrier as is understood in the art. Accordingly, one example of the present disclosure provides a composition (e.g., a pharmaceutical composition) comprising the VLP of the disclosure (and any delivery system e.g. LNP) combined with a pharmaceutically acceptable carrier.

In general terms, by “carrier” is meant a solid or liquid filler, binder, diluent, encapsulating substance, emulsifier, wetting agent, solvent, suspending agent, coating or lubricant that may be safely administered to any subject, e.g., a human. Depending upon the particular route of administration, a variety of acceptable carriers, known in the art may be used, as for example described in Remington's Pharmaceutical Sciences (Mack Publishing Co. N.J. USA, 1991).

A VLP, composition or vaccine of the present disclosure is useful for parenteral, topical, oral, or local administration, intramuscular administration, aerosol administration, or transdermal administration, for prophylactic or for therapeutic treatment. In one example, the VLP, composition or vaccine is administered parenterally, such as intramuscularly, subcutaneously or intravenously. For example, the RNA is administered intramuscularly.

Formulation of a VLP, composition or vaccine of the present disclosure to be administered will vary according to the route of administration and formulation (e.g., solution, emulsion, capsule) selected. An appropriate pharmaceutical composition comprising a VLP, composition or vaccine to be administered can be prepared in a physiologically acceptable carrier. For solutions or emulsions, suitable carriers include, for example, aqueous or alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles can include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. A variety of appropriate aqueous carriers are known to the skilled artisan, including water, buffered water, buffered saline, polyols (e.g., glycerol, propylene glycol, liquid

polyethylene glycol), dextrose solution and glycine. Intravenous vehicles can include various additives, preservatives, or fluid, nutrient or electrolyte replenishers (See, generally, Remington's Pharmaceutical Science, 16th Edition, Mack, Ed. 1980). The compositions can optionally contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents and toxicity adjusting agents, for example, sodium acetate, sodium chloride, potassium chloride, calcium chloride and sodium lactate. The VLP, composition or vaccine can be stored in the liquid stage or can be lyophilized for storage and reconstituted in a suitable carrier prior to use according to art-known lyophilization and reconstitution techniques.

The optimum concentration of the active ingredient(s) in the chosen medium can be determined empirically, according to procedures known to the skilled artisan, and will depend on the ultimate pharmaceutical formulation desired.

Upon formulation, compositions of the present disclosure will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically/prophylactically effective. The dosage ranges for the administration of the molecule of the disclosure are those large enough to produce the desired effect. For example, the composition comprises an effective amount of the VLP, composition or vaccine of the present disclosure. In one example, the composition comprises a therapeutically effective amount of the VLP, composition or vaccine of the present disclosure. In another example, the composition comprises a prophylactically effective amount of the VLP, composition or vaccine of the present disclosure.

The dosage should not be so large as to cause adverse side effects. Generally, the dosage will vary with the age, condition, sex and extent of the disease in the patient and can be determined by one of skill in the art. The dosage can be adjusted by the individual physician in the event of any complication.

Dosage can vary from about 0.1 mg/kg to about 300 mg/kg, e.g., from about 0.2 mg/kg to about 200 mg/kg, such as, from about 0.5 mg/kg to about 20 mg/kg, in one or more dose administrations daily, for one or several days.

In some examples, the VLP, composition or vaccine of the present disclosure is administered at an initial (or loading) dose which is higher than subsequent (maintenance doses). For example, the VLP, composition or vaccine of the present disclosure is administered at an initial dose of between about 10mg/kg to about 30mg/kg. The VLP is then administered at a maintenance dose of between about 0.0001mg/kg to about 10mg/kg. The maintenance doses may be administered every 7-35 days, such as, every 7 or 14 or 28 days.

In some examples, a dose escalation regime is used, in which the VLP, composition or vaccine of the present disclosure is initially administered at a lower dose than used in subsequent doses. This dosage regime is useful in the case of subject's initially suffering adverse events.

In the case of a subject that is not adequately responding to treatment, multiple doses in a week may be administered. Alternatively, or in addition, increasing doses may be administered.

