E484K mutation in RBD, which is of particular concern, and P.1 in particular also contains K417T and N501Y mutations in RBD.

The emergence of multiple strains of the B.1.351 lineage (also known as 501Y.V2), which were first reported in South Africa and have since spread globally, are of great concern. This lineage bears three RBD mutations, K417N, E484K, and N501Y, in addition to several mutations outside of RBD. Using BNT162b2-elicited serum it was reported that, as compared with neutralization of the USA-WA1/2020 strain, neutralization of B.1.1.7-spike and P.1-spike viruses was roughly equivalent, and neutralization of B.1.351-spike virus was robust but lower (Liu Y. et al., 2021, N Engl J Med., doi: 10.1056/NEJMc2102017. Epub ahead of print. PMID: 33684280). In view of the emergence of novel variants that appear to at least partially escape immune responses there is a need for vaccines that are effective against variants of SARS-CoV-2.

The present disclosure provides vaccines that include epitopes of diverse virus protein variants and methods for providing such vaccines. Instead of re-formulating existing vaccines to include diverse virus protein sequences of different virus variants or the coding nucleic acids, the strategy described herein is based on combining diverse epitopes of different virus variants on a limited number of molecules. This allows for the administration of a relatively low dose of the active ingredient, in particular mRNA, while achieving sufficiently high doses of each epitope to induce an effective immune response. It is envisioned that the vaccines described herein are capable of eliciting broadly neutralizing antibodies and thus, are suitable to resolve the ongoing SARS-CoV-2 pandemic.

Summary

The present disclosure generally relates to a vaccine wherein epitopes of different variants of a virus protein are combined on a single molecule. Such molecule comprises at least a portion of a virus protein comprising amino acid modifications present in other variants of the virus protein. The modification generates (additional) epitopes that are specific for such other virus protein variants. Accordingly, the modified virus protein sequences described herein are polyspecific virus protein amino acid sequences. In one aspect, the present disclosure provide a method comprising the steps: a) identifying amino acid positions in a parental virus protein

which are modified compared to the corresponding amino acid positions of one or more virus protein variants; and b) providing an amino acid sequence comprising at least a fragment of the parental virus protein, wherein amino acid positions in the at least a fragment of a parental virus protein are modified to comprise amino acids found in the corresponding amino acid positions of one or more virus protein variants, or a nucleotide sequence encoding the modified amino acid sequence. In another aspect, the present disclosure provides a method comprising the steps: a) identifying amino acid positions in a parental virus protein which are modified compared to the corresponding amino acid positions of one or more virus protein variants; b) substituting codons of a first nucleotide sequence encoding an amino acid sequence comprising at least a fragment of a parental virus protein with other codons to obtain a first mutated nucleotide sequence that encodes a modified amino acid sequence, wherein amino acid positions in the at least a fragment of a parental virus protein are modified to comprise amino acids found in the corresponding amino acid positions of one or more virus protein variants; and c) substituting codons of a second nucleotide sequence encoding an amino acid sequence comprising at least a fragment of a parental virus protein with other codons to obtain a second mutated nucleotide sequence that encodes a modified amino acid sequence, wherein amino acid positions in the at least a fragment of a parental virus protein are modified to comprise amino acids found in the corresponding amino acid positions of one or more virus protein variants, wherein the amino acid modifications in b) at least partially differ from the amino acid modifications in c).

In yet another aspect, the present disclosure provides a method comprising the steps: a) providing a nucleic acid comprising a first nucleotide sequence encoding an amino acid sequence comprising at least a fragment of a parental virus protein, wherein amino acid positions in the at least a fragment of a parental virus protein are modified to comprise amino acids found in the corresponding amino acid positions of one or more virus protein variants; and b) providing a nucleic acid comprising a second nucleotide sequence encoding an amino acid sequence comprising at least a fragment of a parental virus protein, wherein amino acid positions in the at least a fragment of a parental virus protein are modified to comprise amino acids found in the corresponding amino acid positions of one or more virus protein variants,

wherein the amino acid modifications in b) at least partially differ from the amino acid modifications in a).

In yet another aspect, the present disclosure provides a medical preparation comprising: a) a nucleic acid comprising a first nucleotide sequence encoding an amino acid sequence comprising at least a fragment of a parental virus protein, wherein amino acid positions in the at least a fragment of a parental virus protein are modified to comprise amino acids found in the corresponding amino acid positions of one or more virus protein variants; and b) a nucleic acid comprising a second nucleotide sequence encoding an amino acid sequence comprising at least a fragment of a parental virus protein, wherein amino acid positions in the at least a fragment of a parental virus protein are modified to comprise amino acids found in the corresponding amino acid positions of one or more virus protein variants, wherein the amino acid modifications in b) at least partially differ from the amino acid modifications in a).

In a further aspect, the present disclosure provides a use of the medical preparation as described herein in the manufacture of a medicament for inducing an immune response against a virus in a subject. In a further aspect, the present disclosure provides the medical preparation as described herein for use in therapy.

In one example, a polyspecific virus

protein amino acid sequence is a modified full-length virus protein. In one example, a polyspecific virus protein amino acid sequence is a modified portion of a virus protein.

In some examples, a polyspecific virus protein amino acid sequence provided herein may comprise immunoreactive epitopes ((modified) amino acid residues) from a plurality (e.g., at least two or more, including, e.g., at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, etc.) of virus protein variants, e.g., immunoreactive epitopes from the parental virus protein and additionally immunoreactive epitopes from one or more virus protein variants which are different from the parental virus protein. In various examples, a polyspecific virus protein amino acid sequence provided herein may comprise all immunoreactive epitopes ((modified) amino acid residues) from one or more virus protein variants which are different from the parental virus protein, or a portion thereof. In various examples, a polyspecific virus protein amino acid sequence provided herein may comprise a plurality (e.g., at least two or more, including, e.g., at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, etc.) of immunoreactive epitopes ((modified) amino acid residues) from one or more virus protein variants which are different from the parental virus protein.

In some examples, an amino acid sequence comprising a polyspecific virus protein amino acid sequence is encoded by a nucleic acid such as DNA or RNA, in particular RNA. In some examples, a plurality of amino acid sequences each of which comprises a polyspecific virus protein amino acid sequence is encoded by a nucleic acid such as DNA or RNA, in particular RNA. In some examples, a plurality of amino acid sequences each of which comprises a polyspecific virus protein amino acid sequence is encoded by a plurality of nucleic acid molecules such as DNA or RNA molecules, in particular RNA molecules.