A subject may be retreated with the VLP, composition or vaccine of the present disclosure, by being given more than one exposure or set of doses, such as at least about two exposures of the mRNA, for example, from about 2 to 60 exposures, and more particularly about 2 to 40 exposures, most particularly, about 2 to 20 exposures.

In one example, any retreatment may be given when signs or symptoms of disease return.

In one example, any retreatment may be given when there are no signs or symptoms of disease return.

In another example, any retreatment may be given at defined intervals. For example, subsequent exposures may be administered at various intervals, such as, for example, about 3-4 weeks, or 4-12 weeks, or 24-28 weeks, or 48-56 weeks or longer. For example, such exposures are administered at intervals each of about 3-4 weeks, or 4-8 week, or 4-12 weeks, or 24-26 weeks or about 38-42 weeks, or about 50-54 weeks.

In another example, for subjects experiencing an adverse reaction, the initial (or loading) dose may be split over numerous days in one week or over numerous consecutive days.

Administration of the VLP, composition or vaccine of the present disclosure according to the methods of the present disclosure can be continuous or intermittent, depending, for example, on the recipient's physiological condition, whether the purpose of the administration is therapeutic or prophylactic, and other factors known to skilled practitioners. The administration of the VLP may be essentially continuous over a preselected period of time or may be in a series of spaced doses, e.g., either during or after development of a condition.

Screening Assays

Virus-like particle (VLP) antigens

In one example, the composition comprising the VLP is assessed for expression of the antigens. For example, antigen expression is detected using antibodies against the N protein, S protein, M protein and/or E protein. In one example, the number of cells positive for antigen expression is measured by e.g., fluorescence-activated cell sorting (FACS). In another example the mean fluorescence intensity (MFI) is determined using e.g., FACS.

Quantification of Virus-like particle (VLP) release

In one example, the composition comprising VLPs is assessed for formation and release of VLPs from cells. For example, VLPs release from cells is analysed using antibodies against VLP antigens. In a further example, association between VLP antigens is determined using antibody-mediated co-immunoprecipitation and/or detection of the VLP antigens in the co-immunoprecipitation sample by, for example, Western blot analysis.

Microneutralization Assay

In one example, the composition comprising the VLP (naked and/or formulated) is assessed for antibody responses. For example, the composition comprising the VLP is assessed using a microneutralisation assay. Methods of performing a microneutralization assay will be apparent to the skilled person. In one example, the microneutralization assay is a short form assay. For one example, a virus fluorescent focus-based microneutralization assay is performed. In another example, the microneutralization assay is a long form assay.

Antigen Specific T cell Responses

In one example, the composition comprising the VLP (naked and/or formulated) is assessed for its ability to induce antigen specific T cell responses. Methods of assessing induction of antigen specific T cell responses will be apparent to the skilled person and/or are described herein.

For example, antigen-specific T cell detection is performed on splenic cultures. Briefly, splenocyte cultures are established in T cell medium and cell cultures are either stimulated with antigenic peptides or unstimulated. In one example, antigen-specific T cell responses are determined using flow cytometry.

Methods of Treatment or Prevention

The present disclosure provides, for example, methods of treating or preventing or delaying progression of COVID-19, caused by SARS-CoV-2. The present disclosure also provides, for example, methods of treating or preventing or delaying progression of a SARS-CoV-2 infection. In some examples of the present disclosure the subject has a SARS-CoV-2 infection but does not have clinically diagnosed COVID-19. Thus in an example, the subject may exhibit one or more symptoms of a SARS-CoV-2 infection but COVID-19 is not yet clinically detectable.

Coronavirus Disease 2019 (COVID-19)

The present disclosure provides methods of treating, preventing or delaying the progression of COVID-19 or a SARS-CoV-2 infection in a subject.

COVID-19 is an infectious disease caused by SARS-CoV-2. Common symptoms include fever, cough, fatigue, shortness of breath, and loss of smell and taste. While the majority of cases result in mild symptoms, some progress to ARDS or may result in one or more complications including pneumonia or sepsis. Thus the present disclosure contemplates the treating, preventing or delaying the progression of mild COVID-19 or moderate to severe COVID-19 and includes treating, preventing or delaying the progression of ARDS, pneumonia or sepsis in a subject.

Mild COVID-19 may be considered to include symptoms including fever, cough, fatigue, shortness of breath, and loss of smell and taste. In particular, mild COVID-19 is defined as a SARS-CoV-2 positive RT-PCR or molecular test result, and one of the following symptoms:

- fever;
- sore throat;
- headache;
- muscle pain (myalgia);
- gastrointestinal symptoms;
- cough;
- chest congestion;
- runny nose;
- wheezing;
- skin rash;
- eye irritation or discharge;
- chills;
- new or changing olfactory or taste disorders;
- red or bruised looking feet or toes;
- shaking chills or rigors;
- malaise (loss of appetite, generally unwell, fatigue, physical weakness).

A case is considered mild when it meets the above case definition but not the moderate to severe/critical definition.

Moderate COVID-19 may be defined as: a SARS-CoV-2 positive RT-PCR or molecular test result, AND any one of the following new or worsening signs or symptoms:

- respiratory rate $2 \geq 20$ breaths/minute;
- abnormal saturation of oxygen (SpO_2) but still $\geq 93\%$ on room air at sea level;
- clinical or radiologic evidence of pneumonia;
- Radiologic evidence of DVT;
- shortness of breath or difficulty breathing;

or any 2 of the following new or worsening signs or symptoms:

- fever;
- heart rate $2 \geq 90$ beats/minute;
- shaking chills or rigors;
- new or changing olfactory or taste disorders;
- sore throat;
- malaise;
- headache;
- cough;
- muscle pain (myalgia);

- gastrointestinal symptoms;
- red or bruised looking feet or toes.

Severe/critical COVID-19 is defined as: a SARS-CoV-2 positive RT-PCR or molecular test result; and any one or more of the following:

- clinical signs at rest indicative of severe systemic illness (respiratory rate ≥ 30 breaths/minute, heart rate ≥ 125 beats/minute, $SpO_2 \leq 93\%$ on room air at sea level, or $PaO_2/FiO_2 \leq 300$ mmHg);
- respiratory failure (defined as needing high-flow oxygen, non-invasive ventilation, mechanical ventilation, or ECMO [extracorporeal membrane oxygenation])
- evidence of shock (defined as systolic blood pressure ≤ 90 mmHg, diastolic blood pressure ≤ 60 mmHg, or requiring vasopressors);
- significant acute renal, hepatic, or neurologic dysfunction;
- admission to the ICU;
- death.

The time from exposure to onset of symptoms is typically around five days, but may range from two to fourteen days. Complications of a SARS-CoV-2 infection may include viral pneumonia, secondary bacterial pneumonia, sinus infections, and worsening of previous health problems such as asthma or heart failure. Viral pneumonia may also lead to acute respiratory distress syndrome (ARDS).

Thus, in some examples of the present disclosure, the methods or uses of the present disclosure can be used to treat, prevent or delay progress of ARDS in a subject suffering from COVID-19. In one example, the subject is at risk of having COVID-19 caused by a SARS-CoV-2. In one example, the methods of the present disclosure can be used to treat ARDS in a subject suffering from a SARS-CoV-2 infection. In one example, the methods of the present disclosure can be used to prevent ARDS in a subject suffering from SARS-CoV-2 infection. In one example, the methods of the present disclosure can be used to delay progression of ARDS in a subject suffering from SARS-CoV-2 infection.

A subject in need thereof may be an individual who is displaying a symptom of a SARS-CoV-2 infection or who has been diagnosed with a SARS-CoV-2 infection and/or has COVID-19. Further, a subject in need thereof may be one who has been clinically or biochemically determined to be infected with a SARS-CoV-2 infection or COVID-19. In one embodiment, the subject may be asymptomatic.

A reduction in SARS-CoV-2 infection may be determined using any method known in the art or described herein, including measuring viral load in a sample from the subject after treatment and comparing it to viral load in a sample from the same subject before treatment. Preferably, the sample is taken from the respiratory tract, preferably the upper respiratory tract, for example the nose or pharynx (i.e. throat). Alternatively, responsiveness to a treatment may result in lessening of the severity of one or more of the symptoms described herein.

Acute Respiratory Distress Syndrome (ARDS)

The present disclosure provides methods of treating, preventing or delaying the progression of ARDS in a subject.