The amino acid sequences comprising polyspecific virus protein amino acid sequences described herein and nucleic acids encoding these amino acid sequences are particularly useful, when considering the genetic diversity of viruses such as RNA viruses, to provide protection against a plurality of virus variants. The amino acid sequences comprising polyspecific virus protein amino acid sequences described herein and nucleic acids encoding these amino acid sequences may offer an opportunity for development of a diverse and/or otherwise robust (e.g., persistent, e.g., detectable about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50,

55, 60 or more days after administration of one or more doses) neutralizing antibody and/or T cell response, e.g., TH1-type T cell (e.g., CD4+ and/or CD8+ T cell) response. The amino acid sequences comprising polyspecific virus protein amino acid sequences described herein and nucleic acids encoding the amino acid sequences are expected to elicit immune responses, in particular antibody response, that broadly and specifically neutralize a plurality (e.g., at least two or more, including, e.g., at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, etc.) of virus variant strains and have the potential to generate protective immune responses to a range of virus variant strains in a limited number of constructs. In general, the limited number of constructs includes a number of molecules (protein and/or nucleic acid molecules) which is less than the number of the different virus protein variants, e.g., 1, 2, 3, or 4 molecules, such that each molecule includes epitope sequences that correspond to a plurality of virus protein variants. In general, the amino acid sequences comprising polyspecific virus protein amino acid sequences described herein , i.e., vaccine antigens, and nucleic acids encoding these amino acid sequences are useful for the immunotherapeutic treatment of a subject. A vaccine antigen comprises virus protein epitopes that are derived from and specific for inducing an immune response against a plurality of virus protein variants, and thus, virus variant strains, in the subject. In one example, the present disclosure comprises the administration of nucleic acid such as RNA, i.e., vaccine RNA, encoding one or more of the vaccine antigens described herein. In one example, the present disclosure comprises the administration of a plurality, e.g., 2, 3, or 4, of nucleic acid molecules such as RNA molecules encoding different vaccine antigens. The different vaccine antigens (potentially based on the same parental virus protein sequence) may comprise different modifications (optionally from different virus strains) and thus, different immunogenic spectra. RNA encoding vaccine antigen may be administered to provide (following expression of the RNA by appropriate target cells) antigen for induction, i.e., stimulation, priming and/or expansion, of an immune response, e.g., antibodies and/or immune effector cells, which is targeted to target antigen (virus protein, in particular different virus protein variants) or a procession product thereof. In one example, the immune response which is to be induced according to the present disclosure is a B cell-mediated immune response, i.e., an antibody-mediated immune response. Additionally or alternatively,

in one example, the immune response which is to be induced according to the present disclosure is a T cell-mediated immune response. In one example, the immune response is an anti-virus immune response. In one example, the immune response is an immune response which is directed against a plurality of virus strains.

In one example, the disclosure relates to a method comprising the steps:

a) identifying amino acid positions in a parental virus protein which are modified compared to the corresponding amino acid positions of one or more virus protein variants; and

b) providing an amino acid sequence comprising at least a fragment of the parental virus protein, wherein amino acid positions in the at least a fragment of a parental virus protein are modified to comprise amino acids found in the corresponding amino acid positions of one or more virus protein variants, or a nucleotide sequence encoding the modified amino acid sequence.

In one example, the method comprises repeating step b) to provide two or more of the modified amino acid sequences, or two or more of the nucleotide sequences encoding two or more of the modified amino acid sequences.

In one example, the two or more modified amino acid sequences are based on the same parental virus protein.

In one example, the amino acid modifications in the two or more modified amino acid sequences are at least partially different.

In one example, providing the nucleotide sequence comprises:

b') substituting codons of a nucleotide sequence encoding an amino acid sequence comprising at least a fragment of a parental virus protein with other codons to obtain a mutated nucleotide sequence that encodes a modified amino acid sequence, wherein amino acid positions in the at least a fragment of a parental virus protein are modified to comprise amino acids found in the corresponding amino acid positions of one or more virus protein variants.

In one example, the method comprises repeating step b') to provide two or more of the mutated nucleotide sequences encoding two or more of the modified amino acid sequences.

In one example, the two or more modified amino acid sequences are based on the same parental virus protein.

In one example, the amino acid modifications in the two or more modified amino acid sequences are at least partially different.

In one example, the disclosure relates to a method comprising the steps:

a) identifying amino acid positions in a parental virus protein which are modified compared to the corresponding amino acid positions of one or more virus protein variants;

b) substituting codons of a first nucleotide sequence encoding an amino acid sequence comprising at least a fragment of a parental virus protein with other codons to obtain a first mutated nucleotide sequence that encodes a modified amino acid sequence, wherein amino acid positions in the at least a fragment of a parental virus protein are modified to comprise amino acids found in the corresponding amino acid positions of one or more virus protein variants; and

c) substituting codons of a second nucleotide sequence encoding an amino acid sequence comprising at least a fragment of a parental virus protein with other codons to obtain a second mutated nucleotide sequence that encodes a modified amino acid sequence, wherein amino acid positions in the at least a fragment of a parental virus protein are modified to comprise amino acids found in the corresponding amino acid positions of one or more virus protein variants,

wherein the amino acid modifications in b) at least partially differ from the amino acid modifications in c).

In one example, the method may comprise one or more further such steps of substituting codons to obtain a mutated nucleotide sequence encoding at least partially different amino acid modifications.

In one example, the at least a fragment of a parental virus protein comprised in the amino acid sequence encoded by the first nucleotide sequence and the at least a fragment of a parental virus protein comprised in the amino acid sequence encoded by the second nucleotide sequence are identical.

In one example, the amino acid sequence encoded by the first nucleotide sequence and the amino acid sequence encoded by the second nucleotide sequence are identical.

In one example, the first nucleotide sequence and the second nucleotide sequence are identical.

In one example, one or more of the modified amino acid positions in the modified amino acid sequence encoded by the first mutated nucleotide sequence differ from the modified amino acid positions in the modified amino acid sequence encoded by the second mutated nucleotide sequence, i.e., one or more different positions are modified.

In one example, one or more amino acids in modified amino acid positions modified in the modified amino acid sequence encoded by the first mutated nucleotide sequence and in the modified amino acid sequence encoded by the second mutated nucleotide sequence differ from each other, i.e., one or more of the same positions are modified, however, with different amino acid residues.

In one example, the amino acid sequence comprising at least a fragment of a parental virus protein comprises the amino acid sequence of a full-length virus protein.

In one example, the method further comprises providing nucleic acid comprising the nucleotide sequence encoding a modified amino acid sequence.

In one example, the method further comprises providing a vaccine comprising nucleic acid comprising the nucleotide sequence encoding a modified amino acid sequence.

In one example, the nucleic acid is RNA.

In one example, the method is a method of generating a vaccine.

In one example, the vaccine is an RNA vaccine.

In one example, the vaccine has a reduced risk for immune escape.