ARDS is a life-threatening condition characterized by bilateral pulmonary infiltrates, severe hypoxemia, and disruption of the alveolar-capillary membrane barrier (i.e., pulmonary vascular leak), leading to non-cardiogenic pulmonary edema. There is currently no effective pharmacological therapy.

Infectious etiologies, including influenza, are leading causes of ARDS. Accordingly, in one example of the present disclosure, the ARDS is associated with an SARS-CoV-2 infection. For example, the ARDS is associated with SARS-CoV-2 or COVID-19.

ARDS is classified according to the Berlin Definition, which includes:

- (1) presentation within 1 week of clinical insult or onset of respiratory symptoms;
- (2) acute hypoxemic respiratory failure, as determined by a $\text{PaO}_2/\text{FiO}_2$ ratio of 300 mmHg or less on at least 5 cm of continuous positive airway pressure (CPAP) or positive end expiratory pressure (PEEP), where PaO_2 is the partial pressure of oxygen in arterial blood and the FiO_2 is the fraction of inspired oxygen;
- (3) bilateral opacities on lung radiographs not fully explained by effusions, consolidation, or atelectasis; and
- (4) edema/respiratory failure not fully explained by cardiac failure or fluid overload.

In one example, the subject has or suffers from ARDS (i.e., the subject satisfies the Berlin definition of ARDS). For example, the subject is in need of treatment (i.e., in need thereof).

In one example, the subject has or suffers from a symptom associated with ARDS. Symptoms associated with ARDS and methods of identifying subjects at risk of developing ARDS will be apparent to the skilled person and/or are described herein. For example, the subject has one or more or all of the following symptoms:

- a) a respiratory frequency of greater than 30 breaths per minute;
- b) an oxygen saturation (SpO_2) of 93% or less on room air;
- c) a ratio of arterial partial pressure of oxygen to fraction of inspired oxygen ($\text{PaO}_2/\text{FiO}_2$) of less than 300 mmHg;
- d) a $\text{SpO}_2/\text{FiO}_2$ ratio of less than 218; and
- e) radiographic lung infiltrates in an amount of greater than 50%.

Currently, ARDS is classified as mild, moderate or severe with an associated increased mortality. The severity of ARDS can be categorized according to the Berlin definition as follows:

- (i) Mild ARDS: $\text{PaO}_2/\text{FiO}_2$ of 200-300 mmHg on at least 5 cm CPAP or PEEP;
- (ii) Moderate ARDS: $\text{PaO}_2/\text{FiO}_2$ of 100-200 mmHg on at least 5 cm PEEP; and
- (iii) Severe ARDS: $\text{PaO}_2/\text{FiO}_2$ of less than or equal to 100 mmHg on at least 5 cm PEEP.

In one example, the ARDS is mild ARDS. In another example, the ARDS is moderate ARDS. In a further example, the ARDS is severe ARDS.

The methods of the present disclosure can, in addition to treatment of existing ARDS, be used to prevent or delay the onset of ARDS. Thus, in one example, the subject does not have ARDS.

In one example, the subject is at risk of developing one or more symptom(s) associated with ARDS.

Kits

In one example, the kit comprises a composition or vaccine of the present disclosure, optionally in a delivery system and/or a pharmaceutically acceptable carrier or diluent, packaged with instructions for use in treating or preventing or delaying progression of a SARS-CoV-2 infection and/or a COVID-19 infection in a subject in need thereof. In an example, composition or vaccine comprises a VLP comprising an antigen from each of the S protein, M protein, and E protein of the SARS-CoV-2 and optionally an adjuvant such as MF59. In another example, composition or vaccine comprises a VLP comprising an antigen from each of the S protein, M protein, E protein and N protein of the SARS-CoV-2 and optionally an adjuvant such as MF59. In another example, the kit further comprises a delivery system and/or a pharmaceutically acceptable carrier or diluent, packaged with instructions to administer the composition or vaccine to a subject who is suffering from or at risk of suffering from a SARS-CoV-2 infection or COVID-19.