In one example, the modified amino acid positions are amino acid positions at which the amino acid sequence of the one or more virus protein variants differs from the amino acid sequence of the parental virus protein.

In one example, the modified amino acid positions are amino acid positions at which the amino acid sequence of the one or more virus protein variants differs from the amino acid sequence of the wildtype virus protein.

In one example, the modified amino acid positions are potential sites for escape mutants of the virus.

In one example, the escape mutants of the virus are antibody escape mutants of the virus.

In one example, the escape mutants of the virus are resistant to neutralization by antibody against the virus protein.

In one example, the virus protein of the escape mutants of the virus shows reduced antibody binding.

In one example, the antibody is used for treating a patient infected with the virus.

In one example, the antibody is generated in a patient that has been treated with a vaccine against infection with the virus.

In one example, the parental virus protein is modified compared to the wildtype virus protein.

In one example, in the modified amino acid sequence amino acid positions in the parental virus protein which are modified compared to the wildtype virus protein are not modified.

In one example, the parental virus protein is the virus protein of a parental virus strain.

In one example, the parental virus strain is a natural isolate, or the parental virus strain is a mutant of a natural isolate.

In one example, the parental virus strain is a virus variant strain that is prevalent or rapidly spreading.

In one example, the parental virus strain is a virus variant that is a variant of concern.

In one example, the one or more virus protein variants are modified compared to the wildtype virus protein.

In one example, the one or more virus protein variants are modified compared to the parental virus protein.

In one example, in the modified amino acid sequence amino acid positions in the one or more virus protein variants which are modified compared to the wildtype virus protein and/or the parental virus protein are modified.

In one example, one or more (e.g., all) of the one or more virus protein variants are the virus proteins of one or more virus strains.

In one example, one or more (e.g., all) of the one or more virus strains are natural isolates, or one or more (e.g., all) of the one or more virus strains are mutants of a natural isolate.

In one example, one or more (e.g., all) of the one or more virus strains are virus variant strains that are prevalent or rapidly spreading.

In one example, one or more (e.g., all) of the one or more virus strains are virus variant strains that are variants of concern.

In one example, the parental virus strain and the one or more virus strains are virus variant strains that are prevalent or rapidly spreading.

In one example, the parental virus strain and the one or more virus strains are virus variant strains that are variants of concern.

In one example, the parental virus protein and the one or more virus protein variants are modified compared to the wildtype virus protein.

In one example, the one or more virus protein variants comprise virus protein variants of at least two virus strains.

In one example, the one or more virus protein variants in b) are different from the one or more virus protein variants in c).

In one example, in the modified amino acid sequence amino acid modifications in the parental virus protein compared to the wildtype virus protein do not interfere with amino acid modifications in the modified amino acid positions.

In one example, in the modified amino acid sequence amino acid modifications in the parental virus protein compared to the wildtype virus protein are not in close spatial distance to modified amino acid positions or amino acid modifications in the modified amino acid positions.

In one example, in the modified amino acid sequence modifications in the modified amino acid positions do not result in major structural rearrangements.

In one example, the amino acids in the modified amino acid positions are surface exposed.

In one example, the modified amino acid positions comprise one or more, e.g., at least two amino acid positions. The modifications may correspond to one or more virus protein variants/virus strains and may encompass all modifications present in the one or more virus protein variants/virus strains compared to the parental virus protein/virus strain, or a portion thereof.

In one example, the modified amino acid positions in b) and c) each comprise one or more, e.g., at least two amino acid positions.

In one example, the disclosure relates to a method comprising the steps:

a) providing a nucleic acid comprising a first nucleotide sequence encoding an amino acid sequence comprising at least a fragment of a parental virus protein, wherein amino acid

positions in the at least a fragment of a parental virus protein are modified to comprise amino acids found in the corresponding amino acid positions of one or more SARS-CoV-2 S protein variants; and

b) providing a nucleic acid comprising a second nucleotide sequence encoding an amino acid sequence comprising at least a fragment of a parental virus protein, wherein amino acid positions in the at least a fragment of a parental virus protein are modified to comprise amino acids found in the corresponding amino acid positions of one or more virus protein variants,

wherein the amino acid modifications in b) at least partially differ from the amino acid modifications in a).

In one example, the method may comprise one or more further such steps of providing a nucleic acid encoding at least partially different amino acid modifications.

In one example, the nucleic acid is RNA.

Further examples are as described herein.

In one example, the disclosure relates to a medical preparation comprising:

a) a nucleic acid comprising a first nucleotide sequence encoding an amino acid sequence comprising at least a fragment of a parental virus protein, wherein amino acid positions in the at least a fragment of a parental virus protein are modified to comprise amino acids found in the corresponding amino acid positions of one or more virus protein variants; and

b) a nucleic acid comprising a second nucleotide sequence encoding an amino acid sequence comprising at least a fragment of a parental virus protein, wherein amino acid positions in the at least a fragment of a parental virus protein are modified to comprise amino acids found in the corresponding amino acid positions of one or more virus protein variants,

wherein the amino acid modifications in b) at least partially differ from the amino acid modifications in a).

In one example, the medical preparation may comprise one or more further nucleic acids encoding at least partially different amino acid modifications.

In one example, the nucleic acid is RNA.

In one example, the RNA is formulated in lipid nanoparticles (LNP).

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

In one example, the medical preparation is a vaccine.

In one example, the medical preparation is a kit.

In one example, the medical preparation further comprises instructions for use of the medical preparation for vaccination against infection with the virus.

In one example, the disclosure relates to the medical preparation for pharmaceutical use.

In one example, the pharmaceutical use comprises vaccination against infection with the virus.

In one example, the disclosure relates to a method of inducing an immune response against the virus in a subject comprising administering to the subject the medical preparation.

In one example, the method is a method for prophylactic treatment against infection with the virus.

In one example, the method is a method for vaccination against infection with the virus.

Further examples of the above examples are as described herein.

In one example, the virus described herein is SARS-CoV-2. In one example, the virus protein described herein is SARS-CoV-2 spike protein (S protein).

In one example, the disclosure relates to a method comprising the steps:

a) identifying amino acid positions in a parental SARS-CoV-2 spike protein (S protein) which are modified compared to the corresponding amino acid positions of one or more SARS-CoV-2 S protein variants; and

b) providing an amino acid sequence comprising at least a fragment of the parental SARS-CoV-2 S protein, wherein amino acid positions in the at least a fragment of a parental SARS-CoV-2 S protein are modified to comprise amino acids found in the corresponding amino acid positions of one or more SARS-CoV-2 S protein variants, or a nucleotide sequence encoding the modified amino acid sequence.

In one example, the method comprises repeating step b) to provide two or more of the modified amino acid sequences, or two or more of the nucleotide sequences encoding two or more of the modified amino acid sequences.

In one example, the two or more modified amino acid sequences are based on the same parental SARS-CoV-2 S protein.

In one example, the amino acid modifications in the two or more modified amino acid sequences are at least partially different.

In one example, providing the nucleotide sequence comprises:

b') substituting codons of a nucleotide sequence encoding an amino acid sequence comprising at least a fragment of a parental SARS-CoV-2 S protein with other codons to obtain a mutated nucleotide sequence that encodes a modified amino acid sequence, wherein amino acid positions in the at least a fragment of a parental SARS-CoV-2 S protein are modified to comprise amino acids found in the corresponding amino acid positions of one or more SARS-CoV-2 S protein variants.

In one example, the method comprises repeating step b') to provide two or more of the mutated nucleotide sequences encoding two or more of the modified amino acid sequences.

In one example, the two or more modified amino acid sequences are based on the same parental SARS-CoV-2 S protein.

In one example, the amino acid modifications in the two or more modified amino acid sequences are at least partially different.

In one example, the disclosure relates to a method comprising the steps:

a) identifying amino acid positions in a parental SARS-CoV-2 spike protein (S protein) which are modified compared to the corresponding amino acid positions of one or more SARS-CoV-2 S protein variants;

b) substituting codons of a first nucleotide sequence encoding an amino acid sequence comprising at least a fragment of a parental SARS-CoV-2 S protein with other codons to obtain a first mutated nucleotide sequence that encodes a modified amino acid sequence, wherein amino acid positions in the at least a fragment of a parental SARS-CoV-2 S protein are modified to comprise amino acids found in the corresponding amino acid positions of one or more SARS-CoV-2 S protein variants; and

c) substituting codons of a second nucleotide sequence encoding an amino acid sequence comprising at least a fragment of a parental SARS-CoV-2 S protein with other codons to obtain a second mutated nucleotide sequence that encodes a modified amino acid sequence, wherein amino acid positions in the at least a fragment of a parental SARS-CoV-2 S protein are

modified to comprise amino acids found in the corresponding amino acid positions of one or more SARS-CoV-2 S protein variants,

wherein the amino acid modifications in b) at least partially differ from the amino acid modifications in c).

In one example, the method may comprise one or more further such steps of substituting codons to obtain a mutated nucleotide sequence encoding at least partially different amino acid modifications.

In one example, the at least a fragment of a parental SARS-CoV-2 S protein comprised in the amino acid sequence encoded by the first nucleotide sequence and the at least a fragment of a parental SARS-CoV-2 S protein comprised in the amino acid sequence encoded by the second nucleotide sequence are identical.

In one example, the amino acid sequence encoded by the first nucleotide sequence and the amino acid sequence encoded by the second nucleotide sequence are identical.

In one example, the first nucleotide sequence and the second nucleotide sequence are identical.

In one example, one or more of the modified amino acid positions in the modified amino acid sequence encoded by the first mutated nucleotide sequence differ from the modified amino acid positions in the modified amino acid sequence encoded by the second mutated nucleotide sequence.

In one example, one or more amino acids in modified amino acid positions modified in the modified amino acid sequence encoded by the first mutated nucleotide sequence and in the modified amino acid sequence encoded by the second mutated nucleotide sequence differ from each other.

In one example, the amino acid sequence comprising at least a fragment of a parental SARS-CoV-2 S protein comprises the amino acid sequence of the N-terminal domain (NTD) and/or receptor binding domain (RBD) of a SARS-CoV-2 S protein.

In one example, the amino acid sequence comprising at least a fragment of a parental SARS-CoV-2 S protein comprises the amino acid sequence of a full-length SARS-CoV-2 S protein.

In one example, the method further comprises providing nucleic acid comprising the nucleotide sequence encoding a modified amino acid sequence.

In one example, the method further comprises providing a vaccine comprising nucleic acid comprising the nucleotide sequence encoding a modified amino acid sequence.

In one example, the nucleic acid is RNA.

In one example, the method is a method of generating a SARS-CoV-2 vaccine.

In one example, the vaccine is an RNA vaccine.

In one example, the vaccine has a reduced risk for immune escape.

In one example, one or more of the modified amino acid positions are located within the N-terminal domain (NTD) and/or the receptor binding domain (RBD) of a SARS-CoV-2 S protein.

In one example, the modified amino acid positions are amino acid positions at which the amino acid sequence of the one or more SARS-CoV-2 S protein variants differs from the amino acid sequence of the parental SARS-CoV-2 S protein.

In one example, the modified amino acid positions are amino acid positions at which the amino acid sequence of the one or more SARS-CoV-2 S protein variants differs from the amino acid sequence of wildtype SARS-CoV-2 S protein.

In one example, the modified amino acid positions are potential sites for escape mutants of SARS-CoV-2.

In one example, the escape mutants of SARS-CoV-2 are antibody escape mutants of SARS-CoV-2.

In one example, the escape mutants of SARS-CoV-2 are resistant to neutralization by antibody against SARS-CoV-2 S protein.

In one example, the SARS-CoV-2 S protein of the escape mutants of SARS-CoV-2 shows reduced antibody binding.

In one example, the antibody is used for treating a patient infected with SARS-CoV-2.

In one example, the antibody is generated in a patient that has been treated with a SARS-CoV-2 vaccine.

In one example, the parental SARS-CoV-2 S protein is modified compared to wildtype SARS-CoV-2 S protein.

In one example, in the modified amino acid sequence amino acid positions in the parental SARS-CoV-2 S protein which are modified compared to wildtype SARS-CoV-2 S protein are not modified.

In one example, the parental SARS-CoV-2 S protein is the S protein of a parental SARS-CoV-2 strain.

In one example, the parental SARS-CoV-2 strain is a natural isolate, or the parental SARS-CoV-2 strain is a mutant of a natural isolate.

In one example, the parental SARS-CoV-2 strain is a SARS-CoV-2 variant strain that is prevalent or rapidly spreading.

In one example, the parental SARS-CoV-2 strain is a SARS-CoV-2 variant that is a variant of concern.

In one example, the parental SARS-CoV-2 strain is B.1.1.7.

In one example, the one or more SARS-CoV-2 S protein variants are modified compared to wildtype SARS-CoV-2 S protein.

In one example, the one or more SARS-CoV-2 S protein variants are modified compared to the parental SARS-CoV-2 S protein.

In one example, in the modified amino acid sequence amino acid positions in the one or more SARS-CoV-2 S protein variants which are modified compared to wildtype SARS-CoV-2 S protein and/or the parental SARS-CoV-2 S protein are modified.

In one example, one or more (e.g., all) of the one or more SARS-CoV-2 S protein variants are the S proteins of one or more SARS-CoV-2 strains.