Thus, in one example, the kit comprises:

- (a) a VLP disclosed herein, a pharmaceutical composition disclosed herein or a vaccine disclosed herein;
- (b) instructions for use thereof; and optionally
- (c) a pharmaceutically acceptable carrier, excipient or diluent.

In accordance with this example of the disclosure, the package insert is on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds or contains a composition that is effective for a disease or disorder of the disclosure and may have a sterile access port (for example, the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the composition is the VLP. The label or package insert indicates that the composition is used for treating a subject eligible for treatment, e.g., one having or predisposed to developing SARS-CoV-2 infection or COVID-19, and/or ARDS, pneumonia or sepsis in a subject having COVID-19, with specific guidance regarding dosing amounts and intervals of treatment and any other medicament being provided. The kit may further comprise an additional container comprising a pharmaceutically acceptable diluent buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution, and/or dextrose solution. The kit may further

include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

The present disclosure includes the following non-limiting Examples.

EXAMPLES

Example 1: Generation of VLPs

VLPs for use in the invention described herein were synthesized through the individual expression of viral structural proteins, which can then self-assemble into the virus-like structure. Combinations of structural capsid proteins from different viruses, and antigens derived from SARS-CoV-2 (e.g. SARS-CoV-2 S, M, E and/or N protein) were used to create the recombinant VLPs. The VLPs lack infectious machinery and express the relevant SARS-CoV-2 antigens (**Figure 1**).

VLPs generated in accordance with the invention include:

VLP1: comprising N protein, S protein, M protein and E protein antigens from the omicron strain of SARS-CoV-2 with or without adjuvant MF59;

VLP2: comprising S protein, M protein and E protein antigens from the omicron strain of SARS-CoV-2 with or without adjuvant MF59.

Example 2: Effect of VLPs on immunogenicity *in vitro*

The inventors sought to test the effect of VLP1, comprising N protein, S protein, M protein and E protein antigens from the omicron strain of SARS-CoV-2 and VLP2, comprising S protein, M protein and E protein antigens from the omicron strain of SARS-CoV-2, on LV microneutralisation, PV microneutralisation and ACE-2 binding inhibition. The effect of VLP-1 and VLP-2 were also tested in the presence of adjuvant MF-59.

Methods

Microneutralization Assay

The VLPs were assessed for antibody responses using a short form microneutralisation assay.

Microneutralization assay short form (MN Assay SF)

Virus fluorescent focus-based microneutralization (FFA MN) assay was performed using an in house developed protocol. RDE treated test mouse samples and positive control sera was heat inactivated, diluted to a starting dilution of 1:40 with PBS, and fourfold serial diluted using the U-Bottom 96 well plate (BD Falcon) in neutralization medium (comprised of minimum essential medium D-MEM (GIBCO), supplemented with 1% BSA (Rockland, BSA-30), 100 U/mL penicillin and 100 ug/mL streptomycin (GIBCO)). SARS-CoV-2 virus was diluted to ~

1,000 – 1,500 fluorescent focus-forming units (FFU)/well (20,000 – 30,000 FFU/mL) in neutralization medium and added in a 1:1 ratio to diluted serum.

After incubation for 2 h at 37°C, 5% CO₂, plates (Half Area 96 well plate, Corning) containing MDCK 33016-PF cells were inoculated with this mixture and incubated overnight for 16 – 18 h at 37°C with 5% CO₂. MDCK 33016-PF cells are seeded as 3.0E4/well (3.0E6/plate) at 6-8h earlier in the cell growth medium (comprised of D-MEM, supplemented with 10% HyClone fetal bovine serum - FBS (Gibco), 100 U/mL penicillin and 100 ug/mL streptomycin). Following the overnight incubation and prior to immunostaining, cells were fixed with cold mixture of acetone and methanol.

The virus was visualized using separate 1 h incubations at room temperature of monoclonal antibodies specific to the virus proteins of interest and Alexa Fluor 488 Goat Anti-Mouse IgG (H+L) Ab (Invitrogen cat. no. A11001) diluted in PBS buffer containing 0.05% tween-20 (Sigma) and 2% BSA (Fraction V, Calbiochem, 2960, 1194C175). Viral protein was quantified by a CTL Immunospot analyzer (Cellular Technology Limited, Shaker Heights, Cleveland, OH), using a fluorescein isothiocyanate (FITC) fluorescence filter set with excitation and emission wavelengths of 482 and 536 nm. Fluorescent foci were enumerated by use of software Immunospot 7.0.12.1 professional analyzer DC, using a custom analysis module.