In one example, one or more (e.g., all) of the one or more SARS-CoV-2 strains are natural isolates, or one or more (e.g., all) of the one or more SARS-CoV-2 strains are mutants of a natural isolate.

In one example, one or more (e.g., all) of the one or more SARS-CoV-2 strains are SARS-CoV-2 variant strains that are prevalent or rapidly spreading.

In one example, one or more (e.g., all) of the one or more SARS-CoV-2 strains are SARS-CoV-2 variant strains that are variants of concern.

In one example, one or more of the one or more SARS-CoV-2 strains are selected from the group consisting of B.1.351, B.1.1.298, B.1.427/B.1.429, B.1.526, and P1.

In one example, the parental SARS-CoV-2 strain and the one or more SARS-CoV-2 strains are SARS-CoV-2 variant strains that are prevalent or rapidly spreading.

In one example, the parental SARS-CoV-2 strain and the one or more SARS-CoV-2 strains are SARS-CoV-2 variant strains that are variants of concern.

In one example, the parental SARS-CoV-2 S protein and the one or more SARS-CoV-2 S protein variants are modified compared to wildtype SARS-CoV-2 S protein.

In one example, the parental SARS-CoV-2 strain is B.1.1.7 and the one or more SARS-CoV-2 strains are selected from the group consisting of B.1.351, B.1.1.298, B.1.427/B.1.429, B.1.526, P1.

In one example, the one or more SARS-CoV-2 S protein variants comprise SARS-CoV-2 S protein variants of at least two SARS-CoV-2 strains.

In one example, the one or more SARS-CoV-2 S protein variants in b) are different from the one or more SARS-CoV-2 S protein variants in c).

In one example, the one or more SARS-CoV-2 S protein variants in b) are the SARS-CoV-2 S protein variants of B.1.427/B.1.429, and B.1.526, and the one or more SARS-CoV-2 S protein variants in c) are the SARS-CoV-2 S protein variants of B.1.351, P.1, and B.1.1.298.

In one example, in the modified amino acid sequence amino acid modifications in the parental SARS-CoV-2 S protein compared to wildtype SARS-CoV-2 S protein do not interfere with amino acid modifications in the modified amino acid positions.

In one example, in the modified amino acid sequence amino acid modifications in the parental SARS-CoV-2 S protein compared to wildtype SARS-CoV-2 S protein are not in close spatial distance to modified amino acid positions or amino acid modifications in the modified amino acid positions.

In one example, in the modified amino acid sequence modifications in the modified amino acid positions do not result in major structural rearrangements.

In one example, the amino acids in the modified amino acid positions are surface exposed.

In one example, the modified amino acid positions comprise one or more, e.g., at least two amino acid positions. The modifications may correspond to one or more SARS-CoV-2 S protein variants/SARS-CoV-2 strains and may encompass all modifications present in the one

or more SARS-CoV-2 S protein variants/SARS-CoV-2 strains compared to the parental SARS-CoV-2 S protein/SARS-CoV-2 strain, or a portion thereof.

In one example, the modified amino acid positions in b) and c) each comprise one or more, e.g., at least two amino acid positions.

In one example, the modified amino acid positions comprise two or more selected from the group consisting of:

18, 20, 26, 80, 138, 144, 190, 215, 246, 253, 417, 439, 452, 453, 477, 484, 501, 570, 701, 716, 140, 345, 346, 352, 378, 406, 420, 440, 441, 444, 445, 446, 450, 455, 460, 475, 478, 485, 486, 487, 489, 490, 493, 494, 499,

142, 145, 146, 147, 150, 152, 154, 156, 157, 158, 164, 247, 248, 249, 250, 251, 252, 254, 255, 258, 365, 369, 370, 374, 376, 384, 405, 408, 415, 421, 443, 447, 448, 456, 472, 473, 476, 496, 498, 500, 504.

In one example, the modifications in the modified amino acid positions comprise two or more selected from the group consisting of:

18F, 20N, 26S, 80Y, 138Y, 144F, 190S, 215A, 246I, 253G, 417N, 439K, 452R, 453F, 477N, 484K, 501Y, 570D, 701V, 716I,

140L, 345A, 346K, 352S, 378N, 406Q, 420, 440K, 441F, 444, 445A, 446V, 450K, 455F, 460I, 475V, 478I, 485V, 486L, 487D, 489, 490S, 493L, 494P, 499H,

142S, 145H, 146Y, 147N, 150R, 152C, 154Q, 156A, 157L, 158G, 164T, 247G, 248H, 249S, 250N, 251S, 252V, 254F, 255F, 258L, 365D, 369C, 370S, 374L, 376I, 384L, 405Y, 408I, 415N, 421, 443A, 447V, 448Y, 456L, 472V, 473F, 476S, 496C, 498H, 500I, 504D.

In one example, the modifications in the modified amino acid positions comprise two or more selected from the group consisting of:

L18F, T20N, P26S, D80Y, D138Y, Y144F, R190S, D215A, R246I, D253G, K417N, N439K, L452R, Y453F, S477N, E484K, N501Y, A570D, A701V, T716I,

F140L, T345A, R346K, A352S, K378N, E406Q, D420, N440K, L441F, K444, V445A, G446V, N450K, L455F, N460I, A475V, T478I, G485V, F486L, N487D, Y489, F490S, Q493L, S494P, P499H,

G142S, Y145H, H146Y, K147N, K150R, W152C, E154Q, E156A, F157L, R158G, N164T, S247G, Y248H, L249S, T250N, P251S, G252V, S254F, S255F, W258L, Y365D, Y369C, N370S, F374L,

T376I, P384L, D405Y, R408I, T415N, Y421, S443A, G447V, N448Y, F456L, I472V, Y473F, G476S, G496C, Q498H, T500I, G504D.

In one example, the disclosure relates to a method comprising the steps:

a) providing a nucleic acid comprising a first nucleotide sequence encoding an amino acid sequence comprising at least a fragment of a parental SARS-CoV-2 S protein, wherein amino acid positions in the at least a fragment of a parental SARS-CoV-2 S protein are modified to comprise amino acids found in the corresponding amino acid positions of one or more SARS-CoV-2 S protein variants; and

b) providing a nucleic acid comprising a second nucleotide sequence encoding an amino acid sequence comprising at least a fragment of a parental SARS-CoV-2 S protein, wherein amino acid positions in the at least a fragment of a parental SARS-CoV-2 S protein are modified to comprise amino acids found in the corresponding amino acid positions of one or more SARS-CoV-2 S protein variants,

wherein the amino acid modifications in b) at least partially differ from the amino acid modifications in a).