Results

The inventors first sought to determine the effect of VLP1 and VLP2 on neutralising titers and ACE-2 binding inhibition. As shown in **Figure 2**, both VLP1 and VLP2 had a significant effect on neutralising titers and ACE-2 binding inhibition in the presence of adjuvant MF59. An analysis was then conducted to determine the effect of VLP1 and VLP2 on neutralising titers in different substrains of the omicron strain. As shown in **Figure 3**, the effect on neutralising titer was strongest against BA.1, followed by BA.2. These results demonstrate the utility of the VLPs disclosed herein for use as vaccines to treat or prevent the omicron strain of SARS-CoV-2.

CLAIMS

1. A recombinant virus-like particle (VLP) comprising one or more antigens from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), wherein the one or more antigens are selected from:

- (a) a spike (S) protein;
- (b) a membrane (M) protein;
- (c) an envelope (E) protein;
- (d) a nucleocapsid (N) protein,

wherein at least one antigen is from the omicron strain of SARS-CoV-2.

2. The recombinant VLP of claim 1, wherein the VLP comprises an antigen from the S protein of the omicron strain of a SARS-CoV-2.

3. The recombinant VLP of claim 1 or 2, wherein the VLP comprises an antigen from each of the S protein, M protein, and E protein of a SARS-CoV-2.

4. The recombinant VLP of claim 3, wherein the VLP comprises an antigen from each of the S protein, M protein, and E protein of the omicron strain of a SARS-CoV-2.

5. The recombinant VLP of any one of claims 1 to 4, wherein the VLP comprises an antigen from the S protein from an omicron variant of SARS-CoV-2 selected from the group consisting of B.1.1.529, BA.1, BA.2, BA.4, BA.5, BA.2.12.1 and BA.2.75.

6. The recombinant VLP of claim 5, wherein the S protein is from omicron variant BA.1 of SARS-CoV-2 and comprises one or more or all of the mutations selected from the group consisting of A67V, T95I, Y145D, L212L, S371L, G446S, G496S, T547K, N856K, L981F, G142D, Q493R, G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K and del69-70.

7. The recombinant VLP of claim 5, wherein the S protein is from omicron variant BA.2 of SARS-CoV-2 and comprises one or more or all of the mutations selected from the group consisting of G142D, Q493R, del24-26, G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K, T19I, A27S, G142D, V213G, S371F, T376A, D405N and R408S.

8. The recombinant VLP of claim 5, wherein the S protein is from omicron variant BA.4 or BA.5 of SARS-CoV-2 and comprises one or more or all of the mutations selected from the group consisting of L452R, F486V, R493Q, del24-26, del69-70, G339D, S373P, S375F, K417N, N440K, S477N, T478K, E484A, Q498R, N501Y, Y505H, D614G, H655Y, N679K, P681H, N764K, D796Y, Q954H, N969K, T19I, A27S, G142D, V213G, S371F, T376A, D405N and R408S.
9. The recombinant VLP of claim 3 or 4, wherein each of the S protein, M protein and E protein are provided as a fusion polypeptide.
10. The recombinant VLP of any one of claims 1 to 9, wherein each of the antigens are formulated in separate VLPs or the same VLP.
11. The recombinant VLP of any one of claims 1 to 3 or 5 to 10, wherein at least one of the antigens is from the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of SARS-CoV-2.
12. The recombinant VLP of claim 3 or 4, wherein the VLP further comprises an antigen from the N protein of SARS-CoV-2.
13. The recombinant VLP of any one of claims 1 to 12, wherein each of the S protein, E protein, M protein and optionally the N protein are provided as a fusion polypeptide.
14. The recombinant VLP of claim 13, wherein each of the antigens are formulated in separate VLPs or the same VLP.
15. The recombinant VLP of any one of claims 1 to 14, wherein the VLP has a diameter of between about 70nm and 160nm.
16. The recombinant VLP of claim 15, wherein the VLP has a diameter of about 80nm.
17. The recombinant VLP of any one of claims 1 to 16, wherein the VLP is formulated in a lipid nanoparticle (LNP).
18. An isolated, recombinant or synthetic nucleotide sequence encoding the VLP of any one of claims 1 to 17.
19. An expression vector comprising the nucleotide sequence of claim 18.