In one example, the method may comprise one or more further such steps of providing a nucleic acid encoding at least partially different amino acid modifications.

In one example, the nucleic acid is RNA.

Further examples are as described herein.

In one example, the disclosure relates to a medical preparation comprising:

a) a nucleic acid comprising a first nucleotide sequence encoding an amino acid sequence comprising at least a fragment of a parental SARS-CoV-2 S protein, wherein amino acid positions in the at least a fragment of a parental SARS-CoV-2 S protein are modified to comprise amino acids found in the corresponding amino acid positions of one or more SARS-CoV-2 S protein variants; and

b) a nucleic acid comprising a second nucleotide sequence encoding an amino acid sequence comprising at least a fragment of a parental SARS-CoV-2 S protein, wherein amino acid positions in the at least a fragment of a parental SARS-CoV-2 S protein are modified to comprise amino acids found in the corresponding amino acid positions of one or more SARS-CoV-2 S protein variants,

wherein the amino acid modifications in b) at least partially differ from the amino acid modifications in a).

In one example, the medical preparation may comprise one or more further nucleic acids encoding at least partially different amino acid modifications.

In one example, the nucleic acid is RNA.

In one example, the RNA is formulated in lipid nanoparticles (LNP).

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

In one example, the medical preparation is a vaccine.

In one example, the medical preparation is a kit.

In one example, the medical preparation further comprises instructions for use of the medical preparation for vaccination against SARS-CoV-2 infection.

In one example, the disclosure relates to the medical preparation for pharmaceutical use.

In one example, the pharmaceutical use comprises vaccination against SARS-CoV-2 infection.

In one example, the disclosure relates to a method of inducing an immune response against SARS-CoV-2 in a subject comprising administering to the subject the medical preparation.

In one example, the method is a method for prophylactic treatment against SARS-CoV-2 infection.

In one example, the method is a method for vaccination against SARS-CoV-2 infection.

Further examples of the above examples are as described herein.

The nucleic acid described herein may be single-stranded RNA and the medical preparation, e.g., vaccine, described herein may comprise as the active principle single-stranded RNA that may be translated into the respective protein upon entering cells of a recipient. In addition to wildtype or codon-optimized sequences encoding the antigen sequence, the RNA may contain one or more structural elements optimized for maximal efficacy of the RNA with respect to stability and translational efficiency (5' cap, 5' UTR, 3' UTR, poly(A)-tail). In one example, the RNA contains all of these elements. In one example, beta-S-ARCA(D1) ($m_2^{7,2'\text{-}O}$GppSpG) or $m_2^{7,3'\text{-}O}$Gppp($m_1^{2'\text{-}O}$)ApG may be utilized as specific capping structure at the 5'-end of the RNA drug substances. As 5'-UTR sequence, the 5'-UTR sequence of the human

alpha-globin mRNA, optionally with an optimized 'Kozak sequence' to increase translational efficiency (e.g., SEQ ID NO: 12) may be used. As 3'-UTR sequence, a combination of two sequence elements (FI element) derived from the "amino terminal enhancer of split" (AES) mRNA (called F) and the mitochondrial encoded 12S ribosomal RNA (called I) (e.g., SEQ ID NO: 13) placed between the coding sequence and the poly(A)-tail to assure higher maximum protein levels and prolonged persistence of the mRNA may be used. These were identified by an *ex vivo* selection process for sequences that confer RNA stability and augment total protein expression (see WO 2017/060314, herein incorporated by reference). Alternatively, the 3′ UTR may be two re-iterated 3'-UTRs of the human beta-globin mRNA. Furthermore, a poly(A)-tail measuring 110 nucleotides in length, consisting of a stretch of 30 adenosine residues, followed by a 10 nucleotide linker sequence (of random nucleotides) and another 70 adenosine residues (e.g., SEQ ID NO: 14) may be used. This poly(A)-tail sequence was designed to enhance RNA stability and translational efficiency.

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

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

In one example, the vaccine antigens comprising polyspecific SARS-CoV-2 S protein amino acid sequences described herein are able to form a multimeric complex, in particular a trimeric complex. In one example, vaccine antigens comprising different polyspecific SARS-CoV-2 S protein amino acid sequences described herein are able to form a multimeric complex, in particular a trimeric complex. Thus, in examples of the disclosure which comprise provision of vaccine antigens comprising different polyspecific SARS-CoV-2 S protein amino acid sequences described herein to a subject, e.g., by administering different nucleic acids

encoding the vaccine antigens comprising different polyspecific SARS-CoV-2 S protein amino acid sequences, the different polyspecific SARS-CoV-2 S protein amino acid sequences may be able to form a multimeric complex, in particular a trimeric complex. To this end, the polyspecific SARS-CoV-2 S protein amino acid sequences described herein may comprise a domain allowing the formation of a multimeric complex, in particular a trimeric complex of the polyspecific SARS-CoV-2 S protein amino acid sequences. In one example, the domain allowing the formation of a multimeric complex comprises a trimerization domain, for example, a trimerization domain as described herein, e.g., SARS-CoV-2 S protein trimerization domain. In one example, trimerization is achieved by addition of a trimerization domain, e.g., a T4-fibritin-derived "foldon" trimerization domain (e.g., SEQ ID NO: 10), to the polyspecific SARS-CoV-2 S protein amino acid sequence, in particular if the polyspecific SARS-CoV-2 S protein amino acid sequence corresponds to a portion of a SARS-CoV-2 S protein that does not comprise the SARS-CoV-2 S protein trimerization domain.

In one example, a vaccine antigen comprising a polyspecific virus protein amino acid sequence described herein is encoded by a coding sequence which is codon-optimized and/or the G/C content of which is increased compared to wild type coding sequence, wherein the codon-optimization and/or the increase in the G/C content preferably does not change the sequence of the encoded amino acid sequence.

In one example, a vaccine antigen comprising a polyspecific virus protein amino acid sequence is encoded by RNA. In a particularly preferred example, a vaccine antigen comprising a polyspecific virus protein amino acid sequence is encoded by an isolated messenger ribonucleic acid (mRNA) polynucleotide, wherein the isolated mRNA polynucleotide comprises an open reading frame encoding a polypeptide that comprises the vaccine antigen. In one example, the isolated mRNA polynucleotide is formulated in at least one lipid nanoparticle. For example, in some examples, such a lipid nanoparticle may comprise a molar ratio of 20-60% ionizable cationic lipid, 5-25% non-cationic lipid (e.g., neutral lipid), 25-55% sterol or steroid, and 0.5-15% polymer-conjugated lipid (e.g., PEG-modified lipid). In some examples, a sterol or steroid included in a lipid nanoparticle may be or comprise cholesterol. In some examples, a neutral lipid may be or comprise 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC). In some examples, a polymer-conjugated

lipid may be or comprise PEG2000 DMG. In some examples, an immunogenic composition may comprise a total lipid content of about 1 mg to 10 mg, or 3 mg to 8 mg, or 4 mg to 6 mg. In some examples, such an immunogenic composition may comprise a total lipid content of about 5 mg/mL-15 mg/mL or 7.5 mg/mL-12.5 mg/mL or 9-11 mg/mL. In some examples, such an isolated mRNA polynucleotide is provided in an effective amount to induce an immune response in a subject administered at least one dose of the immunogenic composition. In some examples, such an isolated mRNA polynucleotide provided in an immunogenic composition is not self-replicating RNA.