20. An immunogenic composition comprising a VLP of any one of claims 1 to 17.
21. A pharmaceutical composition comprising a VLP of any one of claims 1 to 17 and a pharmaceutically acceptable carrier.
22. The pharmaceutical composition of claim 21 for use as a vaccine.
23. A vaccine comprising the pharmaceutical composition of claim 22.
24. The immunogenic composition of claim 20, the pharmaceutical composition of claim 21 or the vaccine of claim 23, further comprising an adjuvant.
25. The immunogenic composition, pharmaceutical composition or the vaccine of claim 24, wherein the adjuvant is MF59.
26. A method of treating or preventing or delaying progression of a SARS-CoV-2 infection in a subject in need thereof, the method comprising administering the VLP of any one of claims 1 to 17, the immunogenic composition of any one of claims 20, 24 or 25, the pharmaceutical composition of any one of claims 21, 22, 24 or 25, or the vaccine of any one of claims 23 to 25 to the subject.
27. Use of the VLP of any one of claims 1 to 17, the immunogenic composition of any one of claims 20, 24 or 25, the pharmaceutical composition of any one of claims 21, 22, 24 or 25, or the vaccine of any one of claims 23 to 25 in the manufacture of a medicament for treating or preventing or delaying progression of a SARS-CoV-2 infection in a subject.
28. The VLP of any one of claims 1 to 17, the immunogenic composition of any one of claims 20, 24 or 25, the pharmaceutical composition of any one of claims 21, 22, 24 or 25, or the vaccine of any one of claims 23 to 25 for use in the treatment or prevention or delaying progression of a SARS-CoV-2 infection.
29. The method of claim 26, the use of claim 27 or the VLP, composition or vaccine for use of claim 28, wherein the subject has, or is suspected of having, COVID-19.
30. A method of inducing an immune response in a subject, the method comprising administering the VLP of any one of claims 1 to 17, the immunogenic composition of any one of claims 20, 24 or 25, the pharmaceutical composition of any one of claims 21, 22, 24 or 25, or the vaccine of any one of claims 23 to 25 to the subject in need thereof.

31. Use of the VLP of any one of claims 1 to 17, the immunogenic composition of any one of claims 20, 24 or 25, the pharmaceutical composition of any one of claims 21, 22, 24 or 25, or the vaccine of any one of claims 23 to 25 in the manufacture of a medicament for inducing an immune response in a subject in need thereof.
32. The VLP of any one of claims 1 to 17, the immunogenic composition of any one of claims 20, 24 or 25, the pharmaceutical composition of any one of claims 21, 22, 24 or 25, or the vaccine of any one of claims 23 to 25 for use in inducing an immune response in a subject in need thereof.
33. The method of claim 30, the use of claim 31, or the VLP, composition or vaccine for use of claim 32, wherein the immune response is a humoral and/or a cell-mediated immune response.
34. The method, the use or the VLP, composition or vaccine for use of claim 33, wherein the immune response is sufficient to treat, prevent or delay progression of at least one symptom of a SARS-CoV-2 infection caused by the omicron strain of a SARS-CoV-2.
35. The method, the use or the VLP, composition or vaccine for use of claim 33, wherein the immune response is sufficient to treat, prevent or delay progression of at least one symptom of a SARS-CoV-2 infection caused by the delta, beta, alpha, gamma or 2019-nCoV/USA-WA1/2020 strain of a SARS-CoV-2.
36. A method for reducing SARS-CoV-2 viral load in a subject with coronavirus disease 2019 (COVID-19) comprising administering the VLP of any one of claims 1 to 17, the immunogenic composition of any one of claims 20, 24 or 25, the pharmaceutical composition of any one of claims 21, 22, 24 or 25, or the vaccine of any one of claims 23 to 25 to the subject in need thereof.
37. Use of the VLP of any one of claims 1 to 17, the immunogenic composition of any one of claims 20, 24 or 25, the pharmaceutical composition of any one of claims 21, 22, 24 or 25, or the vaccine of any one of claims 23 to 25 in the preparation of a medicament for reducing SARS-CoV-2 viral load in a subject with coronavirus disease 2019 (COVID-19).
38. The VLP of any one of claims 1 to 17, the immunogenic composition of any one of claims 20, 24 or 25, the pharmaceutical composition of any one of claims 21, 22, 24 or 25, or the vaccine of any one of claims 23 to 25 for use in reducing SARS-CoV-2 viral load in a subject with coronavirus disease 2019 (COVID-19).
39. A method for treating or preventing or delaying progression of acute respiratory distress syndrome in a subject with coronavirus disease 2019 (COVID-19) comprising administering the