In one example, the RNA described herein is a modified RNA, in particular a stabilized mRNA.

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

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

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

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

In one example, the RNA comprises a 5’ UTR comprising the nucleotide sequence of SEQ ID NO: 12, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 12.

In one example, the RNA comprises a 3’ UTR comprising the nucleotide sequence of SEQ ID NO: 13, or a nucleotide sequence having at least 99%, 98%, 97%, 96%, 95%, 90%, 85%, or 80% identity to the nucleotide sequence of SEQ ID NO: 13.

In one example, the RNA comprises a poly-A sequence.

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

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

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

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

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

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

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

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

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

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

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

In one example, the RNA is mRNA or saRNA.

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

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

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

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

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

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

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

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

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

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

In one example, the coronavirus is a betacoronavirus.

In one example, the coronavirus is a sarbecovirus.

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

In one example, the disclosure relates to a method of inducing an immune response against coronavirus in a subject comprising administering to the subject a composition described herein, e.g., a vaccine antigen comprising a polyspecific SARS-CoV-2 S protein amino acid sequence or a nucleic acid, e.g., RNA, encoding the vaccine antigen. In one example, the method comprises administration of a plurality of such vaccine antigens or nucleic acids.

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

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

In one example, the subject is a human.

In one example, the coronavirus is a betacoronavirus.

In one example, the coronavirus is a sarbecovirus.

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

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

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

In one example, the disclosure relates to a composition, e.g., protein or nucleic acid composition, obtainable by practicing a method described herein.

Claims

1. A method comprising the steps:
a) identifying amino acid positions in a parental virus protein which are modified compared to the corresponding amino acid positions of one or more virus protein variants; and
b) providing an amino acid sequence comprising at least a fragment of the parental virus protein, wherein amino acid positions in the at least a fragment of a parental virus protein are modified to comprise amino acids found in the corresponding amino acid positions of one or more virus protein variants, or a nucleotide sequence encoding the modified amino acid sequence.

2. The method of claim 1, which comprises repeating step b) to provide two or more of the modified amino acid sequences, or two or more of the nucleotide sequences encoding two or more of the modified amino acid sequences.

3. The method of claim 2, wherein the two or more modified amino acid sequences are based on the same parental virus protein.

4. The method of claim 2 or 3, wherein the amino acid modifications in the two or more modified amino acid sequences are at least partially different.

5. The method of any one of claims 1 to 4, wherein providing the nucleotide sequence comprises:
b') substituting codons of a nucleotide sequence encoding an amino acid sequence comprising at least a fragment of a parental virus protein with other codons to obtain a mutated nucleotide sequence that encodes a modified amino acid sequence, wherein amino acid positions in the at least a fragment of a parental virus protein are modified to comprise amino acids found in the corresponding amino acid positions of one or more virus protein variants.

6. The method of claim 5, which comprises repeating step b') to provide two or more of the mutated nucleotide sequences encoding two or more of the modified amino acid sequences.

7. The method of claim 6, wherein the two or more modified amino acid sequences are based on the same parental virus protein.

8. The method of claim 6 or 7, wherein the amino acid modifications in the two or more modified amino acid sequences are at least partially different.

9. A method comprising the steps:
a) identifying amino acid positions in a parental virus protein which are modified compared to the corresponding amino acid positions of one or more virus protein variants;
b) substituting codons of a first nucleotide sequence encoding an amino acid sequence comprising at least a fragment of a parental virus protein with other codons to obtain a first mutated nucleotide sequence that encodes a modified amino acid sequence, wherein amino acid positions in the at least a fragment of a parental virus protein are modified to comprise amino acids found in the corresponding amino acid positions of one or more virus protein variants; and
c) substituting codons of a second nucleotide sequence encoding an amino acid sequence comprising at least a fragment of a parental virus protein with other codons to obtain a second mutated nucleotide sequence that encodes a modified amino acid sequence, wherein amino acid positions in the at least a fragment of a parental virus protein are modified to comprise amino acids found in the corresponding amino acid positions of one or more virus protein variants,
wherein the amino acid modifications in b) at least partially differ from the amino acid modifications in c).

10. The method of claim 9, wherein the at least a fragment of a parental virus protein comprised in the amino acid sequence encoded by the first nucleotide sequence and the at least a fragment of a parental virus protein comprised in the amino acid sequence encoded by the second nucleotide sequence are identical.

11. The method of claim 9 or 10, wherein the amino acid sequence encoded by the first nucleotide sequence and the amino acid sequence encoded by the second nucleotide sequence are identical.

12. The method of any one of claims 9 to 11, wherein the first nucleotide sequence and the second nucleotide sequence are identical.

13. The method of any one of claims 9 to 12, wherein one or more of the modified amino acid positions in the modified amino acid sequence encoded by the first mutated nucleotide sequence differ from the modified amino acid positions in the modified amino acid sequence encoded by the second mutated nucleotide sequence.

14. The method of any one of claims 9 to 13, wherein one or more amino acids in modified amino acid positions modified in the modified amino acid sequence encoded by the first mutated nucleotide sequence and in the modified amino acid sequence encoded by the second mutated nucleotide sequence differ from each other.

15. The method of any one of claims 1 to 14, wherein the amino acid sequence comprising at least a fragment of a parental virus protein comprises the amino acid sequence of a full-length virus protein.

16. The method of any one of claims 1 to 15, further comprising providing nucleic acid comprising the nucleotide sequence encoding a modified amino acid sequence.

17. The method of any one of claims 1 to 16, further comprising providing a vaccine comprising nucleic acid comprising the nucleotide sequence encoding a modified amino acid sequence.

18. The method of claim 16 or 17, wherein the nucleic acid is RNA.

19. The method of any one of claims 1 to 18, which is a method of generating a vaccine.

20. The method of any one of claims 17 to 19, wherein the vaccine is an RNA vaccine.

21. The method of any one of claims 17 to 20, wherein the vaccine has a reduced risk for immune escape.

22. The method of any one of claims 1 to 21, wherein the modified amino acid positions are amino acid positions at which the amino acid sequence of the one or more virus protein variants differs from the amino acid sequence of the parental virus protein.