VLP of any one of claims 1 to 17, the immunogenic composition of any one of claims 20, 24 or 25, the pharmaceutical composition of any one of claims 21, 22, 24 or 25, or the vaccine of any one of claims 23 to 25 to the subject in need thereof.

40. Use of the VLP of any one of claims 1 to 17, the immunogenic composition of any one of claims 20, 24 or 25, the pharmaceutical composition of any one of claims 21, 22, 24 or 25, or the vaccine of any one of claims 23 to 25 in the preparation of a medicament for treating or preventing or delaying progression of acute respiratory distress syndrome in a subject with coronavirus disease 2019 (COVID-19).

41. The VLP of any one of claims 1 to 17, the immunogenic composition of any one of claims 20, 24 or 25, the pharmaceutical composition of any one of claims 21, 22, 24 or 25, or the vaccine of any one of claims 23 to 25 for use in treating or preventing or delaying progression of acute respiratory distress syndrome in a subject with coronavirus disease 2019 (COVID-19).

42. The method of any one of claims 26, 29, 30, 33 to 36 or 39, the use of any one of claims 27, 31, 33 to 35, 37 or 40 or the VLP, vaccine or composition for use of any one of claims 28, 32 to 35, 38 or 41, wherein the subject is a human of 18 years of age or older.

43. The method, the use or the VLP vaccine or composition for use of claim 42, wherein the VLP, vaccine or composition is administered in a one dose regimen.

44. The method, the use or the VLP, vaccine or composition for use of claim 42, wherein the VLP, vaccine or composition is administered in a two, three or four dose regimen, wherein the doses are administered about 1, 2 or 3 months apart.

45. A eukaryotic cell for expressing the VLP of any one of claims 1 to 17.

46. A method for producing a VLP comprising:

(a) providing an expression vector comprising polynucleotides encoding one or more antigens from a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), wherein the antigens are selected from:

- (i) a spike (S) protein;
- (ii) a membrane (M) protein;
- (iii) an envelope (E) protein;
- (iv) a nucleocapsid (N) protein

wherein at least one antigen is from the omicron strain of SARS-Cov-2;

(b) providing a host cell, and

(c) transfecting said host cell with said vector to produce virus-like particles (VLPs) comprising the one or more antigens,
wherein the polynucleotides are expressed under conditions sufficient for the formation of VLPs.

47. The method according to claim 46, further comprising purifying the VLPs.

48. The method according to claim 46 or 47, wherein the polynucleotides encode antigens from each of the S protein, M protein and E protein expressed from monocistronic polynucleotides, wherein the polynucleotides are operably linked to a promoter sufficient to drive expression of the antigens encoded by the polynucleotides.

49. The method according to claim 46 or 47, wherein the polynucleotides encode antigens from each of the S protein, M protein and E protein expressed from a polycistronic polynucleotide, wherein the polynucleotides are operably linked to a promoter sufficient to drive expression of the antigens encoded by the polynucleotides.

50. A kit comprising:

(a) the VLP of any one of claims 1 to 17, the pharmaceutical composition of any one of claims 21, 22 or 24 to 25, the immunogenic composition of any one of claims 20, 24 or 25 or the vaccine of any one of claims 23 to 25;

(b) instructions for use thereof; and optionally

(c) a pharmaceutically acceptable carrier, excipient or diluent.