23. The method of any one of claims 1 to 22, wherein the modified amino acid positions are amino acid positions at which the amino acid sequence of the one or more virus protein variants differs from the amino acid sequence of the wildtype virus protein.

24. The method of any one of claims 1 to 23, wherein the modified amino acid positions are potential sites for escape mutants of the virus.

25. The method of claim 24, wherein the escape mutants of the virus are antibody escape mutants of the virus.

26. The method of claim 24 or 25, wherein the escape mutants of the virus are resistant to neutralization by antibody against the virus protein.

27. The method of any one of claims 24 to 26, wherein the virus protein of the escape mutants of the virus shows reduced antibody binding.

28. The method of any one of claims 25 to 27, wherein the antibody is used for treating a patient infected with the virus.

29. The method of any one of claims 25 to 27, wherein the antibody is generated in a patient that has been treated with a vaccine against infection with the virus.

30. The method of any one of claims 1 to 29, wherein the parental virus protein is modified compared to the wildtype virus protein.

31. The method of any one of claims 1 to 30, wherein in the modified amino acid sequence amino acid positions in the parental virus protein which are modified compared to the wildtype virus protein are not modified.

32. The method of any one of claims 1 to 31, wherein the parental virus protein is the virus protein of a parental virus strain.

33. The method of claim 32, wherein the parental virus strain is a natural isolate, or the parental virus strain is a mutant of a natural isolate.

34. The method of claim 32 or 33, wherein the parental virus strain is a virus variant strain that is prevalent or rapidly spreading.

35. The method of any one of claims 32 to 34, wherein the parental virus strain is a virus variant that is a variant of concern.

36. The method of any one of claims 1 to 35, wherein the one or more virus protein variants are modified compared to the wildtype virus protein.

37. The method of any one of claims 1 to 36, wherein the one or more virus protein variants are modified compared to the parental virus protein.

38. The method of any one of claims 1 to 37, wherein in the modified amino acid sequence amino acid positions in the one or more virus protein variants which are modified compared to the wildtype virus protein and/or the parental virus protein are modified.

39. The method of any one of claims 1 to 38, wherein one or more of the one or more virus protein variants are the virus proteins of one or more virus strains.

40. The method of claim 39, wherein one or more of the one or more virus strains are natural isolates, or one or more of the one or more virus strains are mutants of a natural isolate.

41. The method of claim 39 or 40, wherein one or more of the one or more virus strains are virus variant strains that are prevalent or rapidly spreading.

42. The method of any one of claims 39 to 41, wherein one or more of the one or more virus strains are virus variant strains that are variants of concern.

43. The method of any one of claims 39 to 42, wherein the parental virus strain and the one or more virus strains are virus variant strains that are prevalent or rapidly spreading.

44. The method of any one of claims 39 to 43, wherein the parental virus strain and the one or more virus strains are virus variant strains that are variants of concern.

45. The method of any one of claims 1 to 44, wherein the parental virus protein and the one or more virus protein variants are modified compared to the wildtype virus protein.

46. The method of any one of claims 1 to 45, wherein the one or more virus protein variants comprise virus protein variants of at least two virus strains.

47. The method of any one of claims 9 to 46, wherein the one or more virus protein variants in b) are different from the one or more virus protein variants in c).

48. The method of any one of claims 1 to 47, wherein in the modified amino acid sequence amino acid modifications in the parental virus protein compared to the wildtype virus protein do not interfere with amino acid modifications in the modified amino acid positions.

49. The method of any one of claims 1 to 48, wherein in the modified amino acid sequence amino acid modifications in the parental virus protein compared to the wildtype virus protein are not in close spatial distance to amino acid modifications in the modified amino acid positions.

50. The method of any one of claims 1 to 49, wherein in the modified amino acid sequence modifications in the modified amino acid positions do not result in major structural rearrangements.

51. The method of any one of claims 1 to 50, wherein the amino acids in the modified amino acid positions are surface exposed.

52. The method of any one of claims 1 to 51, wherein the modified amino acid positions comprise at least two amino acid positions.

53. The method of any one of claims 9 to 52, wherein the modified amino acid positions in b) and c) each comprise at least two amino acid positions.

54. A method comprising the steps:
a) providing a nucleic acid comprising a first nucleotide sequence encoding an amino acid sequence comprising at least a fragment of a parental virus protein, wherein amino acid positions in the at least a fragment of a parental virus protein are modified to comprise amino acids found in the corresponding amino acid positions of one or more virus protein variants; and
b) providing a nucleic acid comprising a second nucleotide sequence encoding an amino acid sequence comprising at least a fragment of a parental virus protein, wherein amino acid positions in the at least a fragment of a parental virus protein are modified to comprise amino acids found in the corresponding amino acid positions of one or more virus protein variants,
wherein the amino acid modifications in b) at least partially differ from the amino acid modifications in a).

55. The method of claim 54, wherein the nucleic acid is RNA.

56. A medical preparation comprising:
a) a nucleic acid comprising a first nucleotide sequence encoding an amino acid sequence comprising at least a fragment of a parental virus protein, wherein amino acid positions in the at least a fragment of a parental virus protein are modified to comprise amino acids found in the corresponding amino acid positions of one or more virus protein variants; and
b) a nucleic acid comprising a second nucleotide sequence encoding an amino acid sequence comprising at least a fragment of a parental virus protein, wherein amino acid positions in the

at least a fragment of a parental virus protein are modified to comprise amino acids found in the corresponding amino acid positions of one or more virus protein variants,
wherein the amino acid modifications in b) at least partially differ from the amino acid modifications in a).

57. The medical preparation of claim 56, wherein the nucleic acid is RNA.

58. The medical preparation of claim 57, wherein the RNA is formulated in lipid nanoparticles (LNP).

59. The medical preparation of any one of claims 56 to 58, which is a pharmaceutical composition.

60. The medical preparation of any one of claims 56 to 59, which is a vaccine.

61. The medical preparation of any one of claims 56 to 60, which is a kit.

62. The medical preparation of claim 61, further comprising instructions for use of the medical preparation for vaccination against infection with the virus.

63. The medical preparation of any one of claims 56 to 62 for pharmaceutical use.

64. The medical preparation of claim 63, wherein the pharmaceutical use comprises vaccination against infection with the virus.

65. Use of the medical preparation of any one claims 56 to 64 in the manufacture of a medicament for inducing an immune response against a virus in a subject.

66. The use of claim 65, wherein the use is for prophylactic treatment against infection with the virus.

67. The use of claim 65 or 66, wherein the use is for vaccination against infection with the virus.

68. The medical preparation of any one claims 56 to 64 for use in therapy.